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A Comparative Study of Moderately and Well-Differentiated Oral Squamous Cell Carcinoma: Clinicopathological, Immunohistochemical analysis and Quality of Life Outcomes

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a prevalent malignancy with variable prognosis depending on histopathological differentiation. The present study aimed to comparatively analyse clinicopathological features, immunohistochemical profiles, and quality of life in moderately-differentiated squamous cell carcinoma (MDSCC) and well-differentiated squamous cell carcinoma (WDSCC).

Method: This retrospective study aimed to compare the clinicopathological characteristics, immunohistochemical expression of epidermal growth factor receptor (EGFR) and tumour protein 63 (p63) and quality of life in MDSCC and WDSCC. A total number of 75 cases from each group were analysed for demographic, clinical, and pathological parameters, while immunohistochemical expression of EGFR and p63 was evaluated in 20 tissue samples (10 per group). Postoperative quality of life was assessed using physical, emotional, and functional indices. Statistical analysis was performed using SPSS version 26.0. Descriptive statistics, Student's t-test, and Kaplan–Meier survival analysis were applied, with a *P*-value <0.05 considered to be statistically significant.

Results: MDSCC showed significantly greater tumour thickness, advanced stage, higher incidence of lymph node positivity, Extranodal extension, and microinvasion compared with WDSCC. WDSCC exhibited higher rates of bone invasion. Immunohistochemical analysis revealed significantly higher proportions of EGFR and p63-positive cells in WDSCC. Postoperative functional impairments variably affected speech, swallowing, and social, emotional well-being. Survival analysis indicated a better prognosis in WDSCC.

Conclusion: MDSCC exhibits a more aggressive clinicopathological profile, whereas WDSCC is associated with higher immunohistochemical marker expression, better quality of life, and improved survival rates.

Keywords: Oral squamous cell carcinoma, Human, Health, Mortality, Quality of life

Introduction

According to the recent Global Cancer Observatory (GLOBOCAN) 2022 report, oral squamous cell carcinoma (OSCC) is the 7th most common malignancy globally, and the third most prevalent carcinoma in

India, accounting for about 4.5% of all cancer cases, worldwide. It is responsible for approximately 916,827 deaths each year, with a five-year prevalence estimated at 3,258,518 cases.[1] OSCC constitutes over 90% of all the malignant neoplasms affecting the oral cavity, and its management primarily involves surgical intervention, radiotherapy, and supportive chemotherapy.[1,2] Oral carcinoma ranks among the most prevalent cancers worldwide, with a particularly high burden in developing nations. In India, it remains a leading cause of cancer-related mortality. Notably, human papillomavirus is implicated in nearly 40% of all the oral cancers in India, whereas its contribution is limited to 2%–4% in Western populations. Despite advancements in diagnostic approaches, the prognosis of OSCC continues to be unfavourable, primarily due to the late-stage diagnosis. However, timely diagnosis can significantly enhance the outcome and improve the quality of life of the patients.[3]

In India, cancers of the oral cavity represent the most frequent malignancy among males and ranked as the third most common cancer in females.[4] The oral cavity comprises of the lips, anterior two-thirds of the tongue, floor of the mouth, buccal mucosa, gingiva, hard palate, retromolar trigone, base of the tongue, tonsillar region, and the soft palate. Amongst these, buccal mucosa is the most frequently involved site followed by the floor of the mouth and lateral border of the tongue. The predominant etiological factor for oral carcinoma in India is the habitual placement of pan as a quid in the buccal mucosa, a preparation consisting of betel leaves combined with lime, catechu, and tobacco. Prolonged exposure of the buccal mucosa to these carcinogenic agents significantly contribute to the malignant transformation.[5] The other etiological factors which contribute to the development of oral carcinoma, includes alcohol consumption, tobacco consumption, low socioeconomic status, poor oral hygiene, nutritional deficiencies, viral infections, and chronic irritation caused by ill-fitting dentures or sharp tooth.[6,7]

OSCC is associated with considerable morbidity, causing facial disfigurement and impairments in vital functions such as swallowing, speech, and taste, all of which profoundly affect the patient's quality of life.[8, 9] Clinically, OSCC often appears as a red, white, or mixed red-white lesion with clearly demarcated margins and a slightly irregular surface.[10,11] In the early stages, the lesions are usually asymptomatic; however, as the disease progresses, they may produce discomfort and manifest features like ulceration, nodular growth, and fixation to underlying tissues.[7],[12,13] OSCC primarily metastasizes to the ipsilateral cervical lymph nodes through lymphatic drainage, though involvement of contralateral or bilateral lymph nodes may also occur. Distant metastases most frequently affect the lungs, bone and the liver.[14,15]

Premalignant conditions such as leukoplakia, erythroplakia, and oral submucous fibrosis are well-recognized precursor lesions for the development of OSCC. These lesions are characterized histopathologically by varying degrees of epithelial dysplasia, ranging from mild to severe. Among them, leukoplakia is the most common lesion and carries a relatively high risk of malignant transformation, with rates reported between 0.6% and 18%. The likelihood of progression to carcinoma is closely related to the presence and severity of dysplasia observed microscopically. Histologically, squamous cell carcinoma is graded as: Grade I – well-differentiated, Grade II – moderately-differentiated, Grade III – poorly-differentiated, and Grade IV – anaplastic.[5]

The prognostication and management of OSCC are primarily guided by clinical staging, most commonly assessed using the tumour, node and metastasis (TNM) classification system.[16] This system has long been applied to determine treatment strategies, predict patient outcomes, and assess therapeutic responses. In its 8th

edition, the American Joint Committee on Cancer (AJCC) staging manual introduced depth of invasion and extra nodal extension (ENE) as important modifiers to the tumour and node categories of oral cavity cancer staging, respectively.[17] Notably, histological grading is not included in the current staging framework. While histologic grade has traditionally not been regarded as a reliable prognostic indicator in head and neck squamous cell carcinoma, it is recognized as an important determinant of prognosis in several other solid tumours.[18, 19]

Immunohistochemical markers hold significant potential in improving early detection and prognostic assessment of OSCC. Overexpression of epidermal growth factor receptor (EGFR) (ErbB1/HER1) has been observed in most of the OSCC cases, and elevated expression has been linked to aggressive tumour behaviour, unfavourable prognosis, and resistance to anticancer therapies.[20, 21] p63 marker is essential for maintaining the proliferative potential of progenitor epidermal cells predominantly located in the basal layers. Its expression is important in the pathogenesis of OSCC, as altered p63 activity can contribute to uncontrolled cell growth and tumour development in the oral epithelium.[22, 23, 24] Therefore, the present study aimed to assess and compare the clinicopathological characteristics and immunohistochemical expression of EGFR and p63 in moderately and well-differentiated OSCC. The objective was to identify distinct molecular and histological differences that could enhance the prognostic accuracy and render more targeted treatment approaches.

Materials and Methods

Sample collection

After obtaining approval from the Institutional Ethics Committee (SRB/SDC/OPATH-2404/25/208), histopathological samples were retrieved from the archives of the Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India, for the period between 2019 and 2025. All cases were derived from patients diagnosed and treated at the institution and were included based on the predefined eligibility criteria. The study adopted a retrospective design, and included two groups: Well-differentiated oral squamous cell carcinoma (WDSCC) and moderately-differentiated oral squamous cell carcinoma (MDSCC), and all eligible cases of both the groups were included. A total of 150 cases were analysed, comprising 75 cases each of MDSCC and WDSCC. Since all available eligible archival cases were included, no formal sample size calculation was performed. Furthermore, as this was a retrospective observational study, randomization was not applicable. For both groups, data were collected on various parameters, including demographic details (age and gender), habit history, and associated comorbidities. Detailed histopathological evaluation involved examining lesion characteristics such as tumour thickness, depth of invasion, bone involvement, nodal status, degree of epithelial dysplasia, presence of mitotic figures, lymphovascular invasion (LVI), perineural invasion (PNI), and the extent of skeletal muscle infiltration. The invasive tumour front was further evaluated using Bryne's Histological Malignancy Grading System. The parameters assessed included degree of keratinization, nuclear pleomorphism, pattern of invasion, and host inflammatory response.[25]

Quality of life was primarily assessed using a validated European quality-of-life questionnaire. To capture additional postoperative functional and psychosocial outcomes relevant to the study population, a limited number of supplementary institution-specific questions were included. The standard questionnaire constituted the principal assessment instrument, while the additional questions were used for descriptive evaluation. The

questionnaire was made available in both English and the local language Tamil. Quality-of-life data were collected during the postoperative follow-up period of up to 5 years through structured telephone interviews. Responses were obtained directly from the patients and recorded by the investigator. Patient demographic details, treatment information, follow-up records, and contact information were retrieved from the institutional DIAS database. For deceased patients, survival status and date of death were confirmed through information obtained from the family members.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Inclusion criteria

Only histopathologically confirmed cases of OSCC diagnosed as WDSCC or MDSCC were included in the study. Formalin-fixed, paraffin-embedded tissue blocks archived between 2019 and 2025 with adequate tissue for histopathological and immunohistochemical evaluation were considered. Only cases with complete clinicopathological records and available follow-up information were included in the analysis.

Exclusion criteria

Poorly-differentiated squamous cell carcinoma cases were excluded from the present study. Additionally, specimen with inadequate or poorly preserved tissue that were unsuitable for detailed histopathological evaluation were not included. Cases lacking complete clinical records and essential demographic and habit history data were also excluded. Furthermore, incisional biopsy specimen and samples insufficient to assess the depth of invasion and other key histopathological parameters were not considered for our study.

Immunohistochemistry

Immunohistochemical analysis was performed on twenty tissue blocks, comprising ten samples each from MDSCC and WDSCC. Antibodies against EGFR and p63 were used following standardized, previously established protocols.[26] Sections were cut from formalin-fixed, paraffin-embedded tissue blocks at a thickness of 3 µm and mounted onto poly-L-lysine–coated slides, then incubated overnight at 50 °C. Deparaffinization was performed by immersing the sections in xylene with three successive changes, each lasting 10 minutes. Antigen retrieval was achieved by heating the slides in EDTA buffer using an electric pressure cooker for 20 minutes, followed by a 20-minute cooling period at room temperature. Endogenous peroxidase activity was blocked by incubating the sections with hydrogen peroxide for 30 minutes, followed by two washes with Tris-buffered saline for 3 minutes each. The slides were then incubated with primary antibodies, including EGFR (Pathnsitu Biotechnologies, Hyderabad, India) and tumour protein (p63) (Master Diagnostica, Granada, Spain). Staining procedures were performed in accordance with the manufacturers' instructions. Subsequently, sections were subjected to a secondary antibody for 30 minutes, followed by three washes with Tris buffered saline. Immune reaction was visualized using a 3,3′-diaminobenzidine substrate for 5–10 minutes. Finally, the slides were counterstained with Harris hematoxylin, dehydrated through graded ethanol and xylene, and mounted with DPX medium.

Scoring criteria and analysis

EGFR: EGFR, a transmembrane receptor with tyrosine kinase activity that regulates cell growth, was evaluated using three parameters: (A) percentage of positively stained cells, (B) staining intensity, and (C) a composite immunoreactive score (IRS) calculated by multiplying the values of A and B.[27]

p63: A transcription factor belonging to the p53 gene family, was assessed using the same three parameters—percentage of positive cells, staining intensity, and the composite IRS.[27]

The specific grading criteria for both markers are detailed in Table 1.

Statistical analysis

The results were tabulated and statistical analyses war performed using SPSS version 26.0. Descriptive statistics were used to summarize demographic, clinicopathological, and immunohistochemical data. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. Student's t-test was used for intergroup comparisons, and Kaplan–Meier survival analysis was performed to estimate survival rates. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

From Table 2, patients with MDSCC had a higher mean age (52.40 ± 8.20 years) than those with WDSCC (49.80 ± 7.50 years). Males constituted 64.00% and females 36.00% of the MDSCC group. In the WDSCC group, males accounted for 58.00% and females for 42.00% of the cases.

Figure 1 shows the comparison of clinical and histopathological parameters between MDSCC and WDSCC. Tumour thickness was significantly greater in the MDSCC group (mean = 2.80 mm) than in the WDSCC group (mean = 1.37 mm) (t = 7.932, $P < 0.001$). The mean depth of invasion was 6.50 mm in MDSCC and 6.12 mm in WDSCC, with no statistically significant difference between the groups ($P = 0.432$). Bone invasion was observed in 40.00% of WDSCC cases and 32.00% of MDSCC cases ($P = 0.002$). Advanced-stage tumours (T3/T4) were present in 52.00% of MDSCC cases and 45.00% of WDSCC cases, demonstrating a statistically significant difference between the groups ($P = 0.008$).

Lymph node positivity and ENE were significantly higher in MDSCC (46% and 35%) than WDSCC (38% and 24%), with *P*-values of 0.010 and <0.001, indicative of greater metastatic potential in the former group respectively.

Severe dysplasia was more prevalent among MDSCC cases (88%) versus WDSCC (85%) with a significant difference ($P = 0.043$), highlighting that moderately-differentiated cancers may possess more aggressive cellular atypia despite their differentiation status.

Mitotic figure frequency was marginally higher in MDSCC (35%) compared with WDSCC (32%), although this did not reach statistical significance ($P = 0.078$), suggesting similar proliferative activity levels.

LVI and PNI were significantly elevated in MDSCC (30% and 42%) versus WDSCC (22% and 26%), both with *P*-values <0.001, which underscores a more aggressive tumour microenvironment in moderately-differentiated cases.

Skeletal muscle invasion and involvement of adjacent structures were also significantly higher in MDSCC (46% and 36%) than WDSCC (40% and 18%) ($P = 0.002$ and <0.001), reflecting enhanced locoregional spread in MDSCC.

Bryne's histological score did not differ significantly between the two groups (mean scores 6.50 vs. 6.60, P = not significant), indicating that overall histologic malignancy grading was comparable despite individual feature disparities.

Figures 2a-d, emphasizes that MDSCC exhibits greater tumour thickness and more aggressive pathological features, including enhanced bone invasion, nodal metastasis, extranodal spread, and microscopic invasion of lymphovascular and neural structures, compared with WDSCC. These distinctions have important implications for prognosis and therapeutic treatment planning.

Table 3 shows the comparison of immunohistochemical expression of EGFR and tumour protein 63 (p63) between MDSCC and WDSCC. The mean percentage of EGFR-positive cells was significantly higher in WDSCC (3.60 ± 0.52) than in MDSCC (2.22 ± 0.67) ($P <$

0.001). Similarly, the mean percentage of p63-positive cells was significantly higher in WDSCC (3.70 ± 0.48) than in MDSCC (2.78 ± 0.44) ($P < 0.001$). No statistically significant differences were observed between the groups with respect to staining intensity or IRS for either EGFR or p63 ($P > 0.05$). Overall, a significantly higher proportion of tumour cells expressed EGFR and p63 in WDSCC, whereas staining intensity and IRS scores were comparable between the two groups.

A *P*-value less than or equal to 0.05 was considered to be a statistically significant difference.

Figures 3a–d shows representative photomicrographs demonstrating the immunohistochemical expression patterns of EGFR and p63 in WDSCC and MDSCC.

Quality of life index parameters

Postoperative physical functioning was assessed based on difficulties in speaking, chewing, and swallowing. Among the participants, 40.9% reported no difficulty, 29.5% reported mild difficulty, 6.8% reported moderate difficulty, and 22.7% reported severe difficulty (Figure 4a).

Representative graph regarding eating and nutrition, 47.7% of participants reported no difficulty in swallowing food or liquids, 36.4% reported mild difficulty, and 15.9% reported severe difficulty. With respect to choking or aspiration during eating or drinking, 47.7% reported never experiencing such events, 31.8% reported rare occurrences, and 20.5% reported occasional occurrences (Figure 4b).

Representative graph regarding post-surgical speech and communication assessment showed that 52.3% of participants reported mildly unclear speech, while 47.7% reported moderate speech clarity issues. No participants reported completely clear speech. Regarding slurred speech, 34.1% of participants reported moderate slurring, 25.0% severe slurring, 29.5% slight slurring, and 11.4% slight speech impairment (Figure 4c).

With respect to appearance related concerns following surgery, 31.8% of participants reported frequent concern, 31.8% reported rare concern, 18.2% reported occasional concern, and 18.2% reported no concern regarding changes in appearance (Figure 4d).

Regarding the impact on relationships, work, and social activities, 27.3% of participants reported frequent effects, 25.0% reported rare effects, 25.0% reported no effects, and 22.7% reported occasional effects (Figure 4e).

Regarding fatigue and energy levels following surgery, 25.0% of patients reported frequent fatigue or reduced energy levels, 22.7% reported occasional fatigue, 20.5% reported fatigue rarely, and 31.8% reported no such symptoms (Figure 4f).

Responses regarding pain and discomfort of patients, 20.5% of patients reported frequent persistent pain in the mouth, jaw, or neck that interfered with daily activities. Pain was reported rarely by 47.7% of patients, while 31.8% reported no pain (Figure 5a).

Regarding xerostomia and salivary function, 79.5% of patients reported no symptoms of dry mouth or difficulty in saliva production affecting mastication and speech. Frequent symptoms were reported by 18.2% of patients, whereas 2.3% reported such symptoms rarely (Figure 5b).

Regarding jaw mobility and functional capacity, 40.9% of patients reported no difficulty in mouth opening or mandibular movements. Mild functional limitation was reported by 38.6% of patients, while 9.1% experienced moderate restriction and 11.4% reported severe difficulty (Figure 5c).

With respect to wound healing and post-surgical complications, 40.9% of patients reported no persistent complications. Mild complications were observed in 38.6% of cases, whereas 9.1% experienced moderate complications and 11.4% reported severe complications, including infection or delayed wound healing (Figure 5d).

In terms of emotional well-being, 54.5% of patients reported occasional feelings of anxiety, depression, or emotional strain

following surgery. No such difficulties were reported by 34.1% of patients, whereas 11.4% reported experiencing these emotions frequently (Figure5e).

Overall quality of life

When evaluating overall quality of life following surgery, 31.8% of patients reported a moderate reduction, 27.3% reported a slight reduction, 25.0% reported a considerable decline, and 15.9% reported an extreme deterioration in quality of life. A substantial proportion of patients reported a moderate to severe reduction in quality of life.

Survival analysis

Kaplan–Meier survival analysis showed differences in post-surgical survival between patients with WDSCC and MDSCC (Figure 6). Survival probability remained higher in the WDSCC group throughout the follow-up period. At 2, 4, 6, 8, and 10 months, the estimated survival probabilities for WDSCC were 0.95, 0.90, 0.85, 0.80, and 0.75, respectively, whereas the corresponding survival probabilities for MDSCC were 0.90, 0.75, 0.65, 0.50, and 0.40, respectively.

Discussion

The present study compared the clinicopathological characteristics, immunohistochemical expression of EGFR and tumour protein (p63), quality-of-life outcomes, and survival patterns between MDSCC and WDSCC. Our findings demonstrated that MDSCC exhibited significantly greater tumour thickness, advanced stage, lymph node positivity, ENE, LVI, PNI, and skeletal muscle infiltration, indicating a more aggressive clinicopathological profile. In contrast, WDSCC showed a significantly higher proportion of EGFR and p63-positive tumour cells and demonstrated superior survival outcomes. Furthermore, quality-of-life assessment revealed varying degrees of postoperative functional and psychosocial impairment among affected patients. These findings highlight the importance of integrating histopathological, immunohistochemical, and patient centred outcome measures in the assessment of OSCC.

OSCC is the most prevalent malignancy affecting the oral cavity and it originates from the mucosal epithelium. The incidence of OSCC demonstrates considerable geographic variation, reflecting differences in regional epidemiological trends. Established etiological factors include cigarette smoking, alcohol intake, and the use of smokeless tobacco products, which are often combined with areca nut and various other additives such as slaked lime, betel inflorescence, condiments, sweeteners, and spices to form betel quid. [28]

The present study evaluated 75 cases each of MDSCC and WDSCC to compare their clinicopathological characteristics, immunohistochemical profiles, quality-of-life outcomes, and survival patterns. The findings demonstrated that MDSCC exhibited significantly greater tumour thickness, advanced tumour stage, lymph node positivity, ENE, LVI, PNI, and skeletal muscle infiltration compared with WDSCC. In contrast, WDSCC showed a significantly higher proportion of EGFR and tumour protein (p63)-positive tumour cells and was associated with better survival outcomes. Furthermore, quality-of-life assessment revealed varying degrees of postoperative functional and psychosocial impairment among affected patients. These findings highlight the distinct biological and clinical behaviour of the two histological subtypes of OSCC.

In the present study, a male predominance was observed in both grades of OSCC. The MDSCC group comprised 64.0% males and 36.0% females, whereas the WDSCC group comprised 58.0% males and 42.0% females. The mean age at presentation was 52.4 years in the MDSCC group and 49.8 years in the WDSCC group. These findings are consistent with those reported by Yasin et al. and Singh et al., who also observed a higher incidence of OSCC among males and reported a mean age of presentation of

approximately 51.8 years.[29, 30] Similarly, Tranby et al. reported a higher incidence of oral and oropharyngeal cancers among males and older individuals in a population-based analysis of Medicaid and commercial insurance claims data in the United States.[31] The observed male predominance may be attributed to greater exposure to established risk factors such as tobacco use, areca nut chewing, and alcohol consumption.

An important observation in the present study was the occurrence of OSCC among female patients without conventional risk factors. Among the female patients evaluated, a substantial proportion reported no history of tobacco use, areca nut chewing, or alcohol consumption, suggesting the presence of non-habit-associated OSCC. This finding is consistent with the observations of Foy et al., who reported an increasing incidence of OSCC among non-smoking and non-drinking individuals, particularly women, and proposed alternative etiological mechanisms, including viral infections and genetic susceptibility.[32] Similarly, Koo et al., identified a distinct subgroup of non-smoker, non-drinker patients with OSCC who were predominantly female and exhibited clinicopathological and molecular characteristics that differed from those of habit-associated tumours.[33] These findings support the possibility that factors other than traditional carcinogenic habits may contribute to OSCC development in a subset of patients.

In the present study, the buccal mucosa was the most frequently affected anatomical site of OSCC. This observation may be related to the common practice of placing tobacco or areca nut quid in the buccal vestibule, resulting in prolonged exposure of the mucosa to carcinogenic agents. The lateral border of the tongue was the second most commonly involved site. These findings are in agreement with those reported by Khan et al., who identified the buccal mucosa (66.7%) as the predominant site of involvement and attributed this distribution to the habitual placement of quid in the vestibule, while the tongue (16.67%) was the next most frequently affected site.[19]

The comparative analysis between MDSCC and WDSCC in the present study demonstrated distinct differences in their clinicopathological behaviour. MDSCC exhibited significantly greater tumour thickness, higher frequencies of lymph node metastasis, ENE, LVI, PNI, skeletal muscle infiltration, and involvement of adjacent structures, indicating a more aggressive biological phenotype. These findings suggest an increased propensity for local invasion and regional spread in moderately differentiated tumours. In contrast, although bone invasion was observed more frequently in WDSCC, most adverse pathological parameters were more prevalent in MDSCC. The significantly higher incidence of lymphovascular and PNI in MDSCC may reflect enhanced tumour–stromal interactions and invasive potential, which are recognized predictors of poor clinical outcome. Similarly, the increased occurrence of nodal metastasis and ENE further supports the aggressive behaviour of MDSCC and its association with less favourable prognosis. Despite these differences, Bryne's histological malignancy scores were comparable between the two groups, suggesting that assessment of individual clinicopathological parameters may provide additional prognostic information beyond composite histological grading. Collectively, these findings indicate that MDSCC is associated with a more aggressive clinicopathological profile than WDSCC and highlight the importance of comprehensive pathological evaluation for accurate prognostication and treatment planning.

Our findings are consistent with those reported by Khan et al., who demonstrated a significant association between the histopathological grade of OSCC and regional lymph node metastasis. In their study, 36.67% of cases exhibited lymph node metastasis, with a higher proportion occurring in moderately- and poorly-

differentiated tumours, and the association was statistically significant ($P = 0.001$).[19] Similar observations were reported by Mrosk et al., who identified histopathological risk factors, particularly depth of invasion, as important predictors of cervical lymph node metastasis noted in oral tongue squamous cell carcinoma.[34] Likewise, Viswanatha et al. demonstrated a positive correlation between lymph node metastasis and adverse histopathological features such as LVI and PNI in oral tongue squamous cell carcinoma.[35] The higher frequency of nodal metastasis and ENE observed in the MDSCC group in the present study further supports the role of these pathological parameters as indicators of aggressive tumour behaviour and regional disease spread.

In our study, the immunohistochemical comparison of EGFR and tumour protein (p63) expression between MDSCC and WDSCC revealed a significantly higher proportion of EGFR- and p63-positive tumour cells in WDSCC. The mean percentage scores for EGFR and p63 were significantly greater in WDSCC than in MDSCC ($P < 0.001$). However, no significant differences were observed with respect to staining intensity or IRS, indicating that the proportion of immunopositive cells differed between the groups, whereas the intensity of expression remained comparable. The finding of increased EGFR positivity in WDSCC contrasts with the conventional association of EGFR overexpression with tumour aggressiveness and may reflect differences in tumour differentiation, antibody reactivity, or expression patterns within the tumour cell population. Furthermore, the higher proportion of p63-positive cells observed in WDSCC is consistent with the role of p63 in epithelial differentiation and basal cell maintenance. These observations suggest that assessment of EGFR and p63 expression may provide complementary information regarding tumour microenvironment when interpreted alongside conventional histopathological parameters.

The findings of the present study are supported by previous investigations evaluating the roles of p63 and EGFR in OSCC. Pansini et al., demonstrated that p63 plays a critical role in oral carcinogenesis, with its expression progressively increasing from normal oral epithelium to epithelial dysplasia and reaching peak levels in OSCC, a finding consistently supported by earlier studies.[23] Similarly, Kimura et al., reported a loss of EGFR expression in highly invasive OSCC (HOC313 cell line), suggesting that reduced EGFR expression may be associated with the acquisition of an invasive phenotype.[21] These observations may explain the higher proportion of EGFR-positive cells observed in WDSCC compared with MDSCC in the present study. Collectively, these findings suggest that while p63 overexpression is associated with epithelial proliferation and tumour progression, reduced EGFR expression in less differentiated and more invasive tumours may reflect tumour dedifferentiation and aggressive biological behaviour. This is consistent with the distinct immunohistochemical profiles observed between the MDSCC and WDSCC groups in the present study.

The quality-of-life assessment in the present study demonstrated that a considerable proportion of patients experienced postoperative functional and psychosocial challenges. Difficulties related to speech, swallowing, mastication, appearance, fatigue, and emotional well-being were reported by many participants, reflecting the substantial impact of OSCC and its treatment on daily functioning. Similar findings were reported by Kosgallana et al., who validated the modified Sinhala version of the EORTC QLQ-OH15 and established its reliability and responsiveness in evaluating oral health-related quality of life among oral cancer patients undergoing radiotherapy, particularly in domains related to masticatory function, gum and speech

problems, and oral soreness.[36] Furthermore, Taylor and Singer, in their systematic review of long-term quality of life among head and neck cancer patients, reported persistent deficits in speech, swallowing, social functioning, and emotional health following treatment, even among long-term survivors.[37] The findings of the present study are consistent with these observations and highlight the lasting functional and psychological consequences of OSCC and its management. These results emphasize the importance of incorporating quality-of-life assessment, psychological support, and multidisciplinary rehabilitation into routine postoperative care to improve long-term patient outcomes.

While the present study provides valuable insights into the clinicopathological characteristics, immunohistochemical expression, quality of life, and survival outcomes of WDSCC and MDSCC, certain limitations should be considered. The retrospective single centre study design may limit the generalizability of the findings and introduce selection bias. In addition, immunohistochemical analysis was performed on a relatively small subset of cases. Furthermore, postoperative quality-of-life assessment was based on available follow-up data and may have been influenced by variations in treatment modalities and patient compliance. Future multicentric prospective studies with larger sample sizes and a broader panel of molecular markers are warranted to validate and extend the present findings.

Conclusion

The present study demonstrates that WDSCCs and MDSCCs exhibit distinct clinicopathological, immunohistochemical, and prognostic profiles. Moderately-differentiated tumours were characterized by greater local aggressiveness, including increased tumour thickness, nodal involvement, ENE, LVI, PNI, and skeletal muscle infiltration, whereas well-differentiated tumours showed broader expression of EGFR and tumour protein 63 and were associated with improved survival outcomes. In addition, postoperative quality-of-life assessment revealed persistent functional and psychosocial challenges, highlighting the importance of long-term patient support and rehabilitation. Collectively, these findings indicate that histological differentiation alone may not fully reflect the biological behaviour and clinical outcomes of OSCC. Therefore, integrating conventional histopathological grading with immunohistochemical biomarkers and patient-reported outcome measures may enhance risk stratification, prognostication, and individualized treatment planning.

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Authors' Contributions

Divya Shree S, contributed to data collection, histopathological and immunohistochemical analysis, statistical analysis, interpretation of data, drafting of the manuscript, and final approval of the version to be published. Dr. Gheena S. contributed to study conception and design, supervision of the research, interpretation of data, critical revision of the manuscript for important intellectual content, and final approval of the version to be published. Both authors agreed to be accountable for all aspects of the work and have read and approved the final manuscript.

Conflict of Interest

Nine declared.

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Table 1. Immunohistochemical scoring criteria used for assessment of EGFR and p63 expression according to percentage of positive cells, staining intensity, and calculation of the IRS

A (percentage of positive cells)	**B (intensity of staining)**	**IRS score (multiplication of A and B)**
0 = no colour reaction	0 = no colour reaction	0-1= negative
1 ≤10% of positive cells	1= mild reaction	2-3 = mild
2 = 10-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 = strongly positive
4 ≥80% of positive cells		
	Final IRS score (AXB): 0-12	

A: Percentage of positive cells; B: Intensity of staining; IRS: Immunoreactive score; EGFR: Epidermal growth factor receptor

Table 2. Comparison of mean age and gender distribution between MDSCC and WDSCC case

Group	Mean age (years)	Male (years)	Female (years)
MDSCC	52.40	64.00	36.00
WDSCC	49.80	58.00	42.00

MDSCC: Moderately-differentiated squamous cell carcinoma; WDSCC: Well-differentiated squamous cell carcinoma

Table 3. Comparison of EGFR and tumor protein (p63). Immunohistochemical expression based on percentage of positive cells, staining intensity, and immunoreactive score (IRS) between well-differentiated oral squamous cell carcinoma (WDSCC) and moderately differentiated oral squamous cell carcinoma (MDSCC)

IHC marker	Groups	Mean	S.D	Standard error	Significance
EGFR percentage of positive cells	**MDSCC**	2.22	.67	.22	**0.000***
	WDSCC	3.60	.52	.16	
EGFR Intensity of staining	**MDSCC**	1.56	.53	.18	**0.263**
	WDSCC	1.90	.74	.23	
EGFR IRS score	**MDSCC**	1.44	.53	.18	**0.062**
	WDSCC	2.00	.67	.21	
P 63 percentage of positive cells	**MDSCC**	2.78	.44	.15	**0.000***
	WDSCC	3.70	.48	.15	
P 63 Intensity of staining	**MDSCC**	2.44	.53	.18	**0.821**
	WDSCC	2.50	.53	.17	
P 63 IRS score	**MDSCC**	1.44	.53	.18	**0.821**
	WDSCC	1.50	.53	.17	

MDSCC: Moderately-differentiated squamous cell carcinoma; WDSCC: Well-differentiated squamous cell carcinoma IRS: Immunoreactive score; EGFR: Epidermal growth factor receptor

Table 4. Frequency distribution of patient responses regarding perceived decline in overall health and quality of life following surgery

Questions	**Response**	**Frequency (%)**
Compared to before surgery, my overall health and quality of life have declined.	A little	12 (27.30%)
	Moderately	14 (31.80%)
	Quite a bit	11 (25.00%)
	Extremely	7 (15.90%)

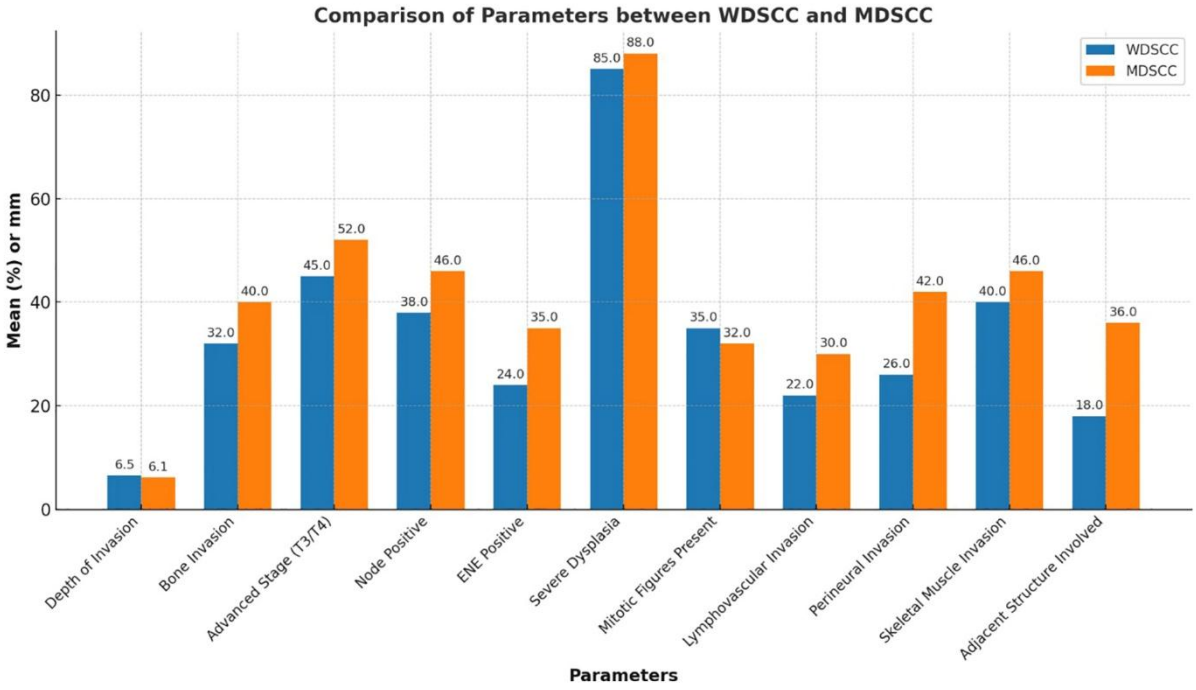

Figure 1. This figure shoes the comparison of clinicopathological characteristics between WDSCC and MDSCC. The graph illustrates the mean depth of invasion (mm) and the proportion of cases (%) exhibiting bone invasion, advanced stage (T3/T4), nodal positivity, ENE, severe dysplasia, mitotic figures, lymphovascular invasion, perineural invasion, skeletal muscle invasion, and adjacent structure involvement in the two study groups.

WDSCC: Well-differentiated oral squamous cell carcinoma; MDSCC: Moderately-differentiated oral squamous cell carcinoma; ENE: Extranodal extension

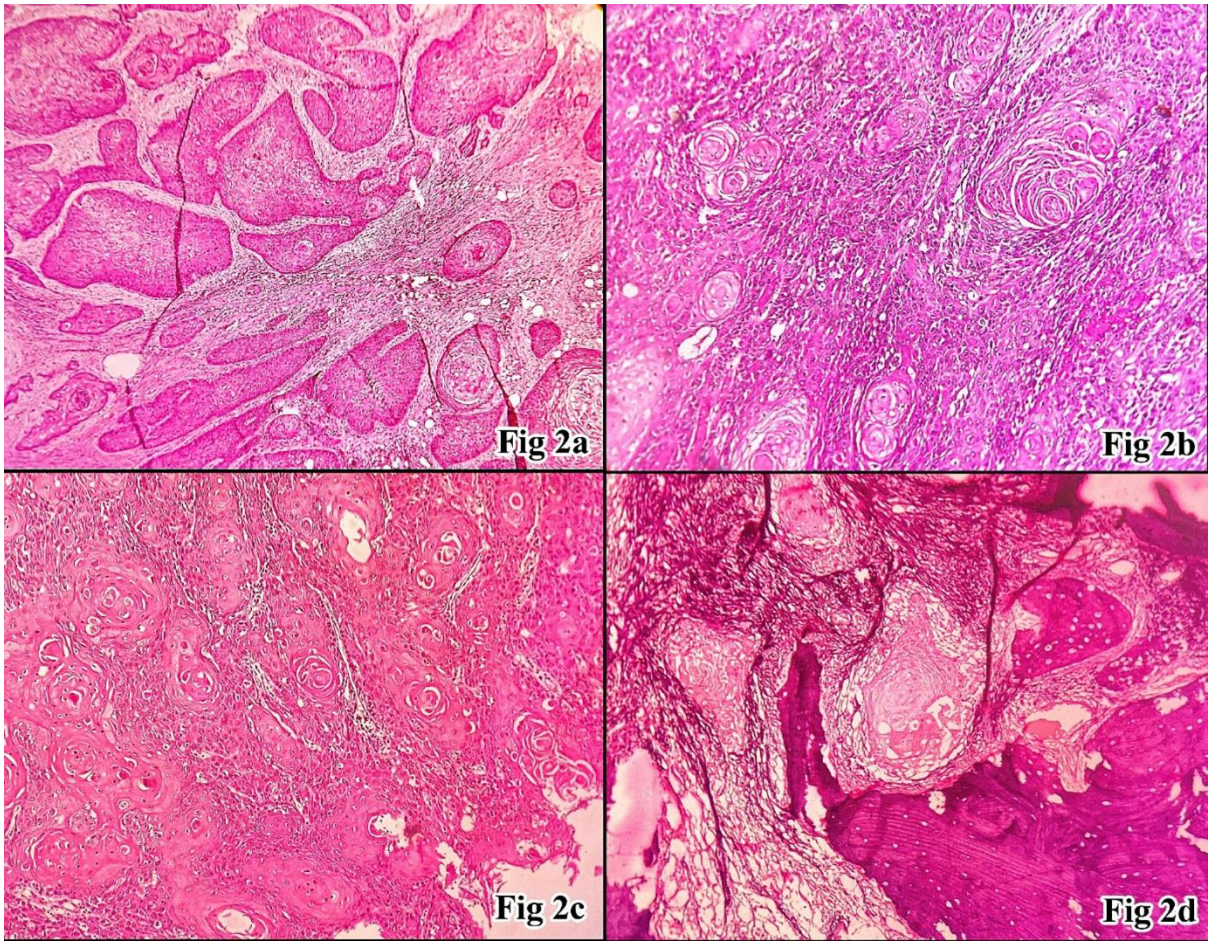

Figure 2. This figure shows the representative hematoxylin and eosin (H&E)-stained photomicrographs illustrating the histopathological features of MDSCC and WDSCC. (a) MDSCC showing irregular nests and cords of malignant squamous epithelial cells infiltrating the connective tissue stroma with moderate keratin pearl formation. (b) MDSCC exhibiting marked cellular pleomorphism, hyperchromatic nuclei, and an increased stromal reaction. (c) WDSCC demonstrating prominent keratin pearl formation and relatively preserved squamous differentiation. (d) WDSCC showing invasive tumor islands infiltrating the adjacent skeletal muscle tissue.

WDSCC: Well-differentiated oral squamous cell carcinoma; MDSCC: Moderately-differentiated oral squamous cell carcinoma

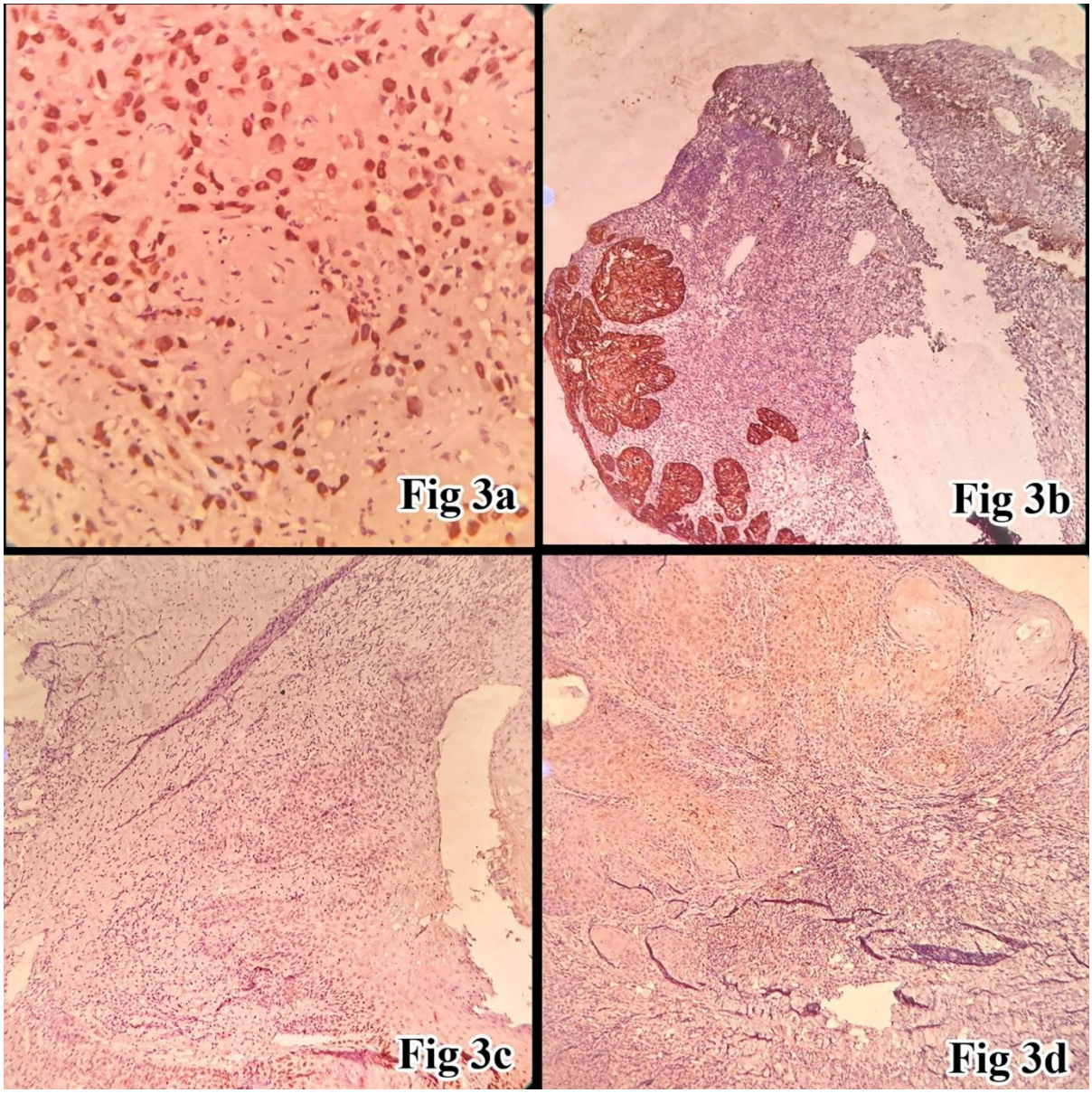

Figure 3. This figure shows the representative immunohistochemical photomicrographs demonstrating the expression of EGFR and p63 in WDSCC and MDSCC. (a) WDSCC showing strong nuclear immunopositivity for p63. (b) WDSCC exhibiting diffuse membranous expression of EGFR. (c) MDSCC demonstrating moderate nuclear immunoreactivity for p63. (d) MDSCC showing reduced membranous expression of EGFR.

EGFR: Epidermal growth factor receptor; WDSCC: Well-differentiated oral squamous cell carcinoma; MDSCC: Moderately-differentiated oral squamous cell carcinoma

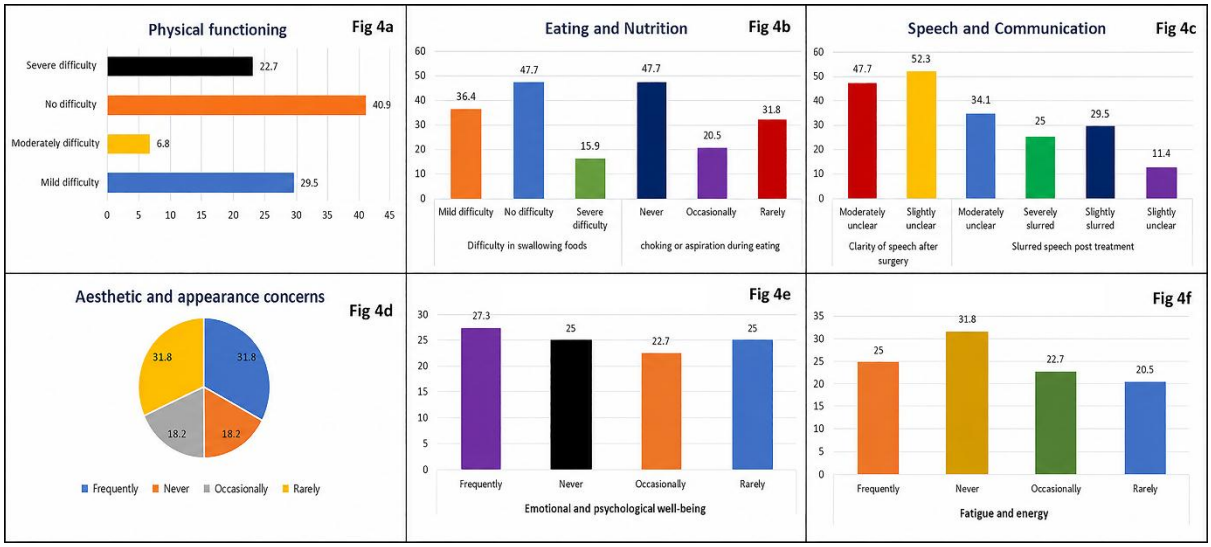

Figure 4. This figure shows graphical representation of the quality-of-life assessment domains among patients with oral squamous cell carcinoma following treatment. The figure depicts the percentage distribution of patient responses across six domains: (a) physical functioning, (b) eating and nutrition, including difficulty in swallowing and aspiration during eating, (c) speech and communication clarity after surgery, (d) aesthetic and appearance concerns, (e) emotional and psychological well-being, and (f) fatigue and energy levels. The responses reflect the perceived impact of the disease and its treatment on various aspects of patients' quality of life.

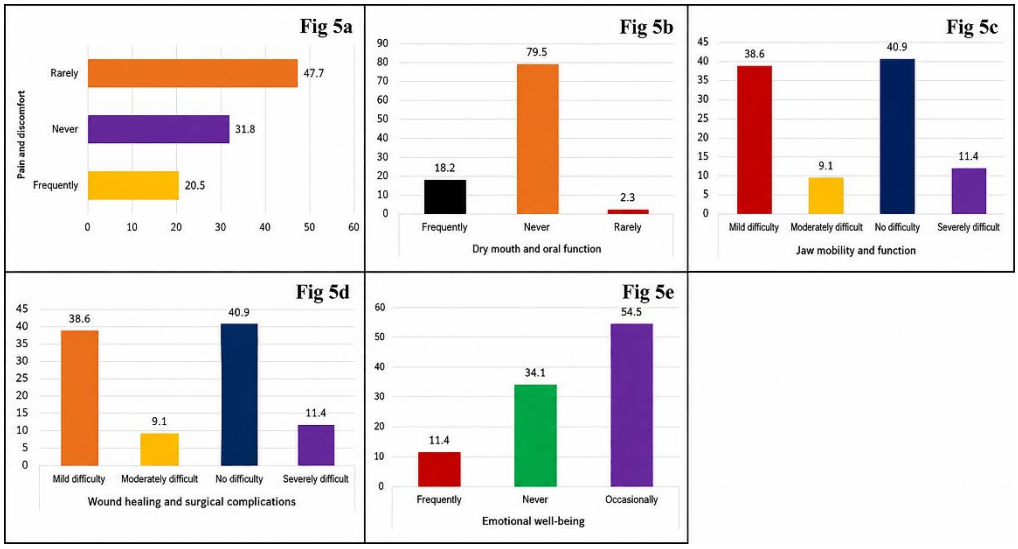

Figure 5. This figure shows the graphical representation of post-treatment functional and psychosocial outcomes among patients with oral squamous cell carcinoma. The figure illustrates the percentage distribution of patient responses regarding (a) pain and discomfort, (b) dry mouth and oral function, (c) jaw mobility and function, (d) wound healing and surgical complications, and (e) emotional well-being following treatment. Most patients reported rare or absent symptoms related to dry mouth and oral dysfunction, while varying degrees of difficulty were observed for jaw mobility and wound healing. Emotional well-being responses ranged from frequent concerns to occasional or no concerns, reflecting the diverse psychosocial impact of treatment. Values are presented as percentages of patient responses.

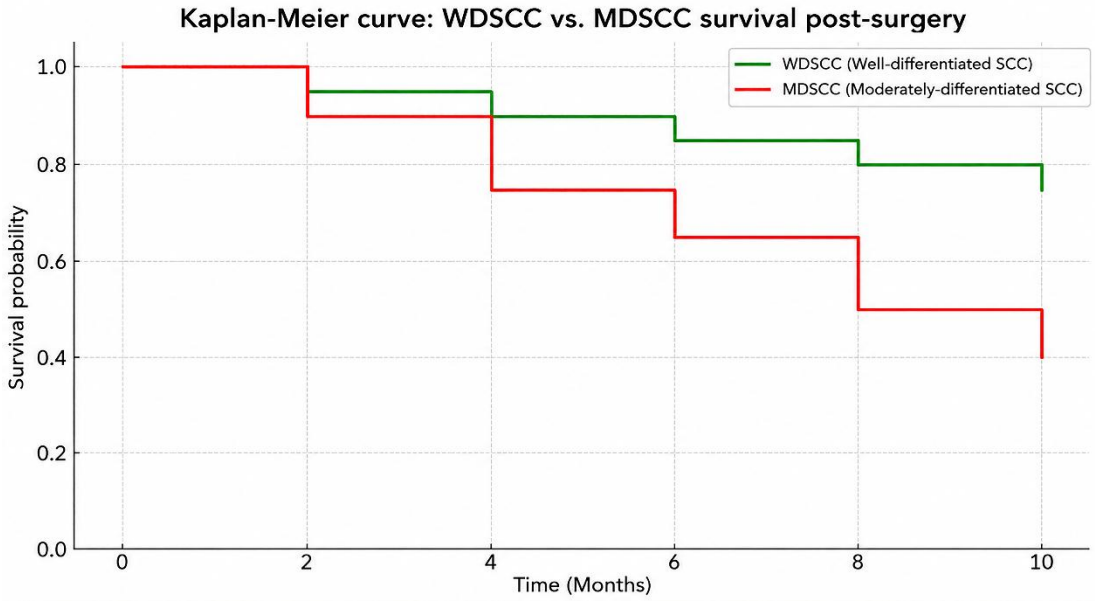

Figure 6. This figure shows the Kaplan–Meier survival curve illustrating the post-surgical survival probabilities of patients with WDSCC and MDSCC during the follow-up period. The WDSCC group demonstrated consistently higher survival probabilities compared with the MDSCC group, indicating a more favorable post-surgical prognosis in patients with well-differentiated tumors. Survival probability is plotted against time (months) following surgical treatment.

WDSCC: Well-differentiated oral squamous cell carcinoma; MDSCC: Moderately-differentiated oral squamous cell carcinoma