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IMMUNOHISTOCHEMICAL EXPRESSION OF OCT4 IN ORAL POTENTIALLY MALIGNANT DISORDERS

Sajda Khan[1], Anish Gupta[2], Shaiq Gajdhar[3]

[1]PhD Scholar, Department of Oral Pathology and Microbiology, Peoples University, Bhopal, India drsajdagajdhar@gmail.com
[2] Department of Oral Pathology and Microbiology, Peoples Dental Academy, Peoples University, Bhopal, Madhya Pradesh, India anishpooja687@yahoo.co.in
[3] PhD scholar, Department of Prosthodontics, Peoples University, Bhopal, Madhya Pradesh, India drshaiqgajdhar@gmail.com
Corresponding Author: Dr. Sajda Khan, PhD Scholar, Department of Oral Pathology and Microbiology, Peoples University, Bhopal, India

Abstract

Background

Oral potentially malignant disorders (OPMDs), including oral leukoplakia (OL) and oral submucous fibrosis (OSMF), are recognized precursor lesions of oral squamous cell carcinoma (OSCC) with varying risks of malignant transformation. Conventional histopathological grading remains the gold standard for assessing malignant potential; however, its predictive value is limited by interobserver variability. Cancer stem cell (CSC) markers have emerged as promising molecular biomarkers for identifying high-risk lesions. Octamer-binding transcription factor 4 (OCT4), a key regulator of stem cell pluripotency and self-renewal, has been implicated in tumor initiation, progression, and resistance to therapy. This study evaluated the immunohistochemical expression of OCT4 in OPMDs and investigated its association with histopathological severity.

Materials and Methods

A retrospective immunohistochemical study was conducted on 40 formalin-fixed, paraffin-embedded tissue specimens comprising 10 cases of normal oral mucosa (NOM) and 30 cases of OPMDs, including oral leukoplakia with mild (n = 9), moderate (n = 4), and severe epithelial dysplasia (n = 4), and oral submucous fibrosis Grade I (n = 3), Grade II (n = 10), and Grade III (n = 11). Immunohistochemical staining was performed using monoclonal anti-OCT4 antibody. The percentage of OCT4-positive cells was evaluated by semi-quantitative analysis, and staining intensity was graded according to the proportion of positively stained cells. Statistical analysis was performed to determine the association between OCT4 expression and histopathological grade.

Results

OCT4 expression showed a progressive increase with increasing severity of oral epithelial dysplasia. The mean OCT4 expression was 1.70 ± 1.25% in normal oral mucosa, 1.78 ± 2.77% in mild dysplasia, 15.50 ± 0.58% in moderate dysplasia, and 37.25 ± 9.50% in severe dysplasia. Within the OSMF group, OCT4 expression was 1.33% in Grade I, 0.00% in Grade II, and increased to 4.50% in Grade III lesions. Overall, 70% of OPMD cases demonstrated negative staining (0–5% positive cells), whereas 30% exhibited varying degrees of positive immunoreactivity. Higher OCT4 expression was associated with increasing dysplastic severity, indicating enhanced cancer stem cell activity during early oral carcinogenesis.

Conclusion

The present study demonstrates that OCT4 expression increases progressively with the severity of oral epithelial dysplasia, suggesting its involvement in the early stages of oral carcinogenesis.

KEYWORDS: OCT4, Oral potentially malignant disorders, Oral leukoplakia, Oral submucous fibrosis, Oral epithelial dysplasia, Cancer stem cells, Immunohistochemistry, Oral carcinogenesis.

INTRODUCTION

Oral cancer is a major global health challenge and remains one of the most common malignancies affecting the head and neck region. More than 90% of oral cancers are histologically classified as oral squamous cell carcinoma (OSCC), which is characterized by aggressive local invasion, regional lymph node metastasis, and relatively poor long-term survival despite advances in diagnosis and treatment.[1,2] Delayed diagnosis continues to be a significant contributor to poor prognosis, emphasizing the need for reliable biomarkers capable of identifying lesions at high risk of malignant transformation.[3]

The majority of OSCCs arise through a multistep process involving the progressive accumulation of genetic, epigenetic, and molecular alterations within clinically identifiable precursor lesions collectively known as oral potentially malignant disorders (OPMDs).[4] The term OPMD, introduced by the World Health Organization (WHO), refers to a group of oral mucosal disorders associated with a statistically increased risk of developing oral cancer.[5] The current WHO classification includes oral leukoplakia (OL), proliferative verrucous leukoplakia, erythroplakia, oral submucous fibrosis (OSMF), oral lichen planus, oral lichenoid lesions, actinic cheilitis, chronic graft-versus-host disease, and several inherited disorders with malignant potential.[5]

Among these disorders, oral leukoplakia and oral submucous fibrosis are the most prevalent in South Asian countries, where tobacco consumption, areca nut chewing, smoking, and alcohol use remain important etiological factors. Oral leukoplakia is clinically defined as a predominantly white plaque that cannot be characterized as any other disease and carries variable malignant transformation potential depending on its clinical presentation and histopathological grade.[6] OSMF is a chronic, progressive, fibrotic disorder strongly associated with areca nut chewing and is characterized by epithelial atrophy, progressive fibrosis of the lamina propria, restricted mouth opening, and an increased risk of malignant transformation.[7,8] The biological behavior of these lesions is highly variable, highlighting the importance of identifying objective biomarkers for risk stratification.[9]

Histopathological assessment of epithelial dysplasia remains the gold standard for evaluating malignant potential in OPMDs. Lesions exhibiting severe epithelial dysplasia have a substantially greater likelihood of progressing to invasive carcinoma than lesions with mild dysplastic changes. However, grading of epithelial dysplasia is subject to considerable interobserver variability, and some lesions lacking significant dysplasia may nevertheless undergo malignant transformation. Consequently, conventional histopathology alone cannot accurately predict biological behavior in every patient, necessitating the identification of complementary molecular biomarkers.[10,11]

Recent advances in molecular oncology have highlighted the pivotal role of cancer stem cells (CSCs) in the initiation and progression of epithelial malignancies. The cancer stem cell hypothesis proposes that only a small subpopulation of tumor cells possesses the capacity for unlimited self-renewal, multilineage differentiation, tumor initiation, metastasis, and therapeutic resistance. These CSCs are believed to survive conventional treatment, contributing to tumor recurrence and disease progression. Increasing evidence suggests that CSC-associated molecular alterations occur early during oral carcinogenesis, even before the development of invasive carcinoma, making CSC markers attractive candidates for early diagnosis and prognostic assessment.[12,13]

Octamer-binding transcription factor 4 (OCT4), encoded by the POU5F1 gene located on chromosome 6p21, is a master regulator of pluripotency and self-renewal in embryonic stem cells. Together with SOX2 and NANOG, OCT4 forms the core transcriptional network responsible for maintaining stem cell identity and preventing cellular differentiation. Under physiological conditions, OCT4 expression is largely restricted to embryonic tissues and germ cells.[14] However, aberrant re-expression of OCT4 has been demonstrated in numerous human malignancies, including cancers of the breast, lung, liver, pancreas, colon, cervix, and oral cavity.[15,16] Overexpression of OCT4 has been associated with increased proliferation, epithelial–mesenchymal transition, invasion, angiogenesis, metastasis, resistance to chemotherapy, and poor patient survival, suggesting its important role in tumor aggressiveness.[17,18]

In oral carcinogenesis, OCT4 has emerged as one of the most promising CSC markers because of its association with stemness characteristics and tumor progression. Several immunohistochemical studies have demonstrated increased OCT4 expression in OSCC compared with normal oral mucosa, while recent investigations suggest that OCT4 expression may also increase during the progression of OPMDs, particularly with increasing grades of epithelial dysplasia.[19,20] These observations support the hypothesis that activation of stem cell-associated pathways represents an early molecular event in malignant transformation.[21] Nevertheless, published studies remain limited and often include relatively small sample sizes or evaluate only a single type of OPMD, resulting in inconsistent conclusions regarding the clinical utility of OCT4 as a predictive biomarker.[22]

Immunohistochemistry (IHC) remains one of the most practical and reproducible methods for evaluating protein expression in routine diagnostic pathology. Quantification of OCT4 immunoexpression enables assessment of the proportion and distribution of stem cell-like cells within dysplastic epithelium and may provide valuable prognostic information beyond conventional histopathological examination. Identification of lesions exhibiting increased OCT4 expression could facilitate early intervention, individualized patient surveillance, and improved clinical decision-making, thereby reducing the burden of advanced oral cancer.[23]

The present study was undertaken to evaluate the immunohistochemical expression of OCT4 in oral potentially malignant disorders, including oral leukoplakia with varying grades of epithelial dysplasia and oral submucous fibrosis, and to compare its expression with normal oral mucosa. Furthermore, the study aimed to determine the association between OCT4 expression and histopathological severity, thereby exploring its potential role as an adjunctive biomarker for assessing the malignant potential of OPMDs and improving early diagnosis of oral carcinogenesis.

MATERIALS AND METHODS

Study Design and Sample Selection

The present retrospective, observational immunohistochemical study was conducted to evaluate the expression of the cancer stem cell marker Octamer-binding Transcription Factor 4 (OCT4) in oral potentially malignant disorders (OPMDs) and to assess its association with histopathological severity. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks archived in the Department of Oral and Maxillofacial Pathology were retrieved after obtaining institutional ethical approval.

A total of 40 tissue specimens were included in the study, comprising 10 cases of normal oral mucosa (NOM), which served as the control group, and 30 histopathologically confirmed cases of OPMDs. The OPMD group consisted of 17 cases of oral leukoplakia (OL) with epithelial dysplasia and 13 cases of oral submucous fibrosis (OSMF). Oral leukoplakia cases were further categorized into mild dysplasia (n = 9), moderate dysplasia (n = 4),

and severe dysplasia (n = 4) according to the World Health Organization (WHO) histopathological criteria. OSMF cases were graded as Grade I (n = 3), Grade II (n = 10), and Grade III (n = 11) based on established histopathological grading criteria. Demographic characteristics and OCT4 expression data for these groups were analyzed.

Inclusion Criteria

The study included:

- Histopathologically confirmed cases of oral leukoplakia with epithelial dysplasia.
- Histopathologically confirmed cases of oral submucous fibrosis.
- Normal oral mucosal tissues obtained from healthy individuals undergoing minor oral surgical procedures.
- Adequately preserved formalin-fixed paraffin-embedded tissue blocks with sufficient epithelial tissue for immunohistochemical evaluation.

Exclusion Criteria

- Tissue blocks with inadequate epithelial tissue.
- Poorly preserved or autolyzed specimens.
- Previously treated OPMD lesions.

Histopathological Evaluation

Archived hematoxylin and eosin (H&E)-stained sections were independently reviewed to confirm the original histopathological diagnosis and grading before immunohistochemical analysis. Oral leukoplakia specimens were graded as mild, moderate, or severe epithelial dysplasia according to the WHO classification. OSMF specimens were categorized into Grades I, II, and III based on the degree of epithelial atrophy, collagen deposition, fibrosis, and associated histopathological changes.

Immunohistochemical Procedure

Sections measuring 4 μm in thickness were cut from each paraffin block using a rotary microtome and mounted on poly-L-lysine-coated glass slides. The sections were deparaffinized in xylene and rehydrated through graded ethanol concentrations to distilled water.

Antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 minutes. After washing with phosphate-buffered saline (PBS), nonspecific protein binding was minimized using a protein blocking solution.

The tissue sections were then incubated with the primary monoclonal anti-OCT4 antibody according to the manufacturer's recommended protocol. Following primary antibody incubation, the slides were treated with a secondary horseradish peroxidase (HRP)-conjugated antibody detection system. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, resulting in a brown reaction product at sites of antigen localization. Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted using DPX mounting medium.

Appropriate positive and negative controls were included in each staining batch to ensure the specificity and reliability of immunostaining.

Evaluation of OCT4 Immunoexpression

Immunohistochemical evaluation was performed independently by two experienced oral pathologists blinded to the clinical and histopathological diagnosis. Nuclear staining was considered positive for OCT4 expression.

For each section, five representative high-power fields (×400 magnification) were selected, and at least 500 epithelial cells were evaluated. The percentage of positively stained epithelial cells was recorded, and immunoreactivity was assessed using a semi-quantitative scoring system based on the proportion of positively stained cells.

The staining scores were categorized as follows:

Score	Percentage of Positive Cells
0	0–5%
+	6–25%
++	26–50%
+++	>51%

The distribution of OCT4 immunostaining among the OPMD cases was recorded and correlated with histopathological grade.

Statistical Analysis

The collected data were entered into IBM Statistical Package for the Social Sciences (SPSS) for statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as frequencies and percentages. Differences in OCT4 expression among study groups were evaluated using appropriate parametric or non-parametric statistical tests based on data distribution. Comparisons between multiple groups were performed using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, followed by post hoc analysis where appropriate. Associations between categorical variables were analyzed using the Chi-square or Fisher's exact test. A p-value < 0.05 was considered statistically significant.

RESULTS

Demographic Characteristics of the Study Population

The present study included 40 formalin-fixed, paraffin-embedded tissue specimens, comprising 10 cases of normal oral mucosa (NOM) and 30 cases of oral potentially malignant disorders (OPMDs). The OPMD group consisted of 17 cases of oral leukoplakia (OL) with epithelial dysplasia and 13 cases of oral submucous fibrosis (OSMF). The oral leukoplakia cases were categorized as mild dysplasia (n = 9), moderate dysplasia (n = 4), and severe dysplasia (n = 4), whereas the OSMF group comprised Grade I (n = 3), Grade II (n = 10), and Grade III (n = 11) lesions.

The overall mean age of patients with OPMDs was 40.5 ± 9.8 years. Patients with oral leukoplakia exhibited a gradual increase in mean age with increasing dysplastic severity, ranging from 35.1 ± 5.4 years in mild dysplasia to 48.0 ± 8.2 years in severe dysplasia. Similarly, patients with OSMF demonstrated increasing mean age from Grade I (32.0 ± 4.5 years) to Grade III (44.1 ± 9.0 years). A marked male predominance was observed across all study groups.

OCT4 Expression in Normal Oral Mucosa

Normal oral mucosa exhibited minimal OCT4 immunoreactivity. The mean percentage of OCT4-positive cells was 1.70 ± 1.25%, indicating negligible expression under normal physiological conditions. Positive staining was limited to occasional basal epithelial cells, while the majority of epithelial cells showed no detectable immunoreactivity.

OCT4 Expression in Oral Leukoplakia

A progressive increase in OCT4 immunoexpression was observed with increasing severity of epithelial dysplasia. Mild epithelial dysplasia demonstrated weak OCT4 immunoreactivity, with a mean expression of 1.78 ± 2.77%. Immunostaining was primarily confined to the basal epithelial layer, with only scattered positive cells.

Moderate epithelial dysplasia exhibited a marked increase in OCT4 expression, with a mean positivity of 15.50 ± 0.58%. Positive staining extended beyond the basal layer into the suprabasal epithelial layers, indicating increased stem cell activity.

The highest OCT4 expression among dysplastic lesions was observed in severe epithelial dysplasia, with a mean positivity of 37.25 ± 9.50%. Strong nuclear immunostaining involved multiple epithelial layers, reflecting progressive activation of cancer stem cell-associated pathways during dysplastic progression (Figure 1).

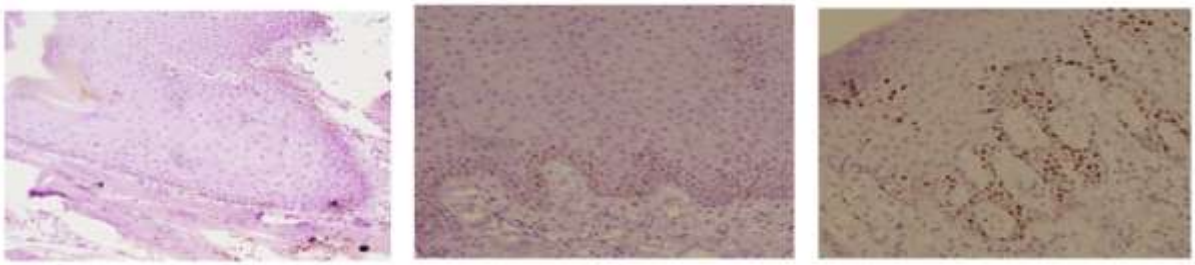

Figure 1: Immunohistochemical Expression of OCT4 in Various Grades of Oral Dysplasia (Leukoplakia) (IHC, x200).

OCT4 Expression in Oral Submucous Fibrosis

Among the OSMF cases, OCT4 expression remained relatively low compared with epithelial dysplasia. Grade I OSMF demonstrated a mean OCT4 expression of 1.33%, whereas Grade II lesions showed negligible immunoreactivity. Grade III OSMF exhibited relatively higher OCT4 expression (4.50%), suggesting a gradual increase in stem cell marker expression with disease progression (Figure 2).

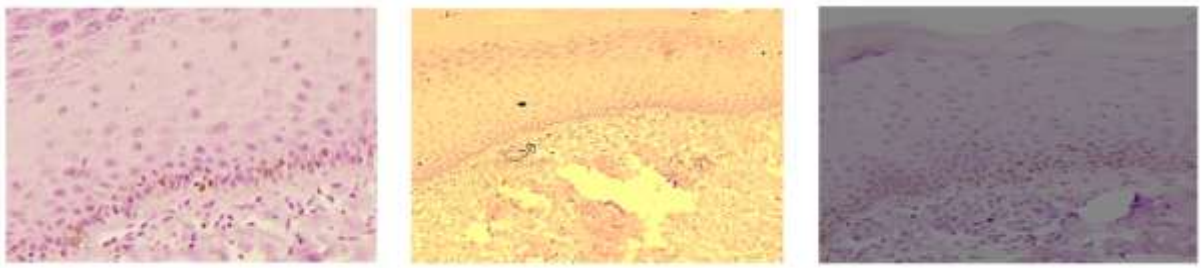

Figure 2: Immunohistochemical Expression of OCT4 in Various Grades of OSMF (IHC, x200)

Distribution of OCT4 Immunostaining

Semi-quantitative analysis demonstrated that 21 of the 30 OPMD cases (70.0%) showed negative immunostaining (0–5% positive cells). Mild positive staining (6–25%) was observed in 5 cases (16.7%), moderate staining (26–50%) in 3 cases (10.0%), and strong staining (>51%) in 1 case (3.3%). The frequency of positive immunostaining increased with advancing histopathological grade (Table 1).

Distribution of OCT4 immunostaining intensity in the OPMD group (n=30)

Scoring Grade	0-5% (Neg.)	6-25% (+)	26-50% (++)	>51% (+++)
No. of Patients (n=30)	21	5	3	1
% of Patients	70.0%	16.7%	10.0%	3.3%

Semi-quantitative scoring: Negative = 0-5% positive cells; (+) = 6-25%; (++) = 26-50%; (+++) = >51%.

Correlation Between OCT4 Expression and Histopathological Grade

Comparison of OCT4 expression among different histopathological grades revealed a clear trend of increasing immunoreactivity with increasing severity of epithelial dysplasia. Mild dysplastic lesions exhibited minimal expression, whereas moderate and severe dysplasia demonstrated substantially higher percentages of OCT4 positive cells. This progressive increase indicates a positive association between OCT4 expression and dysplastic severity, suggesting that activation of stem cell-related pathways occurs during the early stages of oral carcinogenesis.

Similarly, although OCT4 expression in OSMF remained lower than that observed in dysplastic lesions, advanced grades demonstrated relatively greater expression than early-stage lesions, indicating that disease progression may also be accompanied by increased cancer stem cell activity.

DISCUSSION

Oral potentially malignant disorders (OPMDs) represent a heterogeneous group of lesions with varying risks of malignant transformation to oral squamous cell carcinoma (OSCC). Although histopathological grading of epithelial dysplasia remains the cornerstone for risk assessment, its predictive accuracy is limited by subjective interpretation and biological heterogeneity. Consequently, considerable research has focused on identifying molecular biomarkers that reflect early carcinogenic changes and improve the prediction of malignant transformation. Among these biomarkers, cancer stem cell (CSC)-associated proteins have attracted increasing attention because of their central role in tumor initiation, progression, therapeutic resistance, and recurrence. OCT4, a master regulator of pluripotency and self-renewal, is one of the most extensively investigated CSC markers in oral carcinogenesis. Recent reviews emphasize that molecular biomarkers such as CSC markers may complement routine histopathological assessment in stratifying the malignant potential of OPMDs.[24,25]

The present study evaluated OCT4 expression in normal oral mucosa and OPMDs using immunohistochemistry and demonstrated a progressive increase in OCT4 expression with increasing histopathological severity of epithelial dysplasia. Normal oral mucosa exhibited minimal OCT4 immunoreactivity, whereas mild dysplasia showed only occasional positive cells. A marked increase in OCT4 expression was observed in moderate dysplasia, and the highest expression was recorded in severe dysplasia. These findings indicate that activation of stem cell-related pathways occurs early during oral carcinogenesis and becomes increasingly prominent as epithelial atypia progresses. In contrast, OSMF demonstrated relatively low OCT4 expression, although Grade III lesions showed greater immunoreactivity than early-stage disease, suggesting that stem cell activation may accompany disease progression in advanced fibrosis.

The negligible expression of OCT4 observed in normal oral mucosa is consistent with the biological role of OCT4 as a pluripotency-associated transcription factor that is normally restricted to embryonic stem cells and germ cells. Mature oral epithelium contains highly differentiated keratinocytes with limited self-renewal potential; therefore, OCT4 expression is expected to be absent or minimal. Aberrant re-expression of OCT4 in premalignant and malignant oral lesions likely reflects the acquisition of stem cell-like characteristics during neoplastic transformation rather than normal epithelial homeostasis.[26]

One of the principal observations of the present study was the progressive increase in OCT4 expression with increasing grades of oral epithelial dysplasia. Mild dysplastic lesions exhibited minimal staining, whereas moderate and severe dysplasia demonstrated substantially higher immunoreactivity. This trend suggests that accumulation of genetically altered stem-like cells occurs during dysplastic progression and may contribute to increasing malignant potential. Similar observations have been reported by investigators who demonstrated significantly greater OCT4 expression in oral epithelial dysplasia than in normal oral mucosa and proposed OCT4 as a biomarker of early malignant transformation.[27,28] Their findings support the concept that CSC-associated molecular alterations precede the development of invasive carcinoma and may therefore have diagnostic and prognostic significance.[29]

The increase in OCT4 expression observed in severe dysplasia may be explained by the biological functions of OCT4 in maintaining cellular pluripotency, inhibiting differentiation, and promoting unlimited proliferative capacity. Experimental studies have shown that OCT4 regulates multiple signaling pathways involved in stemness, including interactions with SOX2 and NANOG, thereby preserving an undifferentiated cellular phenotype.[30] Aberrant activation of these pathways may permit dysplastic epithelial cells to acquire self-renewal capacity, evade apoptosis, and accumulate additional genetic alterations, ultimately facilitating malignant

transformation. This mechanism provides a plausible explanation for the strong OCT4 immunoreactivity observed in advanced dysplastic lesions.

In the present study, OCT4 expression in OSMF was comparatively lower than that observed in epithelial dysplasia. Nevertheless, Grade III OSMF demonstrated greater expression than Grade I lesions, indicating that OCT4 expression may increase with disease progression. OSMF is characterized primarily by progressive stromal fibrosis and epithelial atrophy rather than marked epithelial proliferation. Consequently, CSC-associated molecular changes may become evident only when dysplastic alterations develop within the overlying epithelium. Recent reviews of OPMDs have similarly emphasized that the biological behavior of OSMF depends not only on fibrosis but also on epithelial molecular alterations that precede malignant transformation.[31]

Semi-quantitative analysis further supported these findings. Most OPMD cases demonstrated negative or weak OCT4 staining, whereas lesions with higher grades of dysplasia showed moderate to strong immunoreactivity. This distribution indicates that OCT4 overexpression is associated primarily with advanced epithelial alterations rather than being uniformly expressed in all OPMDs. Such observations reinforce the concept that OCT4 may serve as a marker of disease progression rather than simply indicating the presence of a potentially malignant lesion.

The present findings are biologically plausible within the framework of the cancer stem cell hypothesis. According to this theory, only a small subset of tumor cells possesses the capacity for unlimited self-renewal, tumor initiation, metastasis, and therapeutic resistance. OCT4-positive cells are believed to represent this stem-like subpopulation and are capable of generating the heterogeneous cell populations characteristic of oral squamous cell carcinoma. As dysplastic severity increases, expansion of this stem cell compartment may contribute to greater malignant potential and progression toward invasive carcinoma.

The clinical implications of these findings are noteworthy. Histopathological grading alone does not always predict malignant transformation accurately, and lesions with similar microscopic appearances may exhibit different biological behavior. Assessment of OCT4 expression may therefore provide additional prognostic information, particularly in lesions with moderate or severe epithelial dysplasia. Patients demonstrating high OCT4 expression may benefit from closer clinical surveillance, complete surgical excision when appropriate, and shorter follow-up intervals. Integration of molecular biomarkers with conventional histopathological evaluation may improve individualized risk assessment and facilitate earlier therapeutic intervention. Current reviews increasingly advocate combining molecular biomarkers with conventional pathology to improve risk stratification in OPMDs.[32,33]

The present study has several strengths. It evaluated OCT4 expression across different categories of OPMDs within a single cohort using a standardized immunohistochemical protocol and semi-quantitative assessment. Inclusion of normal oral mucosa as a control enabled comparison of physiological and pathological expression patterns, while evaluation of different grades of epithelial dysplasia and OSMF provided insight into the progressive changes associated with oral carcinogenesis.

However, certain limitations should be acknowledged. The study was retrospective and involved a relatively limited sample size. Clinical follow-up data regarding malignant transformation were not available, preventing assessment of the predictive value of OCT4 expression over time. In addition, evaluation was restricted to immunohistochemical protein expression without complementary molecular analyses such as quantitative PCR or gene expression profiling. Larger multicenter prospective studies incorporating long-term follow-up and multiple CSC markers are required to validate the clinical utility of OCT4 as a prognostic biomarker.

Overall, the findings of the present study indicate that OCT4 expression increases progressively with the severity of oral epithelial dysplasia and is higher in advanced OPMDs than in normal oral mucosa. These results support the involvement of OCT4 in the early stages of oral carcinogenesis and suggest that OCT4 may serve as a useful adjunctive biomarker for identifying OPMDs at increased risk of malignant transformation. Future prospective studies integrating OCT4 with other cancer stem cell markers and clinicopathological variables may further improve the accuracy of risk prediction and contribute to personalized management strategies for patients with OPMDs.

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