Am J Transl Res 2025;17(6):4278-4287
www.ajtr.org /ISSN:1943-8141/AJTR0163294

Original Article

Predictive value of quantitative DNA analysis in the carcinogenesis of oral submucous fibrosis

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Received January 13, 2025; Accepted May 29, 2025; Epub June 15, 2025; Published June 30, 2025

Abstract: Objective: To explore the diagnostic value of DNA quantitative analysis for the malignant transformation of Oral submucous fibrosis (OSF), as well as the risk factors for OSF carcinogenesis. Methods: Clinical data from 85 OSF patients treated between June 2021 and June 2023 were retrospectively analyzed. Using pathological diagnosis as the gold standard, the diagnostic performance of DNA quantification was evaluated. The relationship between DNA quantitative findings and patients clinicopathological features was analyzed. Meanwhile, factors associated with abnormal DNA content and risk factors for OSF malignant transformation were identified through multivariate logistic regression analysis. The receiver operating characteristic curve (ROC) was employed to evaluate the diagnostic performance of DNA quantitative analysis for predicting malignant transformation. Results: Among the 85 OSF patients, 34 (40.0%) developed malignant transformation. DNA quantitative analysis results revealed 40 (47.1%) had abnormal DNA content, while the other 45 did not. Multivariate Logistic regression analysis identified lesion location and degree of epithelial dysplasia as risk factors for abnormal DNA content; betel-nut chewing frequency, smoking, and the presence of oral leukoplakia (OLK) or oral lichen planus (OLP) were significantly associated with OSF carcinogenesis. The ROC curve for DNA quantitative analysis in diagnosing OSF carcinogenesis showed an area under the curve (AUC) of 0.770, with a sensitivity of 79.4%, specificity of 74.5%, positive predictive value of 67.5%, and negative predictive value of 84.4%. Conclusion: DNA quantitative analysis is a valuable tool for assessing carcinogenic risk of OSF. Lesion location and epithelial dysplasia are significant factors associated with abnormal DNA content, while lifestyle factors and coexisting oral lesions contribute to malignant transformation risk.

Keywords: Oral submucous fibrosis, DNA quantitative analysis, carcinogenesis

Introduction

Oral submucous fibrosis (OSF) is a chronic, insidious disease with malignant potential, commonly attributed to collagen metabolism disorders [1, 2]. Patients typically present with burning sensations in the oral mucosa, accompanied by ulcers, blisters, hypogeusia, xerostomia, and numbness of the lips and tongue. In severe cases, trismus, dysphagia, and restricted tongue mobility may occur [3]. OSF is classified as an oral potentially malignant disorder (OPMD) due to its propensity for malignant transformation [4]. OSF is most prevalent in South and Southeast Asian, such as India, Pakistan, and regional clusters in China, including Hunan and Taiwan [5]. OSF is intimately associated with the development of oral squamous cell carcinoma (OSCC) [6]. According to the World Health Organization (WHO), the global prevalence of OSF reached 4.96% in 2022 [7], and the condition has long been recognized as a precancerous lesion [8]. Despite advances in knowledge about OSCC over the past three decades, the five-year survival rate remains approximately 50% [9]. Therefore, early detection of OSF patients at high risk for malignant transformation, followed by timely and effective intervention, is crucial for reducing the incidence of oral cancer and improving patient outcomes.

Currently, histopathological grading of epithelial dysplasia is the gold standard for assessing the

https://doi.org/10.62347/HMPQ6800

malignant potential of OPMDs [10]. However, biopsy has disadvantages such as invasiveness, procedural complexity, and poor patient compliance. In addition, studies have reported a lack of consistent correlation between dysplasia grade and carcinogenic risk; although high-grade dysplastic lesions may not necessarily progress to malignancy, some low-grade dysplasia or non-dysplastic lesions may undergo malignant transformation [11]. Therefore, there is an urgent need for a reliable, simple, non-invasive, rapid, and patient-friendly diagnostic method to facilitate early detection of oral mucosal carcinogenic risk and improve prognostic accuracy.

Exfoliative cytology of the oral mucosa is a simple, painless, non-invasive, and rapid technique that is well-tolerated by patients, as it poses fewer constraints compared to tissue biopsy. Due to these advantages, it holds great potential for extensive application in population-based screening initiatives [12]. Various diagnostic techniques can be used to analyze exfoliated cells from oral lesions. DNA quantitative analysis, an advancement from traditional morphological pathology to quantitative analysis [13], evaluates cellular physiological and pathological states by measuring nuclear DNA content or chromosomal ploidy. Abnormal DNA content is frequently indicative of malignant transformation and serves as a biomarker of carcinogenesis [14]. However, there are limited studies on the predictive utility of DNA quantitative analysis in OSF carcinogenesis. Therefore, this study aims to assess the diagnostic performance of DNA quantitative analysis in screening for malignant transformation of OSF and predicting its carcinogenic risk.

Materials and methods

Research participants

A retrospective analysis was performed on 85 OSF patients who presented to Changsha Stomatological Hospital between June 2021 and June 2023. Inclusion criteria: (1) Age between 18 and 75 years; (2) Pathologically confirmed OSF, with or without malignant transformation (high-grade dysplasia was considered positive for carcinogenesis); (3) DNA quantitative analysis; (4) No prior treatment involving cryotherapy, laser therapy, radiotherapy, or chemotherapy; (5) No contraindications to examination, with complete medical records and diagnostic data. Exclusion criteria: (1) Age below 18 years or above 75 years; (2) Presence of cardio-cerebrovascular diseases, malignancies in other organs, or systemic disorders affecting respiratory, immune, or endocrine system; (3) Pregnancy or lactation; (4) Non-potentially malignant reactive or reparative hyperplasia due to ulcers, trauma, or nutritional deficiencies; (5) Incomplete medical records. This study was approved by the Ethics Committee of Changsha Stomatological Hospital.

Data collection

Demographic and behavioral data, including age, sex, dietary habits, betel-nut chewing, smoking, and alcohol consumption were collected from electronic medical records and structured questionnaires. Clinical examination included assessment of mouth opening, presence of defective restorations or sharp tooth cusps, oral hygiene status, and coexistence of oral lichen planus (OLP) or oral leukoplakia (OLK). Mouth opening was classified using the Khanna and Andrade system as follows: normal (>35 mm), mildly restricted (26-35 mm), moderately restricted (16-25 mm), and severely restricted (2-15 mm). A flowchart summarizing the study design is presented in **Figure 1**.

DNA quantitative analysis

Patients were instructed to rinse their mouth with clean water for 1 minute to remove food residue. A disposable sterile brush was used to repeatedly brush a 1 cm × 1 cm area at the junction of the lesion and adjacent normal tissue 15-20 times. Thereafter, the brush head was detached and placed into a cell preservation solution, and the specimen was prepared and stained promptly. DNA quantitative analysis was performed using an automatic DNA image analysis system (MOTIC BA600 Mot, Germany), and the results were reported.

A sample was classified as exhibiting abnormal DNA content if it met any of the following criteria: (1) two distinct G0-G1 peaks were observed (diploid G0-G1 peak, with a DNA index of 0.98-1.02; aneuploid G0-G1 peak, with a DNA index of 1.05-1.9 or 2.1-3.8); (2) more than 10% of cells exhibited a DNA index of 1.25-2.3 or ≥2.3; (3) more than 3 cells (≥1% of a minimum of 300 cells) had a DNA index ≥2.3. The presence of

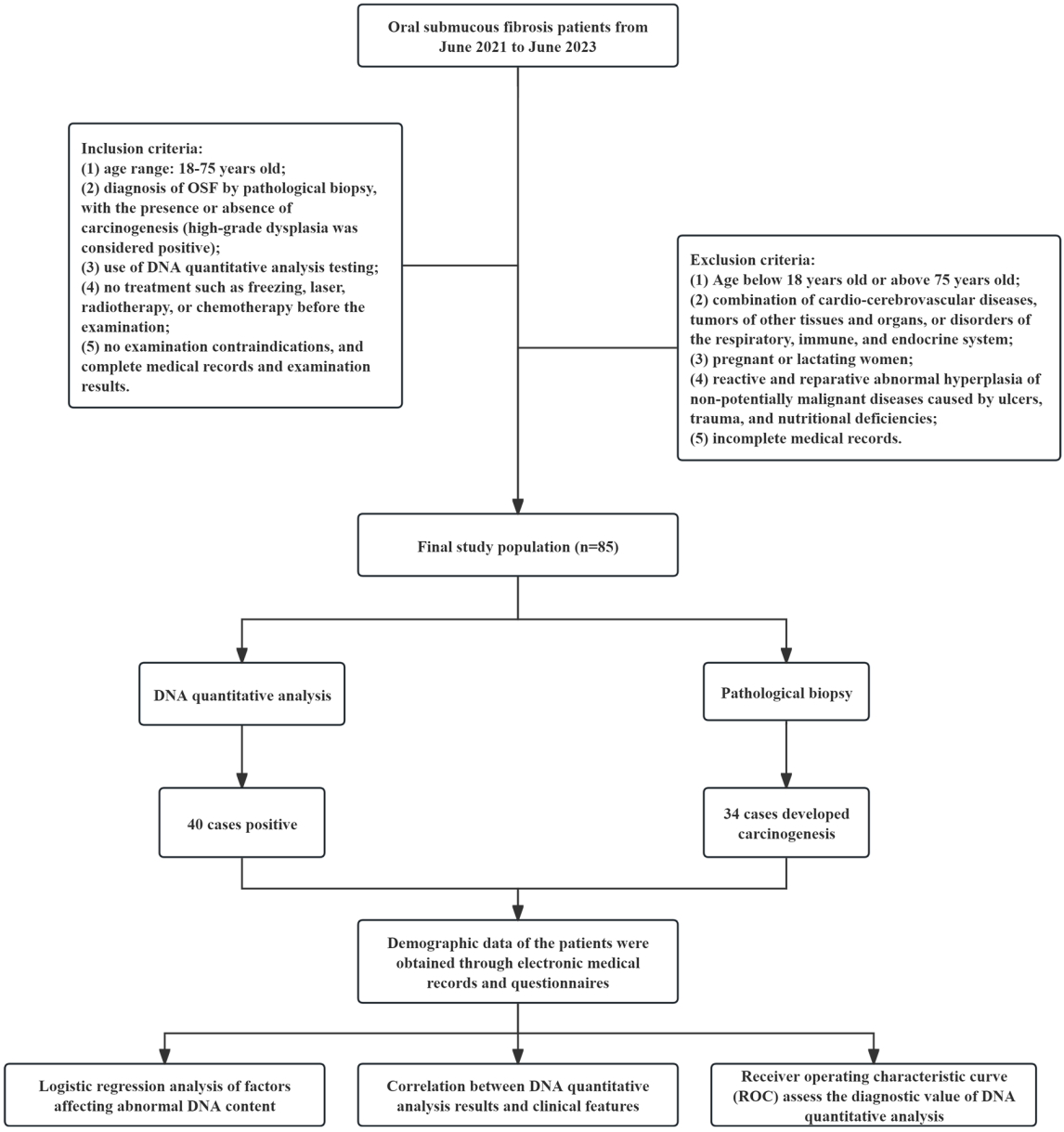

Figure 1. Flow chart of the study.

any of the above findings was considered a positive result for abnormal DNA content.

Pathological examination

All patients underwent incisional biopsy at the same anatomical site where exfoliative cytology was performed. Biopsy specimens were fixed, paraffin-embedded, stained with HE, and examined under a light microscope (Olympus, Japan). Histopathological diagnoses were independently verified by two experienced oral pathologists using a double-blind method. The patients were divided into a either a low-grade dysplasia or a high-grade dysplasia group based on the WHO binary classification system for epithelial dysplasia. Representative histological images are presented in **Figure 2**.

Statistical analysis

SPSS 25.0 was utilized for data processing. Continuous variables were expressed as mean ± standard deviation, and comparisons between groups were conducted using the independent-samples t-test. Categorical variables were presented as frequencies and percentages, with comparisons assessed using the chi-

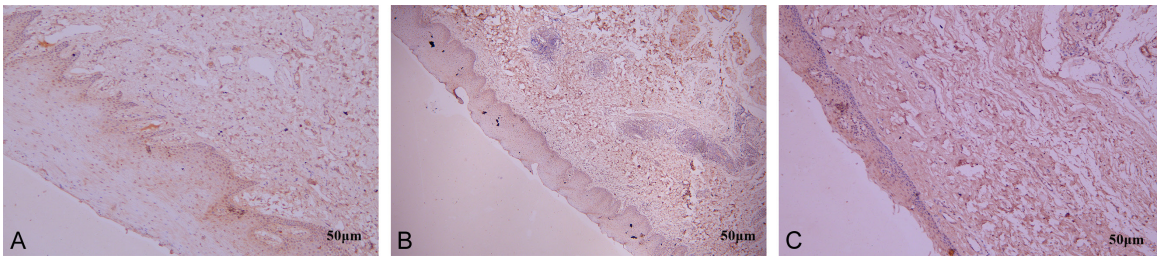

Figure 2. Hematoxylin-eosin staining was performed on specimens from patients with oral submucous fibrosis and oral squamous carcinoma combined with oral submucous fibrosis. A: Early OSF lesions, showing fine collagen fibers, with marked edema and neutrophilic infiltration; B: Early OSF lesions, demonstrating subepithelial bands of vitreous collagen fibers and inter-fibrillar edema accompanied by lymphocytic infiltration; C: Oral squamous cell carcinoma arising in a background of OSF. Note: OSF, oral submucous fibrosis.

Table 1. Carcinogenesis of oral submucous fibrosis

	Pathologically positive	Pathological negative	Total	Positive predictive value (%)	Negative predictive value (%)
DNA-positive	27	13	40	67.5	84.4
DNA-negative	7	38	45		
Total	34	51	85		

square test. Logistic regression analysis was employed to identify risk factors associated with abnormal DNA content. The diagnostic performance of DNA quantitative analysis was evaluated using receiver operating characteristic (ROC) curve analysis. A *P*-value of <0.05 was considered statistically significant.

Results

Diagnostic performance of DNA quantitative analysis for OSF carcinogenesis

As indicated by the histopathological results, among the 85 OSF patients, 34 (40.0%) experienced malignant transformation, while 51 (60%) did not. Based on DNA quantitative analysis, 40 (47.1%) patients were classified as having abnormal DNA content. The positive predictive value (PPV) for detecting carcinogenesis was 67.5%, and the negative predictive value (NPV) was 84.4% (**Table 1**). The distribution of DNA content status and corresponding cytological changes among all patients is summarized in **Table 2**.

Correlation between DNA quantitative analysis results and patient clinical features

Of the 85 patients, 45 (52.9%) exhibited normal DNA content, whereas 40 (47.1%) showed abnormal DNA content. Comparison of clinicopathological characteristics revealed that patients with normal DNA content were significantly younger (P<0.05). In addition, significant inter-group differences were observed between the two groups in terms of lesion location and the degree of epithelial dysplasia (P<0.05), as detailed in **Table 3**.

Logistic regression analysis of factors associated with abnormal DNA content

Variables identified as significant in the univariate analysis were included in the Logistic regression analysis. Age was not found to be an independent risk factor for abnormal DNA content. Instead, lesion location and the degree of epithelial dysplasia were identified as significant risk factors. The risk of abnormal DNA content was 4.273 greater in patients with tongue lesions than those with no tongue lesions (P=0.012), and the risk of abnormal DNA content in patients with high-grade dysplasia was 14.761 times that of low-grade dysplasia (P<0.0001), as shown in **Table 4**.

Univariate analysis of carcinogenesis in OSF

Univariate analysis identified significant associations between OSF carcinogenesis and several factors, including betel-nut chewing, betel-

Table 2. The status of DNA content and cellular changes of 85 patients

Cases	DNA content	Cellular changes	Cases	DNA content	Cellular changes
1	0	0	44	0	0
2	1	0	45	1	1
3	0	0	46	0	0
4	1	1	47	1	0
5	0	0	48	0	1
6	1	0	49	1	1
7	0	0	50	1	0
8	1	1	51	0	0
9	0	0	52	1	1
10	1	1	53	0	0
11	0	0	54	0	0
12	1	1	55	1	0
13	0	0	56	0	0
14	1	0	57	1	1
15	0	1	58	0	0
16	1	1	59	1	1
17	0	0	60	1	1
18	0	0	61	0	0
19	1	1	62	0	0
20	1	1	63	1	0
21	0	0	64	0	0
22	0	1	65	1	1
23	1	1	66	0	0
24	0	0	67	1	0
25	0	0	68	1	1
26	1	1	69	0	0
27	0	0	70	1	0
28	0	0	71	0	0
29	1	1	72	1	1
30	0	0	73	0	0
31	1	0	74	1	1
32	0	1	75	0	0
33	1	1	76	1	1
34	0	0	77	1	1
35	1	0	78	0	0
36	0	0	79	1	0
37	1	1	80	0	0
38	0	1	81	1	1
39	0	0	82	0	0
40	1	1	83	0	1
41	0	1	84	1	1
42	1	0	85	0	0
43	0	0			

nut chewing frequency, smoking, and the coexistence of OLK or OLP, as shown in **Table 5**.

Logistic regression analysis of carcinogenesis in OSF

Multivariate Logistic regression analysis revealed that betel-nut chewing frequency, smoking, and the presence of OLK or OLP were independent predictors of OSF carcinogenesis. Chewing fewer than 10 betel nuts per day was identified as a protective factor, while smoking and coexisting OLK or OLP were significant risk factors for malignant transformation (**Table 6**).

ROC curve analysis of DNA quantitative analysis for diagnosing carcinogenesis

Taking histopathological diagnosis as the reference standard, ROC curve demonstrated that DNA quantitative analysis had an area under the curve (AUC) of 0.770 for detecting OSF carcinogenesis. The sensitivity and specificity were 79.4% of and 74.5%, respectively. Detailed results are shown in **Figure 3** and **Table 7**.

Discussion

DNA quantitative technology has been extensively employed in cervical cancer screening [15]. In recent years, its application has expanded to various non-gynecological fields, including lung cancer, esophageal lesions, oral mucosal cells, sputum, prostate tissue, pleural and peritoneal effusions, and other body fluids [16, 17]. Although this technique has been explored for diagnosing high-risk oral precancerous lesions and oral cancer, a standardized diagnostic criterion for its efficacy remains lacking. This study aimed to evaluate the diagnostic value of DNA quantitative analysis in patients with high-risk oral lesions and OSF-associated carcinogenesis.

In this study, 34 (40.0%) OSF patients developed malignant transformation, while 51 (60%) did not. Based on DNA quantitative analysis, 40 patients (47.1%) had abnormal DNA content. The positive predictive value of DNA quantification for OSF malignant transformation was 67.5% and the negative predictive value was 84.4%. Further analysis revealed that abnormal DNA content was significantly associated with lesion location and the degree of epithelial dysplasia. Specifically, the risk of abnormal DNA content was 4.273 times higher with tongue lesions compared to non-tongue lesions, and 14.761 times higher in patients with high-grade dysplasia than in those with low-

Table 3. Correlation between DNA quantitative analysis results and clinical features

	Normal DNA content (n=45)	Abnormal DNA content (n=40)	χ^2/t	P
Age	50.38±7.58	53.53±6.91	1.992	0.049
Sex			0.178	0.673
Male	29	24		
Female	16	16		
Eating habits			1.214	0.271
Non-irritating	30	22		
Irritating	15	18		
Betel-nut chewing			1.147	0.284
Yes	33	25		
No	12	15		
Smoking			0.016	0.898
Yes	22	19		
No	23	21		
Drinking			1.053	0.305
Yes	22	24		
No	23	16		
Lesion location			8.338	0.004
Tongue	13	24		
Non-tongue	32	16		
Sharp tooth cusps			0.898	0.343
Yes	19	21		
No	26	19		
Mouth opening			0.662	0.416
Normal	37	30		
Limited	8	10		
Epithelial dysplasia			23.811	<0.0001
None/mild	38	13		
Moderate/severe	7	27		

Table 4. Logistic regression analysis of factors associated with abnormal DNA content

	β	SE	Wald	P	HR	95% CI
Age (continuous variable)	0.076	0.039	3.808	0.051	1.079	1.000-1.164
Lesion location (0= non-tongue, 1= tongue)	1.452	0.575	6.369	0.012	4.273	1.383-13.201
Epithelial dysplasia (0= none/mild, 1= moderate/severe)	2.692	0.607	19.646	0.000	14.761	4.489-48.539

grade dysplasia. These results align with previous reports indicating that tongue lesions are more prone to malignant transformation [18]. Xiao et al. [19] found that all 27 patients with simple OSF exhibited normal DNA content, whereas three patients with OSF combined with OLK, two had abnormal DNA content, and one progressed to oral cancer during follow-up. These findings support the notion that coexisting epithelial dysplasia contributes to abnormal DNA content. Epithelial dysplasia typically initiates in the basal and parabasal layers of the epithelium. In contrast, the superficial mucosal layer is often well-differentiated and keratinized. As dysplasia advances, increased cellular DNA synthesis occurs, which will consequently alter the DNA content in the cell nucleus [21].

This study also investigated the risk factors associated with malignant transformation in OSF. The findings indicated that betel-nut chewing frequency, smoking, and the coexistence of

Table 5. Univariate analysis of carcinogenesis in oral submucous fibrosis

	Cancerous OSF (n=34)	Non-cancerous OSF (n=51)	χ^2/t	P
Age	52.00±7.57	51.76±7.35	0.143	0.889
Sex			2.363	0.124
Male	19	34		
Female	15	17		
Eating habits			0.037	0.848
Non-irritating	24	35		
Irritating	10	16		
Betel-nut chewing			5.211	0.022
Yes	28	27		
No	6	24		
Betel-nut chewing frequency			4.615	0.032
<10	7	31		
≥10	27	20		
Smoking			6.296	0.012
Yes	20	16		
No	14	35		
Drinking			0.071	0.790
Yes	19	27		
No	15	24		
Oral health status			2.219	0.528
Good	5	14		
Fair	10	17		
Poor	12	13		
Bad	7	7		
Lesion location			0.965	0.326
Tongue	17	20		
Non-tongue	17	31		
Sharp tooth cusps			0.197	0.657
Yes	15	25		
No	19	26		
Mouth opening			2.302	0.129
Normal	24	43		
Limited	10	8		
Combination of OLK or OLP			9.641	0.002
Yes	23	17		
No	11	34		

Table 6. Multivariate Logistic regression analysis of OSF carcinogenesis

	β	SE	Wald	P	HR	95% CI
Betel-nut chewing (0= no, 1= yes)	-1.329	1.238	1.151	0.283	0.265	0.023-3.000
Betel-nut chewing frequency (0=≥10, 1=<10)	-2.622	1.177	4.957	0.026	0.073	0.007-0.731
Smoking (0= no, 1= yes)	1.535	0.622	6.091	0.014	4.640	1.372-15.700
Combination of OLK or OLP (0= no, 1= yes)	1.946	0.601	10.472	0.001	7.002	2.154-22.756

OLK or OLP were significant contributors to OSF carcinogenesis. Specifically, chewing fewer than 10 betel nuts daily was identified as a protective factor, while smoking and the presence

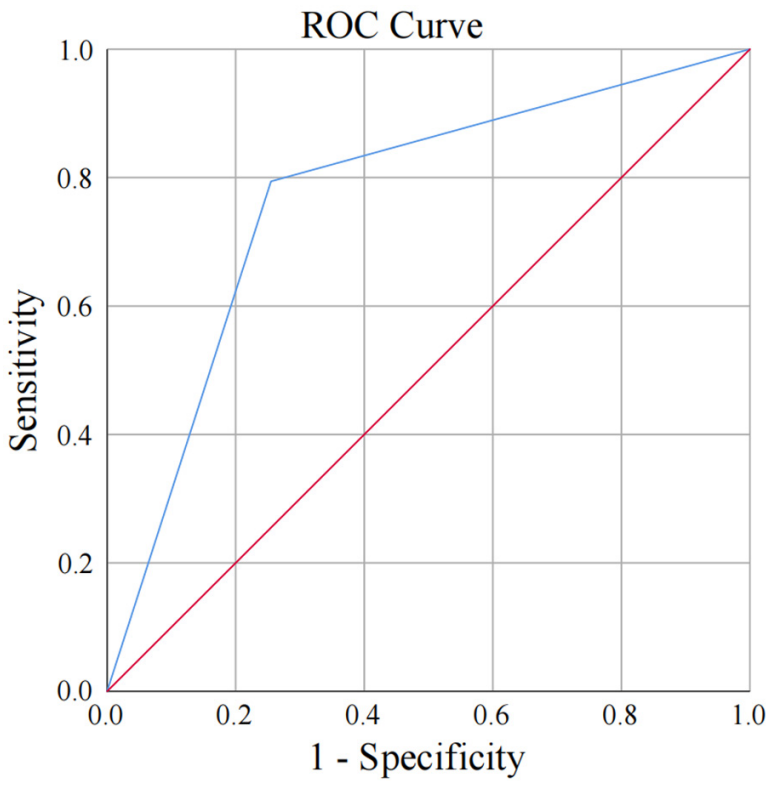

Figure 3. ROC curve of DNA quantitative analysis for diagnosing OSF carcinogenesis. OSF: Oral submucous fibrosis.

of OLK or OLP were independent risk factors. Currently, betel nut (Betel quid, BQ) is widely acknowledged as the primary pathogenic factor for OSF [20]. It is estimated that over 600 million people worldwide chew betel nuts, with a particularly high prevalence in Asia [21]. The International Agency for Research on Cancer (IARC) has classified betel nut as a carcinogen. This has been corroborated in animal studies, where BQ consumption was associated with OSF, oral squamous cell carcinoma (OSCC), and other malignancies. Arecoline, a key alkaloid compound, has been consistently detected in all BQ products and is considered a major pathogenic factor in both OSF and OSCC, regardless of regional differences, product type, or user demographics [22]. Smoking has also been shown to accelerate OSF progression. Cigarette smoke generates a substantial amount of reactive oxygen species (ROS), which damage essential biomolecules such as lipids, proteins, polypeptides, and nucleic acids. This oxidative stress leads to structural and functional alterations, particularly in nucleic acids, promoting genetic instability and cellular damage [23]. Conversely, the accumulation of oxidative damage will trigger cell senescence and further intensify the production of ROS, creating a vicious cycle [24]. The presence of OLK or OLP significantly increases the risk of carcinogenesis in OSF. In a study by Jian et al. [25], clinical features of 11 patients with OSF-related malignant transformation were analyzed, revealing that all had coexisting OLK or OLP. Specifically, 10 cases were associated with OLK, 3 with OLP, and 2 exhibited both concurrently. These findings underscore the synergistic role of OLK and OLP in promoting malignant transformation in OSF patients.

This study does have several limitations. First, as a single-center retrospective study with a relatively small sample size, it may lack sufficient power to detect subtle associations, resulting in biased results. Second, patients with coexisting OLK or OLP were not independently classified or analyzed. Given the potential differences in carcinogenic risk and DNA content abnormalities between patients with isolated OSF and those with combined lesions, separate subgroup analysis is warranted. Third, although certain regions in China have a high incidence of oral cancer, this study did not account for geographic variability, which may influence disease progression and risk profiles. At present, as a cross-sectional investigation, this study cannot determine the prognostic value of exfoliative cytology in OSF. Consequently, multi-center, multi-region, and large-sample-size studies should be carried out in the future.

In conclusion, DNA quantitative analysis can serve as a valuable tool for identifying OSF patients at risk of malignant transformation and for detecting early-stage carcinogenesis. The carcinogenesis of OSF is influenced by multiple factors. Betel-nut chewing, smoking, and the presence of OLK or OLP are identified as significant risk factors. Hence, both self-pre-

Table 7. Diagnostic efficacy of DNA quantitative analysis

	AUC	SE	P	95% CI	Sensitivity (%)	Specificity (%)
DNA quantitative analysis	0.770	0.054	0.000	0.664-0.875	79.4	74.5

vention and clinical treatment of OSF carcinogenesis should focus on mitigating these risk factors to reduce the likelihood of progression to OSCC.

Acknowledgements

This research project was supported by Hunan Provincial Administration of Traditional Chinese Medicine (B2024068); 2025 Hunan Natural Science Foundation University Joint Fund Project: 2025JJ90153. Non-invasive assessment of oral mucosal cancer risk and behavioral and drug improvement.

Disclosure of conflict of interest

None.

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