Malignant Transformation of Oral Submucous Fibrosis

Omar Kujan and Majdy Idrees

Contents

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7.1 Introduction

Oral potentially malignant disorders (OPMDs) include a variety of lesions and conditions with different aetiologies and clinicopathological presentations, but all share an increased risk of malignant transformation [1]. One of the most prevalent OPMDs is oral submucous fibrosis (OSF), which is a relatively widespread disorder in Southeast Asia and in the Pacific [2, 3].

Several chapters in this textbook have addressed significant aspects of this disorder such as epidemiology, aetiology, pathogenesis, clinical and histopathological features, staging, and predictive and prognostic biomarkers. This chapter aims to provide an updated review of the malignant transformation potential of oral submucous fibrosis.

Paymaster, in 1956, first described the potential malignant nature of OSF among a cohort of 650 patients [4]. Since then, many reports have been published to describe the malignant transformation rate of OSF, with estimations ranging from 1.2% to 9.1% [3]. However, these reports are associated with a very high level of heterogeneity and a relatively low quality [3, 5], which may indicate that the actual number is underestimated. A recent meta-analysis published in a special issue by the WHO Collaborating Centre for Oral Cancer on OPMDs revealed that the pooled ratio of malignant transformation among patients with OSF is 4.6%, and the annual malignant transformation rate is 0.73% [3].

Learning Goals

- Provide a global estimation of the malignant transformation rate of oral submucous fibrosis.
- Discuss the related literature in detail and highlight the major outcomes of each individual study.
- Explain the demographic, pathological, and molecular factors contributing to the malignant transformation of oral submucous fibrosis.

7.2 Insight into the Literature

An extensive search of the literature was conducted through various databases (Web of Science, MEDLINE by PubMed, Embase, Scopus) for the period from 1952 to October 2021 using special MeSH terms [3] to find relevant studies that documented the rate and/or risk of malignant transformation of OSF. The search revealed 611 papers, which were screened. Of these, 14 papers underwent further assessment. Of these papers, nine reported the malignant transformation rate of OSF [6–14] (Table 7.1), while the others were either cross-sectional or case-control without further details to assess the malignant transformation rate [15–19] (Table 7.2).

Table 7.1 General description of relevant studies about the malignant transformation rate and/or risk of OSF

Authors/year	Country	Design	Total (N) M:F	Mean age (years)	MT (N)	Overall MT ratio	Annual MT rate	Follow-up (years)	General description/main findings
Jian et al. 2021 [14]	China	Prospective	567	–	32	5.6%	–	–	An institutional prospective study reported from Hunan province in China. All OSF cases were among areca nut chewers. Patients with verrucopapillary lesions or leukoplakia had higher transformation
Chiang et al. 2020 [13]	Taiwan	Prospective	87	–	4	4.6%	0.69%	6.7	To assess the MT rate among a group of OPMDs. The independent risk factor for the MT of OPMDs was heavy betel quid chewing. No data were specifically provided for OSF
Chuang et al. 2018 [12]	Taiwan	Prospective	2333 No females	–	114	4.8%	0.86%	5.7	To assess the MT rate among OPMDs and identify risk factors. The estimated annual malignant risk per 1000 was 8.9 for OSF with betel nut chewing. The risk of OSF MT was higher among patients aged more than 50 years

Table 7.1 (continued)

Authors/ year	Country	Design	Total (N) M:F	Mean age (years)	MT (N)	Overall MT ratio	Annual MT rate	Follow-up (years)	General description/main findings
Yang et al. 2017 [11]	Taiwan	Retrospective	778 678:100	41.8 ± 11.7	71	9.1%	1.4%	6.5	To investigate the MT rate of OSF. Patients with OSF are associated with a significantly higher risk of MT than controls. OLK enhances the MT potential when combined with OSF. This risk of MT was higher among the males in comparison to the females
Chourasia et al., 2015 [10]	India	Retrospective	119 88:31	33.8	5	4.2%	–	–	The incidence of oral cancer concomitant with OSF was 25.77%. The authors concluded that the malignant potential of OSF is underestimated
Wang et al. 2014 [9]	Taiwan	Retrospective	1180 1091:89	44.69 ± 12.43	46	3.9%	–	–	To assess the MT risk among a group of OPMDs. Risk factors were not identified for individual OSF cases. 88.43% and 86.2% of OSF cases were associated with betel quid and smoking, respectively
Hazarey et al. 2007 [8]	India	Retrospective	1000 830: 170	M 27.60 ± 9.58 F 34.78 ± 12.21	33	3.3%	0.66%	5	OSF MT was significantly associated with increased frequency of betel quid chewing and smoking. The majority of the malignant transformed cases were diagnosed as OSCC, 28 out of 33 cases
Hsue et al. 2007 [7]	Taiwan	Prospective	439	–	10	2.3%	0.63%	3.6	To assess the rate of OSF MT. The mean time of MT was shorter for cases with epithelial dysplasia; however, it was not significant
Murti et al. 1985 [6]	India	Prospective	66	–	5	7.6%	0.76%	10[a]	To assess the OSF MT rate. All transformed cases were in women who had habits of chewing tobacco and areca nut with betel leaves and lime
Total (studies that assessed the OSF MT rate)		**6569**	**–**		**320 (4.87%)**		**0.84%**	**6.25**	

M male, *F* female, *MT* malignant transformation, *OLK* oral leukoplakia, *OLP* oral lichen planus, *OSCC* oral squamous cell carcinoma

[a] Median follow up period

Table 7.2 Cross-sectional studies with no information on MT

Authors/year	Country	Design	Total (N) M:F	Mean age (years)	General description/main findings
Srivastava et al. 2020 [15]	India	Cross-sectional	2150 1865:285	Not specified	To assess the prevalence of OPMDs and oral cancers. The major risk factors were tobacco and areca nut, with or without tobacco. The malignant transformation rate of OSF was not assessed
Rangaswamy et al. 2019 [16]	India	Prospective case series	30 23: 7	44.5	To describe the features of oral carcinomas in the background of OSF. No controls. OSF-related carcinomas have distinct clinical presentations The malignant transformation rate of OSF was not assessed
Wang et al. 2018 [17]	Taiwan	Retrospective	181	Not specified	The aim was to study the MT of OLK alone and OLK with other oral lesions. No data was specified for OSF alone. Patients with both OSF and OLK had 58 more risks for MT. The malignant transformation rate of OSF was not assessed
Mohiuddin et al. 2016 [18]	Pakistan	Cross-sectional	765 OSF 472 OSF-OSCC	Not specified	To assess the risk of MT of OSF. 48.3% of transformed cases were related to betel quid with tobacco. The malignant transformation rate of OSF was not assessed
Zhou et al. 2008 [19]	China	Case-control	40 OSF 42 OSF-OSCC	(OSF-OSCC 45) (OSF 38)	To assess the risk of OSF MT. Odds ratios were older age 12.59, duration of chewing 10.15, smoking 7, OSF with OLK or OLP 8.04. The malignant transformation rate of OSF was not assessed

M male, *F* female, *MT* malignant transformation, *OLK* oral leukoplakia, *OLP* oral lichen planus, *OSCC* oral squamous cell carcinoma

The geographic distribution of the reported studies is consistent with the global distribution of OSF, as almost all cases were reported in India and Southeast Asia [20]. Of these studies, six were conducted in India [6, 8, 10, 15, 16, 21] and Taiwan [7, 9, 11–13, 17], followed by two studies in Mainland China [14, 19] and one study in Pakistan [18].

7.3 Malignant Transformation Rate among OSF Patients

Iocca et al., in 2019, conducted a systematic review and meta-analysis to assess the malignant transformation rate among a group of OPMDs [5]. They found that out of 3986 OSF patients, 194 patients exhibited malignant transformation (4.8%) [5]. A comparable result was reported in a more recent meta-analysis by Kujan et al. in 2021: out of 6337 OSF patients, 292 patients underwent malignant transformation (4.6%), with an annual malignant transformation rate of 0.73% [3].

In India, the malignant transformation rate of OSF ranges from 3.3% to 7.6% [6, 8, 10], while studies from Mainland China and Taiwan reveal a malignant transformation rate between 2.3% and 9.1% [7, 9, 11–14]. Previous subgroup analysis showed no significant difference in the malignant transformation rate between "Mainland China and Taiwan" and India, at 4.9% and 3.5%, respectively [3]. Likewise, adjusted odds ratios did not report a significant difference between Mainland China and Taiwan [22]. Based on our literature search, we did not find epidemiological information about the malignant transformation rate of OSF in Vietnam, Pakistan, Thailand, Bangladesh, Sri Lanka, or Nepal.

The malignant potential of OSF is higher or comparable to other OPMDs, such as oral leukoplakia and oral lichenoid lesions; hence, OSF is considered a condition with significant morbidity and mortality rates [5, 23]. However, the malignant transformation potential of OSF has gained less attention among researchers because the literature includes only two meta-analyses in this area [3, 5], in comparison to many studies assessing the malignant transformation of other OPMDs. In addition, studies that report malignant transformation of OSF cases are associated with high heterogeneity and low methodological quality [3, 5]. We believe that the reported number of OSF cases in general, and malig-

nant transformation cases in particular, might be much lower than the real situation.

In India, for example, a recent, nationally representative study in 2021 estimated that the number of areca nut users is more than 223 million people, and the majority of them consume areca nut with tobacco [24]. At the same time, the International Agency for Research on Cancer revealed that more than 35% of global oral and lip cancer cases are in India [25]. This is much higher than in China, including Taiwan (7.9%), although the population in both countries is comparable—around 1.4 billion [26]. Nonetheless, the literature includes only three Indian studies that assessed the malignant transformation rate among cohorts of 3500 OSF patients [6, 8, 10].

We found that the global malignant transformation rate of OSF among nine studies including 6569 OSF patients was 4.87% with an annual malignant transformation rate of 0.84%. Of these studies, five studies were retrospective while the other four were prospective as shown in Table 7.1. The follow-up time ranged from 3 to 10 years with a mean follow-up time of 6.25 years. The age and gender of OSF patients undergoing malignant transformation were only mentioned in one study by Murti et al. [6] The specific descriptions of the included studies with a brief outcome are presented below:

Murti et al. [6] followed up 66 Indian patients with OSF for 17 years with a median observation of 10 years. Patients were diagnosed clinically and followed up annually to detect any malignant transformation changes. The authors mentioned that surgical biopsies were performed according to the patient's consent; however, it was not clear how many patients underwent surgical biopsies during the follow-up time. At the end of the observation period, a malignant transformation of OSF lesions was detected among five female patients aged between 48 and 81 years (average age 64.6 years), giving a malignant transformation rate of 7.6%. The time between the initial diagnosis of OSF and the malignant transformation ranged between 3 and 16 years. All patients with malignant transformation had the habit of chewing areca nut with betel leaves and lime and with or without tobacco.

Hsue et al. [7] followed up a cohort of 1458 Taiwanese patients with various OPMDs including 439 patients with OSF. The patients were followed up for over 10 years, while the mean follow-up time was 42.6 months (3.5 years). Of the patients with OSF, eight cases progressed to carcinoma (2.3%), and two of them were associated with epithelial dysplasia. The mean duration of malignant transformation of the OSF patients with and without epithelial dysplasia was 40 months (3.3 years) and 52.3 months (4.4 years), respectively. However, this difference was not significant.

Hazarey et al. [8] carried out a retrospective hospital-based study among a cohort of 1000 Indian patients with OSF, 830 males, and 170 females. Clinical diagnosis of OSF patients was confirmed by surgical biopsies among a subgroup of cases; however, the number of these cases is unknown. Of the OSF patients, 33 cases (3.3%) transformed to malignancy; most of the malignant cases (28 cases) were diagnosed as OSCCs, while the remaining five cases were diagnosed as verrucous carcinomas. The gender and age of the malignant transformed cases were not mentioned; however, the authors reported significant associations between the malignant transformation of OSF cases and the frequency and duration of smoking and betel quid/tobacco chewing.

Zhou et al. [19] conducted a case-control study among a cohort of 82 Chinese patients (40 with OSCC at the background of OSF and 40 with OSF as controls) to assess the risk factors of OSF malignant transformation. The vast majority of the included subjects were males (97.6%). The mean age of patients in the OSF-OSCC group and OSF control group was 45 years and 38 years, respectively. The most common site of carcinoma involvement was the tongue (61.9%), followed by the buccal mucosa (28.6%) and the gingiva (9.5%). The authors reported significant associations between the malignant transformation of OSF and patient age (p = 0.001, OR 12.59), duration of betel quid chewing (p = 0.008, OR 10.15), duration of cigarette smoking (p = 0.025, OR 7), and concomitant presentations with oral leukoplakia or oral lichen planus (p = 0.019, OR 8.04).

Wang et al. [9] performed a retrospective study among a cohort of 5071 Taiwanese patients with OPMDs; all cases were associated with histopathological assessments. Of these, 1180 patients were diagnosed with OSF (994 OSF cases without epithelial dysplasia and 186 OSF cases with epithelial dysplasia). The mean age of patients at the time of diagnosis was 44.7 ± 12.4 years for OSF patients without dysplasia and 47.7 ± 11.8 years for OSF patients with epithelial dysplasia. Of all OSF cases, the malignant transformation was reported in 46/1180 cases (3.9%); 40 cases of them were diagnosed as OSCCs, and 6 cases were diagnosed as verrucous carcinomas. The rate of the malignant transformation was higher among OSF patients with epithelial dysplasia than OSF cases without epithelial dysplasia, 4.8% vs. 3.7%. The mean duration of the

malignant transformation was higher for OSF patients with epithelial dysplasia (3.6 years) in comparison to those without epithelial dysplasia (3.12 years). Although the authors found that the malignant transformation of OPMDs is statistically significantly associated with patients aged more than 45 years ($p = 0.03$) and male patients ($p = 0.001$), no data were provided specifically for OSF.

Chourasia et al. [10], conducted a retrospective study among a cohort of 344 Indian patients (225 patients with OSCC and 119 patients with OSF) to assess the incidence of OSCC arising secondary to OSF as well as the associated risk factors. Of the OSF patients, there were 88 males and 31 females; more than 97% of them had the habit of areca nut/tobacco chewing. Five OSF patients progressed to carcinoma, giving a malignant transformation rate of 4.2%. However, the mean duration taken for malignant transformation was not specified. The incidence of the concomitant presentations of OSCC with OSF was statistically significant ($p < 0.05$), and it was reported in 25.7% of the OSCC cases.

Mohiuddin et al. [18] carried out a cross-sectional multicentre study among Pakistani patients diagnosed with OSCC and/or OSF between 2004 and 2012 with a major aim to identify the risk factors for the malignant transformation of OSF. The malignant transformation rate of OSF was not assessed in this study. However, a statically significant association between various chewing habits and OSF malignant transformation was reported, $p = 0.001$.

Yang et al. [11] performed a retrospective study to assess the malignant transformation rate of OSF among Taiwanese patients. Data were retrieved from Taiwan's National Health Insurance Research Database and included 778 OSF patients in addition to a control group of 43,568 non-OSF individuals. OSF patients were predominantly males (87.1%), while the mean age of patients was 41.8 ± 11.7 years. The hazard ratios (HRs) were calculated to assess the OSF-associated risk of malignancy. The malignant transformation rate among the OSF patients was higher than that of the control group, 9.1% and 0.3%, respectively. The mean duration of the malignant transformation was 2.5 years for the OSF patients and 5.1 years for the controls. The authors concluded that OSF patients were associated with a higher risk of malignancy in comparison to the control group (adjusted HR: 29.3; 95% CI: 20.5–41.7). The risk of malignancy was higher among the male OSF patients in comparison to the female OSF patients (adjusted HR: 14.5; 95% CI: 3.6–58.6). To further stratify the risk of malignancy among OSF patients, the authors reported that the concomitant presence of oral leukoplakia increased the malignant transformation risk by up to 52.5 times in comparison to the non-OSF patients.

Wang et al. [17] conducted a retrospective study among a cohort of 11,898 Taiwanese patients with oral leukoplakia. Although this study aimed mainly to assess the malignant transformation risk of oral leukoplakia, the possible synergistic effects between oral leukoplakia and OSF in the malignant transformation were analysed. The authors reported that OSF enhances the malignant transformation of oral leukoplakia and increases the risk of malignancy: adjusted HR 27.1 (95% CI: 18.9–38.6) for oral leukoplakia alone in comparison to controls and adjusted HR 53.4 (95% CI: 34.6–98.5) for oral leukoplakia and OSF in comparison to the controls. However, the mean duration of the malignant transformation was lower for the cases of oral leukoplakia alone than the cases with oral leukoplakia and OSF, 1.8 years and 2.6 years, respectively.

Chuang et al. [12] performed a prospective study with an average follow-up time of 5.7 years for a cohort of 8501 Taiwanese patients with OPMDs. Of these, there were 2333 OSF patients and all of them were males. The malignant transformation rate among the OSF patients was 4.9%. The estimated annual malignant transformation risk per 1000 person-years for OSF betel nut chewing was 8.9. This risk was higher for OSF patients with alcohol-drinking habits, 10.0 per 1000. OSF patients aged between 50 and 69 years were associated with a higher annual malignant transformation risk (10.2 per 1000) in comparison to patients in other age groups.

Rangaswamy et al. [16] performed a prospective case series to describe 30 cases of OSCC in the background of OSF related to Indian patients. The mean age of patients was 44.5 years. More than four-quarters of the cases were associated with males, and more than 73% of the cases were linked to a previous history of gutkha usage.

Chiang et al. [13] retrospectively assessed 555 Taiwanese patients with OPMDs for a mean follow-up period of 6.7 years. Out of the 87 OSF cases in this cohort, four patients developed malignancy (4.6%). The duration of the malignant transformation ranged between 6 and 60 months. Heavy betel quid chewing was reported as a significant independent risk for the malignant transformation of OPMDs; however, no specific data were provided for OSF alone.

Srivastava et al. [15] conducted a cross-sectional descriptive study to assess the prevalence of OPMDs among 3735 Indian patients with oral lesions. A group of 9060 healthy subjects without a history of oral lesions were included as a control group. Of the OPMDs group, 2150 patients were diagnosed with OSF. The OSF malignant transformation rate was not assessed in this study;

however, the authors concluded that betel quid chewing, with or without tobacco, is the major risk factor for OPMDs and oral cancers.

Jian et al. [14] reported a prospective study conducted in a single institution in Hunan province in China. Among 567 patients enrolled from 1986 to 2017 and diagnosed with OSF, 32 cases transformed into OSCC (32/567, 5.6%). In 1 patient (3%), the malignant transformation was observed 24 years after the initial diagnosis. The presence of oral leukoplakia increased the rate of malignant transformation.

7.4 Epithelial Dysplasia and OSF

The presence of epithelial dysplasia increases the malignant transformation risk among OPMDs in general [27] and OSF in particular [3, 28–30]. However, among the studies that assessed the malignant transformation rate of OSF, we found that only two studies included the presence or absence of epithelial dysplasia in their cohorts [7, 9]. None of these studies differentiated between the grades of dysplasia. This may be attributed to the classic presumption that fibrosis in OSF starves the tissue and reduces epithelial thickness as a result of blood vessel construction, which in turn reduces the probability of epithelial dysplasia [29]. However, a more logical presumption hypothesizes that the reduction of blood supply would lead to the accumulation of carcinogens in the epithelium for a longer time, which in turn would increase the genotoxicities of these carcinogens and stimulate epithelial changes and malignant transformation [29].

In 2011, Jayasooriya et al. documented a significant increase in the incidence of epithelial dysplasia as the fibrosis thickness increased ($p = 0.004$), and at the same time found that OSF cases with moderate epithelial dysplasia had a significantly thicker fibrous layer than cases with mild epithelial dysplasia ($p < 0.005$) [28]. On the contrary, another study showed that neither the fibrosis thickness nor the grade of epithelial dysplasia is related to each other [31]. In this respect, it is worth noting that in 2021, Sanjai [32] noted that using the WHO grading system of epithelial dysplasia is not possible with all OSF cases, as some features of atypia are not discernible [33]. Instead, the author recommended using the binary system for grading epithelial dysplasia of Kujan et al. [34], with some modifications relevant to OSF [32].

Epithelial dysplasia has been reported in 2.5–43% of OSF cases in various studies [7, 9, 28, 35]. The meta-analysis by Kujan et al. determined that out of 414 patients with OSF and oral leukoplakia or epithelial dysplasia, 40 patients exhibited malignant transformation (9.7%), in comparison to 78 out of 1963 OSF patients without epithelial dysplasia or oral leukoplakia (4%), $p < 0.005$ [3].

7.5 Potential Risk Factors of Malignant Transformation among OSF Patients

7.5.1 Areca Nut Usage as a Major Carcinogen

The significance of this disorder is seen from the global epidemiology of areca nut usage, where it is estimated that this substance is regularly consumed by approximately 600 million people worldwide [36]. However, there are challenges in documenting areca nut usage and its role in malignant transformation in OSF because the areca nut is usually wrapped in the leaf of *Piper betle* and masticated in combination with a huge range of additives, including tobacco [37, 38]. These additives vary between countries, communities, and individuals, to a level that may not be easy to qualify.

It is well established that areca nut is the major etiological factor for OSF [39, 40]. In 2004, the International Agency for Research on Cancer Monographs declared areca nut and betel quid as a "group one carcinogen" [40]. This means that there is enough evidence to conclude that it can cause cancer in humans. Areca nut is consumed either alone or as betel quid, where the latter refers to any chewing materials that contain areca nut besides a wide range of additives [38]. One of the significant pathways for carcinogenesis in subjects chewing area nut is oxidative DNA damage [41, 42]. This is supported by statistically significant serum uric acid values in OSF patients compared to controls [43].

While it has been estimated that the risk of malignancy is 35-fold higher among those who smoke and drink alcohol, a report shows that this risk jumps to be 123-fold among those who smoke and drink alcohol besides chewing betel quid [44]. A previous retrospective study revealed that 68% of women with buccal cancer and 84% of women with tongue cancer are non-smokers and only chew betel quid [45]. According to that study, the elimination of chewing habits would substantially reduce this risk of malignancy by up to 91% [45].

In general, areca nut can contribute to malignant transformation through two major pathways: alkaloids and trace elements. Four major alkaloids have been specified in areca nut: arecoline, guvacine, arecaidine, and guvacoline. Of these, arecoline (1,2,4,5-tetrahydro-1-methyl-pyridine carboxylic acid) is the most potent, as it has cytotoxic and genotoxic properties on oral mucosal fibroblasts and keratinocytes. A dose-dependent effect of arecoline in inhibiting gingival fibroblast attachment and migration in vivo was documented [46].

Furthermore, collagen synthesis was significantly inhibited by the action of arecoline [46]. A previous study to identify a possible mechanism of arecoline-induced carcinogenesis found that arecoline downregulated p21 and p27 through the reactive oxygen species/mTOR complex 1 and facilitated G [1]/S transition of the cell cycle, which subsequently led to error-prone DNA replication [47]. Another animal study found that chronic exposure to arecoline downregulates tumour suppressor genes BRCA1 and BRCA2 and increases the risk of cancer formation [48].

On the other hand, a high copper concentration in the areca nut could play a potential role in the malignant transformation of OSF. A previous study reported a higher mean salivary copper level in OSF than oral leukoplakia and oral lichen planus [49]. Another study found a significant increase in serum copper level among patients with OSF and oral cancers in comparison to normal controls [50]. Likewise, it has been reported that the level of serum copper increased gradually from OSF to oral cancer as the duration of areca nut consumption increased [51]. The high copper content of areca nut has been shown to stimulate tumour angiogenesis by activating several angiogenic molecules, such as vascular endothelial growth factor (VEGF), tumour necrosis factor-α (TNF-α), and interleukin-1 (IL-1) [52].

7.6 Pathological Mechanisms of the Malignant Transformation of OSF

Although the potential of malignancy of OSF was first described in 1956 [4], the pathological mechanisms implicated have yet to be elucidated. However, the pathogenesis of this disease is believed to be multifactorial, where numerous pathways and molecules are implicated. These pathways are among the hallmarks of cancer, namely hypoxia, angiogenesis, alterations in the cell cycle, and epithelial-mesenchymal transition [52–54].

7.6.1 Hypoxia

It is widely accepted that connective tissue fibrosis, as a characteristic pathological feature for OSF, i.e. narrow blood vessels, further results in compromised blood supply and lesional hypoxia. Therefore, several previous reports have highlighted the possible role of hypoxia in the malignant transformation of OSF [55]. Hypoxia-inducible factor 1α (HIF-1α), a known transcription factor induced by hypoxic conditions, is significantly upregulated at both the protein and mRNA levels in OSF cases with epithelial dysplasia [35]. Therefore, HIF-1α was proposed for use as a marker of malignant transformation in OSF cases [35]. Moreover, a previous study by Jayasooriya et al. in 2011 revealed that the severity of epithelial dysplasia grade was significantly associated with the thickness of fibrosis in OSF cases [28]. This finding was attributed to the advancement of fibrosis in OSF, which reduces local vascularity, resulting in hypoxia, and in turn increases the incidence and the grade of epithelial dysplasia [28]. Based on these findings, Ye et al. proposed using hyperbaric oxygen as a supplementary therapy to treat OSF, as it can increase oxygen tension at the cellular level and reduce the expression of HIF-1α at the molecular level [56].

7.6.2 Angiogenesis

In environments with reduced oxygen concentrations, HIF-1α activates several hypoxia-related genes and upregulates VEGF, which in turn promotes angiogenesis in an attempt to compensate for the oxygen reduction [57]. However, once the malignant process begins, angiogenesis promotes malignant proliferation by causing normally quiescent vasculature to continue growing to assist growing neoplastic mass [58]. A previous study in 2014 performed computer-aided quantification of immunohistochemical images and proposed using HIF-1α as a strong screening marker of OSF and VEGF for risk stratification [59].

7.6.3 Alterations in Cell Cycle

Proliferating cell nuclear antigen (PCNA) acts as a central coordinator of DNA transactions involved in DNA replication, repair, chromatin dynamics, and cell cycle regulation [60]. PCNA is used to assess proliferative activities and is known to be highly expressed in malignancies. However, several previous reports revealed a significantly higher expression of PCNA in OSF cases than in normal tissues, in addition to a significant increase of PCNA expression in dysplastic OSF cases in comparison with non-dysplastic OSF [60].

7.6.4 Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a complex biological process in which polarized epithelial cells undergo multiple biochemical changes to gain the migratory and invasive properties of mesenchymal stem cells [61]. The most characteristic feature associated with EMT is the loss of E-cadherin expression [61]. E-cadherin is considered an important tumour suppres-

sor gene that is localized on the surfaces of epithelial cells and responsible for adherens junctions [62]. The reduction of E-cadherin expression has been correlated with a poor OSCC prognosis in many previous studies [63]. Transcription factors such as Snail (SNAI1), Slug (SNAI2), and Twist, as known repressors for E-cadherin, are involved in the pathogenesis of areca nut-related OSF. In a recent study (2021), the authors found that the expressions of Snail and Twist were significantly higher in OSF than in normal tissues. This association was concomitant with a significant loss of E-cadherin expressions among OSF with dysplasia [64]. This suggests a possible role for this mechanism in the malignant transformation of OSF [64]. Another study revealed that Twist transcript and protein expression were higher in areca nut-associated OSF than in normal tissues [65]. A previous in vivo experiment reported that treatment of human primary buccal mucosal fibroblasts with arecoline increased the Twist expression transcript and protein levels in a dose-dependent manner, while this phenomenon was reversed by knocking down Twist [65].

7.7 Prognosis of OSF Malignant Transformation

OSF is not only a condition with high morbidity and mortality rates; it is believed that OSF-associated malignancy represents a distinctive clinicopathological, morphological, and histological disease [66]. This has been attributed to the distinct biochemical mechanisms of the areca nut [52]. Many studies have shown that OSF-associated malignancy has a younger age of onset than other non-OSF-associated malignancies [16, 66–68]. Moreover, OSF-associated malignancy was associated with more aggressive pathological behaviours, although this association was not significant [67]. The metastasis and recurrence rates of OSF-associated malignancy were 13.5% and 39.1%, respectively, while these rates were lower in non-OSF-associated malignancies, 7.6% and 27.8% [67]. On the contrary, other studies found that regional lymph node metastasis and 3-year disease-free survival were significantly higher in non-OSF-related malignancies compared to OSF-associated malignancies [66, 68]. This was attributed to the potential protective effect of fibrosis in OSF, where collagen with abnormal cross-linking may resist the invasion process. This can also be explained by the ability of fibrosis to reduce and block submucosal lymphatics [66, 68]. Nonetheless, the current evidence is insufficient to support these suppositions.

Regarding the histopathological features, it has been reported that OSF-associated malignancies are significantly associated with a better grade of tumour differentiation, i.e. well-differentiated squamous cell carcinoma. In contrast, non-OSF-related malignancies were significantly associated with moderate- and low-differentiated squamous cell carcinoma [66].

One study found that OSF patients with reduced mouth opening presented with a more advanced tumour stage [16, 69]. Although the evidence is not strong enough to support this association, this can be attributed to the difficulty of earlier diagnosis among patients with limited mouth opening.

7.8 Conclusion

OSF is a widespread condition with very limited published data on malignant transformation. While there is reason to suggest that the malignant transformation potential associated with OSF is underestimated, the current malignant transformation rate based on our analyses is around 4.87%. The risk of malignancy is increased substantially by the concomitant presence of oral leukoplakia and epithelial dysplasia with OSF. The risk of malignancy is directly associated with the dose and duration of areca nut chewing. There is an urgent need to conduct well-designed, multicentre longitudinal studies to further investigate the potential malignancy of OSF.

Summary

The current evidence indicates that oral submucous fibrosis is associated with a malignant transformation rate of 4.87% while the annual malignant transformation rate is 0.84%. The risk of malignancy is directly linked with the concomitant presentation of oral leukoplakia, as well as the dose and duration of areca nut chewing.

References

1. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, et al. Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. Oral Dis. 2021;27(8):1862–80.
2. Mello FW, Miguel AFP, Dutra KL, et al. Prevalence of oral potentially malignant disorders: a systematic review and meta-analysis. J Oral Pathol Med. 2018;47(7):633–40.
3. Kujan O, Mello FW, Warnakulasuriya S. Malignant transformation of oral submucous fibrosis: a systematic review and meta-analysis. Oral Dis. 2021;27(8):1936–46.
4. Paymaster JC. Cancer of the buccal mucosa; a clinical study of 650 cases in Indian patients. Cancer. 1956;9(3):431–5.
5. Iocca O, Sollecito TP, Alawi F, et al. Potentially malignant disorders of the oral cavity and oral dysplasia: a systematic review

and meta-analysis of malignant transformation rate by subtype. Head Neck. 2019;42(3):539–55.
6. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent Oral Epidemiol. 1985;13(6):340–1.
7. Hsue SS, Wang WC, Chen CH, Lin CC, Chen YK, Lin LM. Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. J Oral Pathol Med. 2007;36(1):25–9.
8. Hazarey VK, Erlewad DM, Mundhe KA, Ughade SN. Oral submucous fibrosis: study of 1000 cases from central India. J Oral Pathol Med. 2007;36(1):12–7.
9. Wang YY, Tail YH, Wang WC, et al. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health. 2014;14:99.
10. Chourasia NR, Borle RM, Vastani A. Concomitant Association of Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma and Incidence of Malignant Transformation of Oral Submucous Fibrosis in a Population of Central India: A Retrospective Study. J Maxillofac Oral Surg. 2015;14(4):902–6.
11. Yang PY, Chen YT, Wang YH, Su NY, Yu HC, Chang YC. Malignant transformation of oral submucous fibrosis in Taiwan: a nationwide population-based retrospective cohort study. J Oral Pathol Med. 2017;46(10):1040–5.
12. Chuang TD, Khorram O. Expression profiling of lncRNAs, miRNAs, and mRNAs and their differential expression in leiomyoma using next-generation RNA sequencing. Reprod Sci. 2018;25(2):246–55.
13. Chiang WF, Liu SY, Lin JF, et al. Malignant development in patients with oral potentially malignant disorders detected through nationwide screening: Outcomes of 5-year follow-up at a single hospital. Head Neck. 2020;42(1):67–76.
14. Jian X, Jian Y, Wu X, et al. Oral submucous fibrosis transforming into squamous cell carcinoma: a prospective study over 31 years in mainland China. Clin Oral Investig. 2021;25(4):2249–56.
15. Srivastava R, Sharma L, Pradhan D, Jyoti B, Singh O. Prevalence of oral premalignant lesions and conditions among the population of Kanpur City, India: a cross-sectional study. J Family Med Prim Care. 2020;9(2):1080–5.
16. Rangaswamy S, Chikkalingaiah RG, Sanjeevarayappa PN, Govindraju P. Carcinoma arising in the background of oral submucous fibrosis. Ann Maxillofac Surg. 2019;9(2):247–52.
17. Wang T, Chiu YW, Chen YT, et al. Malignant transformation of Taiwanese patients with oral leukoplakia: a nationwide population-based retrospective cohort study. J Formos Med Assoc. 2018;117(5):374–80.
18. Mohiuddin S, Fatima N, Hosein S, Fatima N. High risk of malignant transformation of oral submucous fibrosis in Pakistani females: a potential national disaster. J Pak Med Assoc. 2016;66(11):1362–6.
19. Zhou S, Guo F, Li L, et al. Multiple logistic regression analysis of risk factors for carcinogenesis of oral submucous fibrosis in mainland China. Int J Oral Maxillofac Surg. 2008;37(12):1094–8.
20. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. Oral Oncol. 2006;42(6):561–8.
21. Singh AK, Chauhan R, Anand K, Singh M, Das SR, Sinha AK. Prevalence and risk factors for oral potentially malignant disorders in Indian Population. J Pharm Bioallied Sci. 2021;13(Suppl 1):S398–401.
22. Li S, Lee YC, Li Q, et al. Oral lesions, chronic diseases and the risk of head and neck cancer. Oral Oncol. 2015;51(12):1082–7.
23. Shearston K, Fateh B, Tai S, Hove D, Farah CS. Malignant transformation rate of oral leukoplakia in an Australian population. J Oral Pathol Med. 2019;48(7):530–7.
24. Singh PK, Yadav A, Singh L, et al. Areca nut consumption with and without tobacco among the adult population: a nationally representative study from India. BMJ Open. 2021;11(6):e043987.
25. Ferlay J EM, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer. https://gco.iarc.fr/today. Published 2020. Accessed September, 2021.
26. The World Bank data. Total Population. https://data.worldbank.org/indicator/SP.POP.TOTL. Accessed 10 September, 2021.
27. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(6):612–27.
28. Jayasooriya PR, Nadeeka Jayasinghe KA, Mudiyanselage TW. Relationship between thickness of fibrosis and epithelial dysplasia in oral submucous fibrosis. J Investig Clin Dent. 2011;2(3):171–5.
29. Ray JG, Ranganathan K, Chattopadhyay A. Malignant transformation of oral submucous fibrosis: overview of histopathological aspects. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):200–9.
30. Ranganathan K, Kavitha L. Oral epithelial dysplasia: classifications and clinical relevance in risk assessment of oral potentially malignant disorders. J Oral Maxillofac Pathol. 2019;23(1):19–27.
31. Illeperuma RP, Ryu MH, Kim KY, Tilakaratne WM, Kim J. Relationship of fibrosis and the expression of TGF-β1, MMP-1, and TIMP-1 with epithelial dysplasia in oral submucous fibrosis. Oral Med Pathol. 2010;15(1):21–8.
32. Sanjai K. Does oral epithelial dysplasia criteria hold true for dysplastic epithelium of oral submucous fibrosis? Oral Surg Oral Med Oral Pathol Oral Radiol. 2021;132(1):e41–2.
33. Barnes L, Eveson J, Reichart P, Sidransky D. Pathology and genetics of head and neck tumours, International Agency for Research on Cancer (IARC), vol. 9. 3rd ed. Lyon: IARC Press; 2005.
34. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006;42(10):987–93.
35. Tilakaratne WM, Iqbal Z, Teh MT, et al. Upregulation of HIF-1alpha in malignant transformation of oral submucous fibrosis. J Oral Pathol Med. 2008;37(6):372–7.
36. Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. Addict Biol. 2002;7(1):77–83.
37. Warnakulasuriya S, Trivedy C, Peters TJ. Areca nut use: an independent risk factor for oral cancer. BMJ. 2002;324(7341):799–800.
38. Sharan RN, Mehrotra R, Choudhury Y, Asotra K. Association of betel nut with carcinogenesis: revisit with a clinical perspective. PLoS One. 2012;7(8):e42759.
39. Amarasinghe HK, Usgodaarachchi US, Johnson NW, Lalloo R, Warnakulasuriya S. Betel-quid chewing with or without tobacco is a major risk factor for oral potentially malignant disorders in Sri Lanka: a case-control study. Oral Oncol. 2010;46(4):297–301.
40. Humans IWGotEoCRt. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum. 2004;85:1–334.

41. Liu TY, Chen CL, Chi CW. Oxidative damage to DNA induced by areca nut extract. Mutat Res. 1996;367(1):25–31.
42. Li YC, Cheng AJ, Lee LY, Huang YC, Chang JT. Multifaceted mechanisms of Areca nuts in oral carcinogenesis: the molecular pathology from precancerous condition to malignant transformation. J Cancer. 2019;10(17):4054–62.
43. Yadav KD, Patil BA, Raheel SA, et al. Serum uric acid levels in patients with oral cancer, leukoplakia and submucous fibrosis: a cross-sectional study. Transl Cancer Res. 2020;9(4):3084–91.
44. Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J Oral Pathol Med. 1995;24(10):450–3.
45. van Wyk CW, Stander I, Padayachee A, Grobler-Rabie AF. The areca nut chewing habit and oral squamous cell carcinoma in South African Indians. A retrospective study. S Afr Med J. 1993;83(6):425–9.
46. Jeng JH, Lan WH, Hahn LJ, Hsieh CC, Kuo MY. Inhibition of the migration, attachment, spreading, growth and collagen synthesis of human gingival fibroblasts by arecoline, a major areca alkaloid, in vitro. J Oral Pathol Med. 1996;25(7):371–5.
47. Ji WT, Yang SR, Chen JY, et al. 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. 2012;103(7):1221–9.
48. Choudhury Y, Sharan RN. 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. 2011;31(1):57–69.
49. Ayinampudi BK, Narsimhan M. Salivary copper and zinc levels in oral pre-malignant and malignant lesions. J Oral Maxillofac Pathol. 2012;16(2):178–82.
50. Khanna S, Udas AC, Kumar GK, Suvarna S, Karjodkar FR. Trace elements (copper, zinc, selenium and molybdenum) as markers in oral sub mucous fibrosis and oral squamous cell carcinoma. J Trace Elem Med Biol. 2013;27(4):307–11.
51. Hosthor SS, Mahesh P, Priya SA, Sharada P, Jyotsna M, Chitra S. Quantitative analysis of serum levels of trace elements in patients with oral submucous fibrosis and oral squamous cell carcinoma: a randomized cross-sectional study. J Oral Maxillofac Pathol. 2014;18(1):46–51.
52. Ekanayaka RP, Tilakaratne WM. Oral submucous fibrosis: review on mechanisms of malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):192–9.
53. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
54. Kujan O, Huang G, Ravindran A, Vijayan M, Farah CS. The role of cyclin-dependent kinases in oral potentially malignant disorders and oral squamous cell carcinoma. J Oral Pathol Med. 2019;48(7):560–5.
55. Kujan O, Shearston K, Farah CS. The role of hypoxia in oral cancer and potentially malignant disorders: a review. J Oral Pathol Med. 2017;46(4):246–52.
56. Ye X, Zhang J, Lu R, Zhou G. HBO: a possible supplementary therapy for oral potentially malignant disorders. Med Hypotheses. 2014;83(2):131–6.
57. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature. 2006;441(7092):437–43.
58. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996;86(3):353–64.
59. Anura A, Conjeti S, Das RK, et al. Computer-aided molecular pathology interpretation in exploring prospective markers for oral submucous fibrosis progression. Head Neck. 2016;38(5):653–69.
60. Chiang CP, Lang MJ, Liu BY, et al. Expression of proliferating cell nuclear antigen (PCNA) in oral submucous fibrosis, oral epithelial hyperkeratosis and oral epithelial dysplasia in Taiwan. Oral Oncol. 2000;36(4):353–9.
61. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420–8.
62. Birchmeier W, Behrens J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta. 1994;1198(1):11–26.
63. Luo SL, Xie YG, Li Z, Ma JH, Xu X. E-cadherin expression and prognosis of oral cancer: a meta-analysis. Tumour Biol. 2014;35(6):5533–7.
64. Hosur MB, Puranik RS, Vanaki SS, Puranik SR, Sudhakara M, Das S. Evaluation of immunohistochemical expression of epithelial- mesenchymal transition markers E-cadherin, Twist and Snail in oral submucous fibrosis and their possible association with malignant transformation. J Oral Maxillofac Pathol. 2021;25(1):97–104.
65. Lee YH, Yang LC, Hu FW, Peng CY, Yu CH, Yu CC. Elevation of Twist expression by arecoline contributes to the pathogenesis of oral submucous fibrosis. J Formos Med Assoc. 2016;115(5):311–7.
66. Gadbail AR, Chaudhary M, Gawande M, et al. Oral squamous cell carcinoma in the background of oral submucous fibrosis is a distinct clinicopathological entity with better prognosis. J Oral Pathol Med. 2017;46(6):448–53.
67. Guo F, Jian XC, Zhou SH, Li N, Hu YJ, Tang ZG. A retrospective study of oral squamous cell carcinomas originated from oral submucous fibrosis. Zhonghua Kou Qiang Yi Xue Za Zhi. 2011;46(8):494–7.
68. Chaturvedi P, Vaishampayan SS, Nair S, et al. Oral squamous cell carcinoma arising in background of oral submucous fibrosis: a clinicopathologically distinct disease. Head Neck. 2013;35(10):1404–9.
69. Xu H, Lyu FY, Song JY, et al. Research achievements of oral submucous fibrosis: progress and prospect. Biomed Res Int. 2021;2021:6631856.