# A STUDY ON CANCER ASSOCIATED FIBROBLAST USING ALPHA SMOOTH MUSCLE ACTIN IMMUNOHISTOCHEMISTRY IN ORAL SQUAMOUS CELL CARCINOMA

# A STUDY ON CANCER ASSOCIATED FIBROBLAST USING ALPHA SMOOTH MUSCLE ACTIN IMMUNOHISTOCHEMISTRY IN ORAL SQUAMOUS CELL CARCINOMA

BY

**DR. AMBIKA KUNHIKANNAN**

**DISSERTATION SUBMITTED TO**
**SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH**

**TAMAKA, KOLAR, KARNATAKA**
**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF**

**DOCTOR OF MEDICINE**
**IN**
**PATHOLOGY**

**UNDER THE GUIDANCE OF**

**DR. T.N. SURESH MD, DNB, MNAMS**
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The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the synopsis entitled **"A study on cancer associated fibroblast using alpha smooth muscle actin immunohistochemistry in oral squamous cell carcinoma"** being investigated by **Dr.Ambika Kunhikannan**, Dr.T.N.Suresh & Dr.S.M.Azeem Mohiyuddin[1] in the Departments of Pathology & ENT[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

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Thank you everyone.

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ABSTRACT Background Oral-squamous cell carcinoma (O-SCC) represents a significant global health burden with complex pathophysiology involving TME interactions. The tumour stroma, particularly CAFs, plays a crucial role in tumour advancement, invasion, as well as metastasis. CAFs, identified by alpha- SMA expression, influence tumour behaviour through ECM remodelling and pro-tumorigenic signalling. Despite emerging evidence of their prognostic significance, the relationship between CAF expression patterns and clinicopathological parameters in O-SCC remains inadequately characterized. Objectives This study aimed to detect CAFs using α-SMA IHC in O-SCC and evaluate their association with LNM and pTNM staging, potentially identifying new prognostic markers for clinical management. Methodology This laboratory-based analytical study was conducted at Sri Devaraj Urs Medical College between September 2022 and December 2023. Histo-pathologically confirmed O-SCC cases (n=88) treated by composite resection and cervical lymph node dissection were included, excluding recurrent cases, patients who received neoadjuvant chemotherapy, and second primary cancers. Haematoxylin and eosin slides were reviewed for LNM and pTNM staging. Immunohistochemical staining for α-SMA was performed on 4µm formalin-fixed paraffin-embedded tissue sections using heat-induced antigen retrieval, followed by incubation with primary antibody and HRP-conjugated secondary antibody. CAF expression was quantified using Kellermann's scoring system (Score 1is less than 1%, Score 2 is between 1 and 50%, Score 3 is more than 50% stained cells) and categorized by distribution pattern (focal, network, or spindle). Additional parameters assessed included TSR, WPOI, T-B, and TILs. Results The study population comprised 88 patients (69.3% female, 30.7% male) with an average age of 56.97 years. The buccal mucosa represented the most frequent site (50%), with well-differentiated tumours predominating (80.7%). Pathological staging revealed stage IVA as most prevalent (33%), followed by stage III (31.8%). Regarding CAF expression, score 3 (abundant CAFs) was observed in 55.7% of cases, Score 2 in 40.9%, and Score 1 in only 3.4%. Network pattern CAF distribution predominated (38.6%), with equal representation of focal and spindle configurations (30.7% each). Statistical analysis revealed no significant association between CAF scores and LNM ($p=0.758$); however, CAF distribution patterns demonstrated a statistically significant association with pTNM staging ($\chi^2=26.716$, $p=0.001$), with advanced stages showing distinct pattern shifts 2 toward network and spindle arrangements. Notably, significant correlations were observed between TSR and CAF score ($p<0.0001$), T-B and CAF score ($p=0.021$), and T-B and LNM ($p=0.047$). More aggressive invasion patterns demonstrated higher CAF scores and increased T-B intensity. Conclusion While CAF scores alone did not predict LNM, CAF architectural patterns demonstrated significant associations with pathological staging in O-SCC. The correlations between CAF expression, T-B, and invasion patterns suggest that CAF distribution, rather than mere presence, may serve as a valuable prognostic indicator. These findings highlight the potential of CAF architectural evaluation as an adjunctive histopathological parameter for risk stratification in O-SCC patients Keywords: Oral Squamous Cell Carcinoma; Cancer-Associated Fibroblasts; Alpha-Smooth Muscle Actin; Tumour Microenvironment; Tumour Budding; Lymph Node Metastasis; Tumour-Stroma Ratio INTRODUCTION O-SCC signifies a significant global health problem, placing as the eighth most widespread cancer worldwide according to GLOBOCAN 2022 estimates, with approximately 500,000 new cases diagnosed annually.1 The disease burden shows marked geographical variation, with particularly high incidence rates in South Asian countries.2 In India, O-SCC accounts for nearly 30% of all cancers, with an age-standardized incidence rate of 12.6 per lakh population, significantly higher than the global average of 4.1 per lakh.2,3 The southern states of India, including Karnataka, report particularly concerning statistics, with O-SCC accounting for up to 40% of all malignancies in some regional cancer centers.2 In Karnataka specifically, oral cancer ranks as the most prevailing cancer among men and the third most prevailing among women, with an age-adjusted incidence rate of 11.5 per lakh in men and 8.9 per lakh in women.2 The high prevalence in this region is attributed to widespread use of tobacco products, betel quid chewing, and alcohol consumption, combined with limited access to early diagnostic facilities and treatment centers.2,3 The progression and prognosis of O-SCC are influenced by complex interactions within the TME, which comprises various cellular and non-cellular components.4 Among these, CAFs have emerged as crucial modulators of tumour behavior.5 CAFs, which develop through the transformation of normal fibroblasts into an 4 activated phenotype, demonstrate remarkable plasticity and heterogeneity in their functions.6 These cells actively participate in multiple aspects of tumour

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# ABSTRACT

## Background

Oral-squamous cell carcinoma (O-SCC) represents a significant global health burden with complex pathophysiology involving TME interactions. The tumour stroma, particularly CAFs, plays a crucial role in tumour advancement, invasion, as well as metastasis. CAFs, identified by alpha- SMA expression, influence tumour behavior through ECM remodelling and pro-tumorigenic signalling. Despite emerging evidence of their prognostic significance, the relationship between CAF expression patterns and clinicopathological parameters in O-SCC remains inadequately characterized.

## Objectives

This study aimed to detect CAFs using α-SMA IHC in O-SCC and evaluate their association with LNM and pTNM staging, potentially identifying new prognostic markers for clinical management.

## Methodology

This laboratory-based analytical study was conducted at Sri Devaraj Urs Medical College between September 2022 and December 2023. Histo-pathologically confirmed O-SCC cases (n=88) treated by composite resection and cervical lymph node dissection were included, excluding recurrent cases, patients who received neoadjuvant chemotherapy, and second primary cancers. Haematoxylin and eosin slides were reviewed for LNM and pTNM staging. Immunohistochemical staining for α-SMA was performed on 4μm formalin-fixed paraffin-embedded tissue sections using heat-induced antigen retrieval, followed by incubation with primary antibody and HRP-conjugated secondary antibody. CAF expression was quantified using Kellermann's scoring system (Score 1is less than 1%, Score 2 is between 1 and 50%, Score 3 is more than

50% stained cells) and categorized by distribution pattern (focal, network, or spindle). Additional parameters assessed included TSR, WPOI, T-B, and TILs.

**Results**

The study population comprised 88 patients (69.3% female, 30.7% male) with an average age of 56.97 years. The buccal mucosa represented the most frequent site (50%), with well-differentiated tumours predominating (80.7%). Pathological staging revealed stage IVA as most prevalent (33%), followed by stage III (31.8%). Regarding CAF expression, score 3 (abundant CAFs) was observed in 55.7% of cases, Score 2 in 40.9%, and Score 1 in only 3.4%. Network pattern CAF distribution predominated (38.6%), with equal representation of focal and spindle configurations (30.7% each). Statistical analysis revealed no significant association between CAF scores and LNM ($p=0.758$); however, CAF distribution patterns demonstrated a statistically significant association with pTNM staging ($\chi^2=26.716$, $p=0.001$), with advanced stages showing distinct pattern shifts toward network and spindle arrangements. Notably, significant correlations were observed between TSR and CAF score ($p<0.0001$), T-B and CAF score ($p=0.021$), and T-B and LNM ($p=0.047$). More aggressive invasion patterns demonstrated higher CAF scores and increased T-B intensity.

**Conclusion**

While CAF scores alone did not predict LNM, CAF architectural patterns demonstrated significant associations with pathological staging in O-SCC. The correlations between CAF expression, T-B, and invasion patterns suggest that CAF distribution, rather than mere presence, may serve as a valuable prognostic indicator. These findings highlight the potential of CAF architectural evaluation as an adjunctive histopathological parameter for risk stratification in O-SCC patients

**Keywords:** Oral Squamous Cell Carcinoma; Cancer-Associated Fibroblasts; Alpha-Smooth Muscle Actin; Tumour Microenvironment; Tumour Budding; Lymph Node Metastasis; Tumour-Stroma Ratio

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# ABBREVIATIONS

| S. No | Abbreviation | Explanation |
|---|---|---|
| 1 | OSCC | Oral Squamous Cell Carcinoma |
| 2 | TMC | Tumour Microenvironment |
| 3 | CAF | Cancer-Associated Fibroblasts |
| 4 | α-SMA | A-Smooth Muscle Actin |
| 5 | HPV | Human Papillomavirus |
| 6 | DNA | Deoxyribonucleic acid |
| 7 | FA | Fanconi Anaemia |
| 8 | OLP | Oral Lichen Planus |
| 9 | OSMF | Oral Submucous Fibrosis |
| 10 | PTV | Pathological Tumour Volume |
| 11 | MTV | Metabolic Tumour Volume |
| 12 | DOI | Depth Of Invasion |
| 13 | AJCC | American Joint Committee On Cancer |
| 14 | WPOI | Worst Pattern Of Invasion |
| 15 | PNI | Perineural Invasion |
| 16 | LVI | Lymphovascular Invasion |
| 17 | TILs | Tumour-Infiltrating Lymphocytes |

| | | |
|---|---|---|
| 18 | TATE | Tumour-Associated Tissue Eosinophilia |
| 19 | LNR | Lymph Node Ratio |
| 20 | ECE | Extracapsular Extension |
| 21 | TME | Tumour Microenvironment |
| 22 | TSR | Tumour-Stroma Ratio |
| 23 | PDGFR | Platelet-Derived Growth Factor Receptors |
| 24 | ECM | Extracellular Matrix |
| 25 | EMT | Epithelial-Mesenchymal Transition |
| 26 | MMPs | Matrix Metalloproteinases |
| 27 | IHC | Immunohistochemistry |
| 28 | FAP | Fibroblast Activation Protein |

# INTRODUCTION

O-SCC signifies a significant global health problem, placing as the eighth most widespread cancer worldwide according to GLOBOCAN 2022 estimates, with approximately 500,000 new cases diagnosed annually.[1] The disease burden shows marked geographical variation, with particularly high incidence rates in South Asian countries.[2] In India, O-SCC accounts for nearly 30% of all cancers, with an age-standardized incidence rate of 12.6 per lakh population, significantly higher than the global average of 4.1 per lakh.[2,3] The southern states of India, including Karnataka, report particularly concerning statistics, with O-SCC accounting for up to 40% of all malignancies in some regional cancer centers.[2] In Karnataka specifically, oral cancer ranks as the most prevailing cancer among men and the third most prevailing among women, with an age-adjusted incidence rate of 11.5 per lakh in men and 8.9 per lakh in women.[2] The high prevalence in this region is attributed to widespread use of tobacco products, betel quid chewing, and alcohol consumption, combined with limited access to early diagnostic facilities and treatment centers.[2,3]

The progression and prognosis of O-SCC are influenced by complex interactions within the TME, which comprises various cellular and non-cellular components.[4] Among these, CAFs have emerged as crucial modulators of tumour behavior.[5] CAFs, which develop through the transformation of normal fibroblasts into an activated phenotype, demonstrate remarkable plasticity and heterogeneity in their functions.[6] These cells actively participate in multiple aspects of tumour progression, including ECM remodelling, angiogenesis promotion, and immune response modulation.[7,8]

The identification and characterization of CAFs typically rely on the expression of specific markers, with α-SMA being one of the most reliable and widely used markers.[9,10] Studies have shown that the presence and distribution pattern of CAFs, as detected by α-SMA IHC, may correlate with various

clinicopathological parameters in O-SCC.[4,11] However, the relationship between CAF presence and crucial foretelling factors such as LNM and pTNM staging remains incompletely understood.[12]

LNM serves as one of the most considerable foretelling indicators in O-SCC, with its presence substantially reducing patient survival rates from approximately 80% to 40% at five years.[13] Regional studies from Karnataka have reported LNM in 40-60% of O-SCC cases at presentation, significantly higher than the global average of 30-35%.[14] The conventional TNM staging practice, while valuable, may not fully portray the biological complexity of tumour progression, particularly regarding the role of stromal elements.[3,15] Recent studies suggest that the incorporation of stromal markers, including CAF assessment, might provide additional prognostic information beyond traditional staging parameters.[16]

The interaction between CAFs as well as tumour cells creates a permissive microenvironment that enables tumour invasion and metastasis.[11,17] Through the secretion of various growth factors, cytokines, and matrix-modifying enzymes, CAF contribute to the formation of pre-metastatic niches and the enhancement of tumour cell invasive capabilities.[18] Furthermore, CAFs have been implicated in the modulation of immune responses within the TME, potentially influencing treatment outcomes and patient prognosis.[19]

Previous studies have demonstrated correlations between CAF density and various clinicopathological parameters in O-SCC.[20,21] However, these investigations have often yielded conflicting results, possibly due to variations in methodology, scoring systems, and patient populations.[9] Additionally, most studies have focused on single aspects of CAF biology, rather than examining their relationship with multiple prognostic parameters simultaneously.[12,22]

## Need for Study

The present understanding of CAFs in O-SCC presents significant knowledge gaps that need further analysis. While the presence of CAFs has been established as a possible prognostic marker, the connection between their distribution patterns and specific clinical consequences remains incompetently explored.[6,7] Mainly, the relationship between CAF scores and LNM, which represents a critical factor of patient existence, needs more complete evaluation.[13]

The existing literature principally focuses on CAF density as a separate parameter, without sufficient consideration of its association with other significant prognostic factors such as tumour depth, pattern of invasion, and ECE.[14,23] This study aims to tackle this gap by examining the association between CAF presence and multiple clinicopathological parameters simultaneously, potentially providing a more comprehensive understanding of their prognostic significance.

Furthermore, while several scoring systems for CAF assessment have been proposed, there is no consensus on the most clinically relevant method for evaluating CAF distribution and its prognostic implications.[9,12] This study will employ a standardized scoring system based on α-SMA IHC, contributing to the establishment of more reliable assessment criteria for future investigations.

Additionally, most existing studies have been conducted in different geographical and ethnic populations, with limited data available from the South Indian context, particularly Karnataka.[2] Given the high prevalence of O-SCC in this region, with an annual incidence of approximately 8,000 new cases in Karnataka alone, and potential variations in tumour biology across different populations, this study will provide valuable insights specific to the local patient population, helping to optimize treatment strategies and improve patient outcomes.

# OBJECTIVES

1. To detect cancer associated fibroblast using alpha α-SMA IHC in O-SCC.
2. To evaluate the association of cancer associated fibroblasts with LNM and pTNM staging in O-SCC.

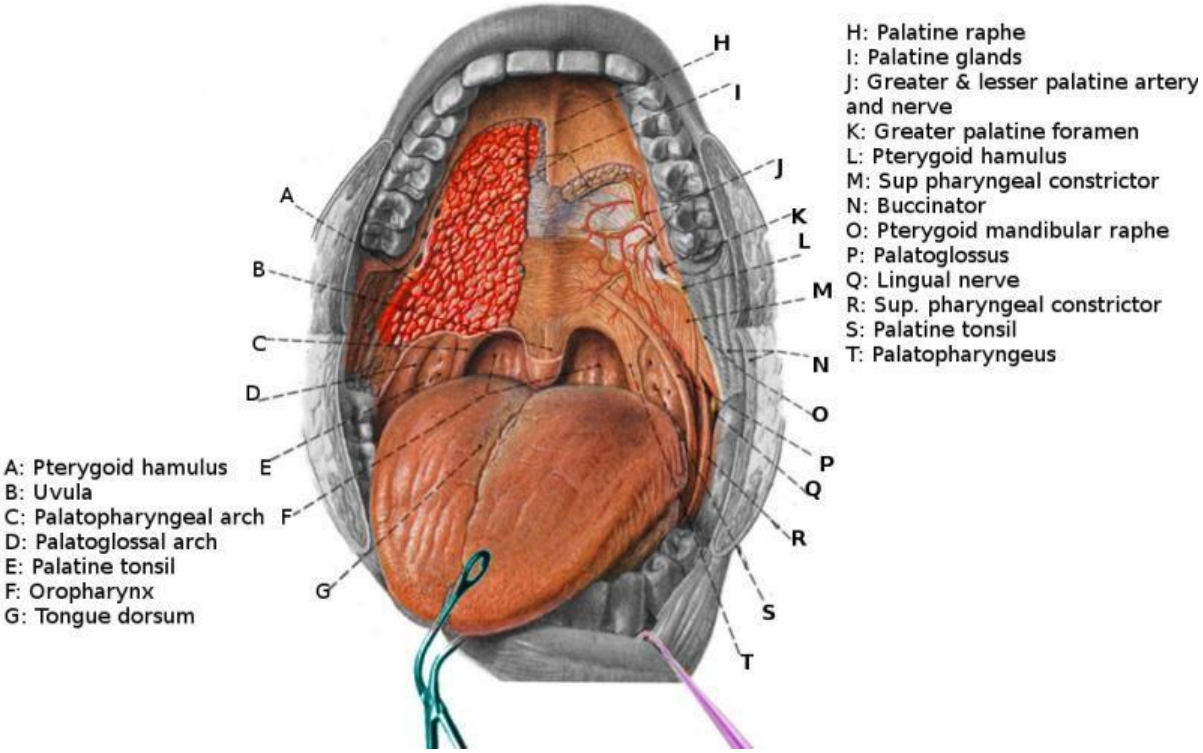
# REVIEW OF LITERATURE

## Embryology of structures in head as well as neck

The embryology of head and neck structures is a complicated process that includes the development of various tissues besides organs from the early embryonic layers. During the fourth week of embryonic growth, the pharyngeal arches, also accepted as branchial arches, develop. These arches contribute to the development of the face, neck, and related structures. Each arch covers an ectodermal outer layer, a mesodermal core, besides an endodermal inner coating. Neural crest cells drift into these arches, playing a central part in the development of craniofacial components, including the bones, cartilage, as well as connective tissues. Moreover, the development of the thyroid, thymus, besides parathyroid glands instigate from the endodermal pouches of these arches. Turbulences in this meticulous process can results in congenital anomalies such as cleft lip or palate.[24,25]

## The oral cavity

The oral cavity encompasses numerous anatomic sites plus subsites vital for understanding O-SCC. These comprise the buccal mucosa, floor of mouth, hard palate, lower gum, oral tongue, retromolar trigone, besides upper gum. Each site plays a different part in oral health plus disease. The mucosa lines the inner cheeks, while the floor of the mouth backs the tongue. The hard palate forms the ridge of the mouth, and the oral tongue is the mobile part of the tongue, crucial for taste and speech. The retromolar trigone is the area behind the last molar, often a site for neoplasms. Understanding these sites is vital for diagnosing and treating oral cancers, as each site may present unique challenges and prognoses.[26,27]




Oral cavity[28]

## Etiological factors predisposing to O-SCC

O-SCC is a significant global health issue with various etiological factors contributing to its development. Among these, smoking, tobacco use, and alcohol consumption are well-documented risk factors. Other factors are diet and nutrition, infections, genetic disorders such as Xeroderma pigmentosa, and conditions like FA.

**Smoking:**

It is one of the most significant risk factors for O-SCC. Tobacco smoke contains numerous carcinogenic compounds, including polycyclic aromatic hydrocarbons and nitrosamines, which can induce mutations in the oral epithelial cells. These mutations can lead to the development of malignant tumours. The risk of O-SCC increases with the duration and intensity of smoking, and even passive smoking has been implicated in increasing risk factors. Smoking not only contributes to the initiation of cancer but also affects

the prognosis, often leading to more aggressive forms of O-SCC. Studies have shown that smoking cessation can significantly reduce the risk of developing O-SCC and improve treatment outcomes for those diagnosed with the disease.[29]

**Tobacco use**:

This use in forms beyond smoking, such as smokeless tobacco (chewing tobacco and snuff), also poses a considerable risk for O-SCC. The carcinogens present in smokeless tobacco are absorbed through the oral mucosa, leading to direct exposure of the oral tissues to these harmful substances. This exposure can result in oral potentially malignant disorders like leucoplakia and erythroplakia, which may progress to O-SCC. The risk associated with smokeless tobacco is compounded when combined with alcohol consumption, as the two substances act synergistically to increase carcinogenic potential. Public health initiatives focusing on reducing tobacco use have been pivotal in decreasing the incidence rates of O-SCC.[30]

**Alcohol use:**

It significantly contributes to the risk of O-SCC, particularly when combined with tobacco use. Alcohol acts as a solvent, enhancing the penetration of tobacco carcinogens into the oral mucosa. Additionally, the metabolism of alcohol produces acetaldehyde, a known carcinogen, which can induce DNA damage and promote carcinogenesis in the oral cavity. Chronic alcohol consumption can lead to nutritional deficiencies and immune suppression, further increasing susceptibility to O-SCC. The risk is dose-dependent, with heavy drinkers facing a markedly increased risk compared to moderate or non-drinkers. Preventive approaches that tackle alcohol consumption, specifically in conjunction with tobacco use, are necessary in reducing O-SCC incidence.[31]

**Diet and Nutrition**:

The starring role of diet in the aetiology of O-SCC is well-deep-rooted. High eating of fruits and vegetables is linked with a decreased risk of O-SCC because of their rich antioxidant content, which can offset oxidative stress besides DNA damage. On the contrary, diets excessive in processed meats as well as low in key nutrients can raise cancer risk. Malnutrition besides deficiencies in micronutrients such as vitamins A, C, plus E, as well as minerals like selenium, have been associated in the pathogenesis of O-SCC. These deficiencies can results in compromised mucosal integrity besides immune function, creating a conducive conditions for carcinogenesis.[32,33]

**Infections**:

Fewer infections are related to O-SCC, with HPV being one of the most investigated. HPV infection, particularly by high-risk types like HPV-16, can lead to malignant transformation of oral epithelial cells. The virus integrates into the host genome, disrupting cell cycle regulation and promoting carcinogenesis. Chronic infections, such as those caused by Candida species, have also been associated with O-SCC, potentially due to chronic inflammation and immune modulation. The presence of specific bacterial profiles in the oral microbiome has been suggested to play a role in O-SCC development, possibly through the production of carcinogenic metabolites.[34,35]

**Xeroderma Pigmentosa (XP)**:

XP is a rare genetic disorder characterized by extreme sensitivity to ultraviolet (UV) rays due to defects in DNA repair mechanisms. Individuals with XP have a markedly increased risk of developing skin cancers, including O-SCC, particularly on sun-exposed areas such as the lips. The inability to repair UV-induced DNA damage results in the accumulation of mutations, leading to carcinogenesis. Although XP primarily affects the skin, the oral mucosa can also be involved, and patients may develop squamous cell carcinomas in the oral cavity, necessitating vigilant monitoring and preventive measures.[36,37]

**Fanconi Anaemia (FA)**:

FA is a hereditary condition that predisposes individuals to cancer due to chromosomal instability and impaired DNA repair. Patients with FA are at a significantly higher risk of developing O-SCC, often at a younger age compared to the general population. The genetic mutations associated with FA lead to defects in the FA/BRCA DNA repair pathway, contributing to genomic instability and tumorigenesis. This condition underscores the importance of genetic counselling and regular surveillance for early detection and management of O-SCC in affected individuals.[38]

**Bloom Syndrome**:

Bloom syndrome is a rare autosomal recessive disorder characterized by genomic instability, predisposing individuals to various cancers, including O-SCC. The syndrome is marked by a higher frequency of chromosomal breaks and rearrangements, which can lead to malignant transformations in tissues, including the oral cavity. Although direct evidence linking Bloom syndrome to O-SCC is limited, the genetic instability inherent in the syndrome suggests a heightened risk for developing oral malignancies. Studies indicate that patients with Bloom syndrome should be monitored closely for early signs of oral cancer, given their increased predisposition.[39,40]

**Immunosuppression**:

Immunosuppression, whether due to medical conditions, treatments, or chronic inflammation, significantly increases the risk of O-SCC. The immune system plays a critical role in identifying and eliminating malignant cells. When compromised, there is an increased likelihood of cancerous transformations within the oral cavity. Conditions such as HIV/AIDS, organ transplantation requiring immunosuppressive drugs, and chronic inflammatory diseases can create an environment conducive to carcinogenesis. Immunosuppression is

often associated with a more aggressive disease course and poorer prognosis in O-SCC patients.[19,41]

**Periodontal Disease**:

Chronic periodontal disease is linked to an increased risk of O-SCC. Periodontal disease results in chronic inflammation and infection, both of which can contribute to carcinogenesis. The presence of periodontal pathogens and the inflammatory mediators they release can lead to DNA damage and promote malignant transformations in the oral epithelium. A systematic review and meta-analysis have shown a significant association between periodontal disease and O-SCC, suggesting that maintaining periodontal health could be a preventive measure against oral cancer.[42,43]

**Oral Hygiene**:

Poor oral hygiene is a modifiable risk factor for O-SCC. Inadequate oral hygiene practices can lead to the accumulation of dental plaque, gingivitis, and periodontitis, all of which contribute to chronic inflammation. This persistent inflammatory state can create conditions favourable for carcinogenesis. Regular oral hygiene practices, including brushing, flossing, and dental check-ups, are associated with a reduced risk of O-SCC. Studies emphasize the importance of oral health care in preventing not only dental diseases but also in potentially reducing the risk of O-SCC development.[44,45]

## Premalignant lesions of oral cavity

The oral cavity is a common site for premalignant lesions, which have the potential to transform into O-SCC. Among these, leucoplakia, erythroplakia, lichen planus, and submucous fibrosis are significant due to their prevalence and potential for malignant transformation.

**Leucoplakia**:

Defined as a white patch or plaque that cannot be characterized as any other disease, leucoplakia is the most prevalent premalignant lesion in the oral cavity. It is often associated with tobacco use, both smoking and smokeless forms, as well as alcohol consumption. The risk of malignancy in leucoplakia varies, with dysplastic lesions having a higher risk. The lesion's appearance can range from homogenous, with a uniform white appearance, to non-homogenous, which includes speckled or nodular forms that are more likely to exhibit dysplasia. Management of leucoplakia includes eliminating risk factors, regular monitoring, and, in some cases, surgical excision to prevent malignant transformation.[46,47]

**Erythroplakia**:

Although less common than leucoplakia, erythroplakia is more concerning due to its higher potential for malignancy. Characterized by a red patch that cannot be attributed to any other condition, erythroplakia often shows significant dysplasia, carcinoma in situ, or invasive carcinoma upon biopsy. The aetiology may be linked to similar risk factors as leucoplakia, such as tobacco and alcohol. Erythroplakia requires careful evaluation and often surgical excision due to its high risk of progression to O-SCC. Early diagnosis and intervention are crucial to preventing malignant transformation.[48,49]

**Oral-Lichen Planus (OLP)**:

OLP is a chronic inflammatory condition that affects the mucous membranes of the oral cavity. It is considered a potentially malignant disorder, with a small percentage of cases transforming into O-SCC. The exact pathogenesis of OLP is unknown, but it is believed to involve an immune-mediated mechanism. Clinically, it presents in various forms, including reticular, erosive, and atrophic, with the erosive form being more symptomatic and potentially more prone to malignant transformation. Management involves symptomatic relief and regular surveillance to monitor for dysplastic changes.[50,51]

**Oral-Submucous Fibrosis (OSMF)**:

OSMF is a chronic, progressive condition characterized by fibrosis of the oral mucosa, leading to stiffness and trismus. It is strongly associated with the use of areca nut, a common habit in South Asia. OSMF is considered a premalignant condition due to its high rate of transformation to O-SCC. The fibrosis leads to a marble-like appearance of the oral mucosa, with symptoms such as burning sensation and difficulty in opening the mouth. Management focuses on cessation of areca nut use, nutritional support, and, in some cases, surgical intervention to relieve trismus.[52,53]

## Oral squamous cell carcinoma (O-SCC)

Oral cancers, particularly O-SCC, are a significant health concern globally, with India being one of the countries most affected. India bears a substantial burden of oral cancer, accounting for one-third of the global cases. Oral cancer ranks as the most prevailing cancer among men and the third most prevailing among women in India. The high prevalence is largely attributed to the widespread use of tobacco, both smoked and smokeless, and betel quid containing areca nut. Cultural practices and socioeconomic factors also contribute to the incidence. The tongue and buccal mucosa are the most frequently affected sites. Despite advancements in treatment, the survival rate remains low due to late-stage diagnosis and limited access to healthcare facilities.[54,55]

**Conventional Squamous Cell Carcinoma**:

Conventional squamous cell carcinoma (SCC) is the most prevailing form of oral cancer, characterized by the proliferation of atypical squamous cells. It is typically categorized into keratinizing and non-keratinizing subtypes based on histological features. The keratinizing type is more common and is associated with better differentiation and prognosis. Conventional SCC is aggressive, often invading local tissues and spreading to regional lymph nodes. Risk factors

include tobacco use, alcohol consumption, and HPV infection. Treatment usually involves a combination of surgery, radiation, and chemotherapy.[56,57]

**Variants of Squamous Cell Carcinoma**:

Several histopathological variants of SCC exist, each with distinct clinical and pathological features. Verrucous carcinoma, a low-grade variant, is characterized by a warty appearance and slow progression. It rarely metastasizes but can cause significant local destruction. Basaloid squamous cell carcinoma is a high-grade variant with a poorer prognosis due to its aggressive nature and propensity for metastasis. Other variants include spindle cell carcinoma, which exhibits both epithelial and mesenchymal characteristics, and adenosquamous carcinoma, which presents both glandular and squamous differentiation. These variants often require specialized management strategies due to their unique behaviors.[58,59]

**Prognostic factors**

O-SCC has several key prognostic factors that influence patient outcomes. The stage of the disease at diagnosis, as per the pTNM staging, is a critical determinant of prognosis. Early-stage cancers (I and II) generally have a better prognosis compared to advanced stages (III and IV), which are often associated with lymph node involvement and distant metastasis. Recurrence of the disease significantly impacts survival rates, making it a vital prognostic factor. Other factors include the patient's age, with younger patients sometimes showing different prognostic patterns, and the anatomical location of the primary tumour. Tumours located in certain areas, such as the tongue or floor of the mouth, may have a poorer prognosis due to the likelihood of deeper tissue invasion and metastasis.[60,61]

**Histological differentiation as a prognostic factor in O-SCC**

Histological differentiation plays a critical role in the prognosis of O-SCC. The degree of differentiation, categorized as well, moderately, or poorly differentiated, is instrumental in predicting clinical outcomes and tailoring treatment strategies.

**Well-differentiated** O-SCCs, characterized by cells that closely resemble normal squamous epithelium, tend to have a better prognosis. These tumours are less aggressive, exhibit slower growth, and have a lower propensity for metastasis.[62] The presence of keratinization and intercellular bridges is indicative of well-differentiated tumours, which are often managed effectively with surgical excision alone.[63]

**Moderately differentiated** O-SCCs present a more varied histological appearance, with noticeable cellular atypia and reduced keratinization. These tumours demonstrate a higher degree of aggression compared to well-differentiated ones, with increased potential for local invasion and regional LNM. As such, they may require a combination of surgery, radiation, and chemotherapy to achieve optimal control.[64]

**Poorly differentiated** O-SCCs are the most hostile, manifested by high cellular atypia, pleomorphism, besides minimal keratinization. These tumours grow promptly, conquering surrounding tissues, and are liable to early metastasis, often causing poor prognosis. The lack of differentiation implies a need for aggressive multimodal management to manage the disease successfully.[65]

Numerous studies have supported the use of histological differentiation as a foretelling factor in O-SCC. Arduino et al. carried out a retrospective study on 334 O-SCC cases, emphasizing that lower histological differentiation is correlated with bigger tumour size and higher repetition rates, accentuation the consequence of differentiation in foreseeing outcomes.[63] Similarly, Massano et al. reviewed the relationship between histological differentiation besides

prognosis, observing that poorly differentiated tumours are linked with worse survival outcomes.[64]

Additionally, in this study, Larsen et al. explained that tumour depth plus grade are strong forecasters of nodal metastasis besides overall survival. Their investigation shows that histological appearances are very significant in deciding the prognosis of O-SCC.[65]

Research by Pereira et al. on the prognostic value of numerous O-SCC histologic subtypes has presented that basaloid squamous cell carcinoma, for example, displays exclusive clinical behaviours besides results. In order to make informed therapeutic and prognosis choices, this research highlights the significance of proper histological examination.[66]

Histological variation remains a keystone in the predictive evaluation of O-SCC. The degree of differentiation specifies precious understandings into tumour behaviour, steering therapeutic approaches besides aiding in predicting patient outcomes. As research remains to evolve, combining molecular along with genetic markers with histological estimation may further improve prognostic models for O-SCC.

**Tumour volume (TV)**

In O-SCC, TV has become a major prognostic marker offering important new perspectives on patient outcomes and disease development. Tumour volume has been shown to be a viable independent prognostic marker in many studies assessing its influence in predicting survival and recurrence.

Mukoyama et al. explored at how PTV linked survival outcomes in O-SCC cases. Larger PTV was linked in the investigation to worse existence, so it is a decisive element of prediction. Though PTV was distinguished, the authors noticed that, given other factors, it was not a separate projecting indicator. This

highlights how uncomprehensible cancer prediction is, in which numerous components unite to influence the results in cancer patients.[67]

By contrast, Tarsitano et al. observed that, figured from CT scans, pre-treatment TV was a consistent predictive forecaster for existence in O-SCC patients. Their investigation emphasized that worse endurance rates were related with greater tumour dimensions, signifying that preoperative valuations should take TV into account to direct treatment decisions. This work promotes the integration of imaging methods in prognosis therefore enabling more exact evaluations[68]

Moreover, validating the prognostic importance of TV, Lin et al. found that in patients with O-SCC TV is an independent predictor of worse survival. Independent of other pathological characteristics like angiolymphatic invasion and lymph node extracapsular dissemination, their results revealed the correlation between rising TV and negative effects. This emphasizes even more the need of include TV into the whole evaluation of cancer prognosis.[69]

In O-SCC cases experiencing primary surgery, Zhang et al. considered the use of metabolic TV (MTV) as a prognostic indicator. Their investigations validated MTV as an indicator; greater MTV resembles to worst existence rates. This work highlights how incorporating anatomical as well as metabolic assessments could better prognostic accurateness in O-SCC patients.[70]

Emphasizing the need of TV comparing to other known factors, Massano et al. provide a thorough impression of prognostic elements in O-SCC cases. Their study emphasized the different character of cancer prediction in those cases, in which TV interacted with other known elements like cancer stages and LNM to affect patient outcomes. This all-encompassing method emphasizes the requirement of integrated prognostic models including many factors to improve forecast accuracy in those cancer patients.[64]

TV is a major prognostic factor in O-SCC; numerous research supports its function in estimating endurance besides recurrence. Although it may not always be a good standalone marker, its interaction with several other prognostic variables improves risk assessment accuracy along with it directs therapy decisions. Incorporating new imaging technologies along with metabolic evaluations as research develops, they will help to further improve the predictive value of tumour volume in O-SCC.

**Tumour thickness**

In O-SCC patients, tumour thickness has been identified as major determinant that greatly affects results including LNM and total survival in these cases. Many researchers have investigated its prognostic consequences, therefore offering strong proof for its inclusion into clinical evaluations of such cancers.

The crucial experiment carried out by Yuen et al. examined the prognostic significance of tumour thickness compared to other dimensions like diameter, length, and volume in oral tongue carcinoma. The findings highlighted that tumour thickness was the most reliable predictor of nodal metastasis, underscoring its vital role in prognostication.[71]

Reviewing studies stressing the significance of tumour thickness and DOI for forecasting nodal involvement as well as prognosis of O-SCC, Pentenero et al These criteria, which provide more reliable prognostic data than tumour size alone, they said should be included into staging systems.[72]

Further encouraging the higher relationship between tumour thickness and LNM along with local recurrence in O-SCC cases was observed Larsen et al. in their study. Their investigate long-established tumour depth along with grade as independent prognostic markers, consequently emphasising the significance of include tumour thickness into clinical assessments in these type of cases.[65]

Lee and their colleagues measured for early-stage O-SCC cases the predictive relevance of DOI against tumour thickness in their retroactive analysis. Their results shows that both tumour thickness plus DOI provide important predictive information; for early-stage instances, tumour thickness is a simpler and more useful metric.[73]

Massano et al. provided a comprehensive review of prognostic factors in O-SCC, highlighting tumour thickness as a critical determinant of disease progression and survival. The review emphasized that greater tumour thickness correlates with adverse outcomes, advocating its use in risk stratification and treatment planning.[64]

These studies when considered taken together support the use of tumour thickness as an indication of prognosis in O-SCC patients. Tumour thickness helps to refine treatment plans and enhance patient outcomes by providing important analysis of the possibility of LNM and recurrence in such cases. Including tumour thickness into staging and prognostic models as research develops would probably improve O-SCC's clinical care.

**DOI**

Established as a predictive factor in O-SCC patients, DOI affects management choices as well as forecasts outcomes including LNM along with survival rates. Several investigations in this regard have shown its significance for O-SCC prognostic assessment in these types of cancers.

Evaluating DOI along with tumour thickness as predictive elements for early stage of O-SCC cases, Lee et al. performed a retroactive research Their results supported its inclusion in the AJCC staging system as DOI seems to be a consistent predictor for nodal metastases and general prognosis.[73]

A meta-analysis by Caldeira et al. underlined the importance of using DOI in early-stage of O-SCC cases. The research underlined the significance of exact

measurement of DOI in surgical pathology to guide clinical choices as it revealed that higher rates of LNM correlate with a larger DOI.[74]

Reviewing the research on prognostic variables in O-SCC cases, Pentenero et al. argued the importance of usage of DOI in staging systems because its great association with lymph-nodal involvement and also survival results. Their study implies that DOI offers more comprehensive forecasting data than tumour size by itself.[72]

Ghazi et al. emphasized the unpredictable behaviour of O-SCC and the role of DOI in prognosis. Their review highlighted that DOI, alongside other histopathological features, significantly impacts treatment outcomes and should be considered in clinical practice.[75]

Almangush et al. investigated DOI in conjunction with T-B and the WPOI in early-stage oral tongue cancer. They found that high DOI correlates with increased mortality, supporting the integration of DOI with other histological markers to enhance prognostic accuracy.[76]

DOI is a critical prognostic factor in O-SCC, providing valuable insights into the likelihood of metastasis and patient survival. Its incorporation into staging and treatment planning can improve the management of O-SCC and support more personalized therapeutic approaches.

**Surgical margins**

Surgical margins play a critical role in the prognosis of O-SCC. The status of these margins—whether they are clear, close, or involved—can significantly impact patient outcomes, including recurrence rates and overall survival.

Sutton et al. explored the prognostic implications of surgical margins in O-SCC. Their study emphasized that involved surgical margins were strongly associated with poorer survival outcomes, underscoring the importance of achieving clear

margins during surgical resection.[77] Similarly, Montoro et al. identified surgical margin status as a key prognostic factor in O-SCC, alongside tumour-related factors like LNM. Their findings highlighted the necessity of meticulous surgical techniques to ensure adequate margins and improve survival rates.[78]

Underscoring that limiting local recurrence along with improving the likelihood of survival in O-SCC patients depends on well-defined resections, Massano et al. examined prognostic and predictive elements in these instances. In their research, their detailed study supports the practice of complete surgical excision to get ideal result.[64]

Binahmed et al. investigated in oral cancer patients the therapeutic relevance of positive surgical margins. The research found that positive margins significantly predicted both local recurring as well as reduced survival, therefore underlining the significance of clean margins to stop disease development over the study period.[79]

Examining prognostic markers in O-SCC of the oral tongue patients, El-Husseiny et al. noticed that one of the few distinct indicators influencing both general and relapse-free survival was involved resection margins in them. The prognostic importance of surgical margins in O-SCC treatment is underlined in this work even further when considered.[80]

All things considered; in O-SCC patients the condition of surgical margins is a major prognostic determinant in them. Reducing recurrence plus raising survival rates depend on precise surgical planning besides execution ensuring clean margins. Maintaining strict criteria for margin evaluation should always be first concern in O-SCC treatment plans as surgical methods and technology develop.

**Tumour invasion**

It is a critical prognostic factor in O-SCC, significantly influencing survival rates and recurrence. The manner in which the tumour invades surrounding tissues provides insights into its aggressiveness and potential for metastasis. Studies have consistently demonstrated the importance of evaluating tumour invasion patterns to predict patient outcomes.

Massano et al. highlighted the role of invasion in O-SCC prognosis, indicating that invasive characteristics correlate with poorer outcomes and should be considered in treatment planning. [64]

El-Husseiny et al. emphasized that invaded surgical margins were one of the few independent prognostic factors affecting both overall and relapse-free survival, underscoring the clinical importance of understanding invasion dynamics.[80]

Montoro et al. discussed the prognostic implications of tumour invasion, noting that its presence significantly affects survival, thus necessitating careful surgical and histopathological evaluation.[78] Mishra et al. concluded that the pattern of invasion is a strong adverse prognostic factor, associated with higher locoregional failure and poor prognosis, highlighting the need for detailed histological assessment.[81]

Brandwein-Gensler et al. developed a classification system for the WPOI in O-SCC, which has been validated as a significant prognostic indicator. There are five types, and each pattern denotes increasing aggressiveness and poorer prognosis.

*Table 1: WPOI classification by Brandwein-Gensler et al. (2005)*

| Pattern of Invasion | Definition |
|---|---|
| WPOI I | Broad pushing tumor front |
| WPOI II | Finger-like pushing pattern of tumor invasion |
| WPOI III | Tumor islands having >15 cells (large tumor islands) |
| WPOI IV | Tumor islands having <15 cells (small tumor islands) |
| WPOI V | Tumor satellites which are away from the main tumor by 1mm |

Chaturvedi et al. validated the Brandwein-Gensler model, demonstrating its predictive capability for locoregional recurrences.[82] Akolkar et al. correlated the WPOI with BI, further supporting its prognostic value in aggressive disease management.[83] A higher invasive front grading (types IV & V) was associated with overall poor survival. The biopsy depth was significantly under-represented than the actual tumour depth.

**T-B**

It is characterized by the presence of isolated single cells or small clusters of cells at the invasive front of tumours, has garnered attention as a significant prognostic factor in O-SCC. Its presence is associated with aggressive tumour behaviour and poorer clinical outcomes, making it a crucial consideration in the prognostic evaluation of O-SCC.

Angadi et al. examined the prognostic significance of T-B in O-SCC, demonstrating that it serves as an independent predictor of LNM. Their study underscores the need for routine assessment of T-B to identify high-risk patients who may benefit from more aggressive treatment strategies.[84]

Togni et al. highlighted T-B as an emerging prognostic marker with independent significance for locoregional recurrence in O-SCC. The researchers underlined that T-B that must be included in histological analyses as its presence corresponds with a greater risk of recurrence in cases besides poor prognosis among them.[85]

Mascitti et al. demonstrated that certain T-B thresholds are correlated with higher locoregional recurrences amongst the cancer patients, therefore supporting the predictive utility of T-B. They have mentioned that T-B quantification could help to improve prognostic evaluations as well as guide treatment decisions in those patients.[86]

Including O-SCC cases, a meta-analysis by Almangush and his colleagues evaluated the prognostic consequences of T-B across many cancer patients. The study demonstrated that T-B is a strong predictive marker associated with negative outcomes, therefore supporting its use in cancer prediction among those cases.[76]

Together with T-B, Dourado et al. investigated the prognostic significance of the tumour–stroma ratio and found that both elements greatly affect prognostic evaluations in O-SCC. Their results underline the need of taking many histological factors into account in order to grasp tumour behaviour completely.[9]

In O-SCC, T-B is a crucial prognostic marker linked with aggressive tumours and worse results. Its examination should be included into standard histological analyses to improve prognosis accuracy and direct therapy decisions.

**PNI and LVI**

The important prognostic markers in O-SCC, PNI and LVI greatly affect cancer patient outcomes including survival as well as recurrence. These invading patterns influence treatment plans besides revealing the aggressiveness of the cancer.

PNI results from cancer cells invading the area around neurons and thereby enabling the tumor cells to migrate via nerve pathways.

Tarsitano et al. showed that in O-SCC PNI is an independent predictive factor for local and regional failure. Their research underlined how PNI is linked to higher risk of recurrence, thereby stressing the significance of include PNI into histological analyses.[68]

By review plus meta-analysis, Binmadi and his colleagues verified that PNI is a prognostic determinant in O-SCC cases. Reiterating PNI's importance as a major factor in prognosis, the researchers included over 26,000 cases and demonstrated that PNI is linked with worse disease-specific survival (DSS) as well as with disease-free survival (DFS).[87]

Park et al. carried a study of how many PNI categorization systems affected O-SCC prognosis in patients. The researchers concluded that the degree of PNI is a more accurate prognostic marker than its simple existence, suggesting that thorough categorization methods could improve prognostic accuracy in those patients.[88]

Alkhadar and his colleagues spoke about the molecular understanding of PNI and also emphasized that in O-SCC cases it is among the most crucial prognostic determinant among them. They concluded that the necessity of molecular investigations in order to grasp the mechanisms behind PNI besides create focused treatments.[89]

Another most important determinant of O-SCC prognosis is lymphatic besides blood vascular invasion, or LVI.

According to Jardim et al., LVI is a major predictor of concealed metastases and also linked to advanced-stage O-SCC, thereby demanding severe therapy techniques in patients.[90]

Mascitti et al. examined in their study, how LVI may be used as a predictive tool in O-SCC patients. The study concluded that LVI is an essential measure for patient stratification along with treatment plan customization, hence optimizing patient outcomes in this study.[91]

Emphasizing their connection with recurrence and survival, Spoerl et al. studied the effects of lymphatic and vascular invasion in O-SCC. Their results imply that thorough LVI evaluation should be included into regular pathological assessments to guide treatment choices.[92]

Important prognostic elements in O-SCC, both PNI and LVI have major effects on survival and recurrence. Including these elements into standard histological evaluations enables more accurate prognostic forecasts and informed clinical decision-making, which finally helps to better manage patients.

**Sarcolemmal spread (SL-S)**

Though less often mentioned about this marker, SL-S is showing up as a possible predictive element in O-SCC cases. It describes the spread of malignant cells along the sarcolemma, the membrane encasing muscle fibres, which might point to aggressive tumour behaviour and worse patient outcomes in O-SCC.

Pallavi et al. investigated, in O-SCC, the predictive relevance of many histological characteristics including SL-S. Their results showed that SL-S is

statistically significant in relation to negative effects including lowered survival rates and more recurrence.[93]

Majumdar et al. reviewed clinically-pathologically based prognosticators in O-SCC holistically. The research underlined the need of examining all patterns of invasion as they together contribute to the general prognosis of the illness, even if they found little evidence especially addressing the prognostic usefulness of SL-S.[3]

In O-SCC, Khan et al. looked at how histopathological grade correlated with invasion patterns. Their retrospective analysis included SL-S and came to the conclusion that prognosis is much influenced by LVI and PNI in addition to other factors.[23]

Although SL-S is not as well investigated as other prognostic variables, new research indicates that in O-SCC it may be very important. Its incorporation in thorough histological analyses might increase prognostic accuracy and direct more sensible therapy development.

**Bone invasion (BI)**

A major prognostic determinant among all, BI in O-SCC points to a more aggressive cancer progression and worse patient outcomes among the patients. Advanced tumours as well as the invasion of cancer cells into the bone are linked and also may complicate therapy as well as lower survival chances.

Emphasizing that BI in O-SCC is an independent prognostic factor even with consideration for other negative factors such tumour size and LVI, Ebrahimi et al. They concluded that the importance of careful assessment in afflicted individuals as it showed that the existence of BI greatly corresponds with worse survival rates.[94]

Jimi and other authors gave understanding of the molecular and cellular processes behind BI by O-SCC. They detailed how cancer cells interact with bone tissue to produce osteoclastogenesis and then bone resorption. Development of focused treatments aiming at reducing BI and enhancing patient outcomes depends on an awareness of these processes.[95]

Specifically in gingival SCC, a subtype of O-SCC, Yoshida and her colleagues investigated the prognostic consequences of BI. The researchers noticed that BI is linked with more locoregional reappearance, and it also greatly influences prognosis in patients. This emphasizes the need of adding BI status into staging along with clinical decision-making.[96]

The use of BI as a prognostic marker in O-SCC was reviewed by Michalek et al., who noted that its presence is usually associated with other aggressive characteristics such PNI and also LVI. The findings of the study imply that thorough evaluation of many elements might improve prognosis accuracy and direct customized treatment plans.[97]

Focusing on mechanisms causing osteoclastogenesis, Vaassen and other authors investigated molecular changes causing BI in O-SCC cases. Knowing these molecular alterations in these patients provides possible targets for therapy intervention, hence maybe reducing the negative impact of BI on prognosis.[98]

In O-SCC, BI is a vital prognostic marker linked to more aggressive disease and worse prognosis. Improving patient prognosis depends on the identification and resolution of BI by means of thorough diagnostic and therapy plans.

**Salivary gland invasion (SGI)**

A major prognostic marker, SGI in O-SCC patients indicates a more aggressive disease progression and also maybe worse prognosis. The existence of this invasion may influence survival chances as well as complicate therapy options in those patients.

Therkildsen et al. included squamous cell carcinoma subtypes as among the prognostic elements in patients with salivary gland carcinomas. They highlighted the need of histological assessment in defining cancer aggressiveness; SGI is a crucial factor influencing the prognosis.[99]

In a study by Montoro et al., the authors explored various prognostic factors in SCC of the oral cavity, including the role of SGI. They found that SGI was associated with advanced disease stages and could serve as a predictor for locoregional recurrence, highlighting its importance in O-SCC prognosis.[78]

Terhaard et al. investigated the prognostic impact of salivary gland carcinoma, including parameters like PNI and LVI. Although their study primarily focused on salivary gland tumours, the findings underscore the importance of gland invasion in assessing tumour behaviour and potential outcomes in O-SCC.[100]

Nishida et al. examined the histopathological aspects of prognostic factors for salivary gland cancers, noting that SGI is frequently associated with LNM and poor prognosis. The researcher emphasized the need for comprehensive histological assessments in O-SCC to better understand the implications of SGI.[101]

Fang et al. evaluated potential prognostic factors in primary SCC of the parotid gland, a salivary gland. Their findings indicated that PNI was a significant predictive factor, suggesting that similar invasive patterns in O-SCC could provide valuable prognostic information.[102]

SGI is a critical prognostic factor in O-SCC, associated with more aggressive disease and worse outcomes. Recognizing and addressing SGI through detailed pathological evaluation is essential for improving prognostic accuracy and guiding treatment strategies.

**TILs**

It has emerged as significant prognostic factors in O-SCC, reflecting the body's immune response to tumour cells. Their presence indicates how the immune system interacts with cancer, influencing both the progression and the potential response to therapies.

Wolf et al. conducted a study on TILs in patients with oral cavity squamous carcinoma, demonstrating that certain lymphocyte subsets, particularly CD4+ cells, were associated with improved survival rates. This study highlights the potential of TILs to serve as biomarkers for patient prognosis and treatment planning.[103]

High numbers of CD56+ and CD8+ lymphocytes in patients were good prognostic markers when Caruntu et al. investigated the forecasting ability of TILs in removable O-SCC. The findings of this study imply that measuring these immune cells can provide important new perspectives on patient outcomes as well as direct individualized therapy plans.[104]

On the function of TILs in O-SCC—De Ruiter and his colleagues conducted a methodical review along with meta-analysis. Their significance in the role of prognostic variables in stratifying patient risk and customizing therapies is underlined by their conclusion that a larger presence of TILs is usually connected with favourable outcome.[105]

Fang and other authors assessed TIL prognostic relevance in O-SCC and they discovered that survival results were much influenced by these immune cells. The findings of this study supports the incorporation of TIL assessment in normal histological assessments to improve prognostic accuracy.[106]

With certain TIL subgroups and combined scores helping to prognostic evaluation, Spector and other authors found TIL levels as independent prognostic indicators in head and neck squamous cell cancer. This supports

TILs' therapeutic importance in guiding therapy choices and assessing patient prognosis.[107]

TILs are indications of immune response and possible survival outcomes, so they are very important for the O-SCC prognosis. Their evaluation in clinical settings may greatly improve the prognostic accuracy and direct therapy plans.

**Tumor-associated tissue eosinophilia (TATE)**

TATE is being known as a major O-SCC prognostic determinant in oral cancer patients. Although their function as a prognostic marker is still unclear and somewhat debatable, eosinophils present in the TME may affect cancer development as well as patient outcomes.

Dorta and their colleagues investigated the impact of TATE on O-SCC prognosis among the study patients. The findings of this study indicated a dual function in cancer growth as a greater eosinophil count within the tumour was linked to both positive as well as negative outcomes. The study's findings also underlined the requirement of a sophisticated knowledge of TATE's influence on O-SCC prediction among those patients.[108]

Mascitti and their colleagues highlighted TATE's prospective as a prognostic marker by offering a thorough assessment in O-SCC patients. TATE may show a strong immunological response, they said, which would be related to better survival rates in those patients. The study did, however, also note variations in results across research, therefore highlighting the intricacy of TATE's influence on cancer biology amongst the study patients.[109]

By means of quantitative examination of TATE in O-SCC diagnosed patients, Siddiqui and the other authors discovered that eosinophil infiltration had a positive prognostic indication in certain instances, thereby confirming its antitumoral function in O-SCC cases. This research proposed that TATE might be a helpful indicator of patients with perhaps improved prognoses.[110]

Through a comprehensive review plus meta-analysis on TATE in O-SCC diagnosed patients, Choudhary et al. found that eosinophil presence may be a prognostic factor, however its predictive efficacy differed between researchers. Their studies underlined the need of consistent approaches in evaluating TATE to improve its prognostic dependability among the O-SCC patients.[111]

Reflecting the intricate interaction between the immune system and tumours dynamics, TATE shows potential as a prognostic factor in O-SCC. Although its function is still complex, including TATE evaluation into clinical practice can provide insightful analysis of patient prognosis and treatment approaches.

**Cellular cannibalism (CC)**

CC, a phenomenon where a cell engulfs another cell within the same species, is emerging as a significant prognostic factor in O-SCC. This process reflects the aggressive nature of the tumour and its potential for metastasis and recurrence.

Examining O-SCC for cannibalistic cells, Jose and their colleagues discovered that these cells linked to a more aggressive tumours profile. They suggested that CC may be a prognostic marker, therefore guiding the aggressive behaviour and possible results of the tumor.[112]

Siquara da Rocha et al. discussed the role of cell-in-cell events, including CC, in O-SCC. Their findings indicated that these events might be associated with tumour cell survival and resistance to treatment. They highlighted the importance of understanding this phenomenon to improve prognostic assessments and therapeutic strategies amongst the study patients.[113]

In early oral tongue cancer, a subtype of O-SCC, Almangush et al. investigated the correlation between cell-in--cell formations and cancer-related death among the study patients. Their research indicated that CC had possible prognostic value as it was connected to aggressive tumour features besides greater death rates in their study patients.[114]

Keerthika et al. gave O-SCC histo-morphological proof of sophisticated cannibalism. Their studies revealed that CC may be a diagnostic as well as prognostic marker, providing insightful analysis of tumour behaviour and supporting the patient outcome prediction in O-SCC patients.[115]

Associated with poor prognostic results, CC in O-SCC demonstrates the aggressive propensity of the cancer. Understanding this phenomenon in clinical settings can help to stratify patient risk and direct treatment decisions.

**LNM**

A major prognostic factor in O-SCC cases, LNM greatly influences the patient outcomes. A key factor in the TNM staging system as the presence of metastatic lymph nodes is linked with worse survival rates as well as higher recurrence risk.

Kikuchi et al. conducted a multicentred study that highlighted the prognostic role of lingual LNM in O-SCC. They found that the presence of metastasis in these nodes was linked to poorer survival outcomes, underscoring the importance of thorough lymph node assessment in managing O-SCC.[116]

Angadi et al. examined the relationship between T-B and cervical LNM in O-SCC. Their study concluded that cervical lymph node involvement is a major prognostic factor, influencing both survival and treatment strategies. The research emphasized the need for precise evaluation of lymph node status to guide clinical decisions.[84]

In O-SCC diagnosed cases, Yamagata and other authors looked at the LNR's prognostic worth. The study concluded that poorer prognosis corresponded with a greater LNR, defined as the ratio of positive lymph nodes to the total number of removed nodes. This result implies that in postoperative care LNR might be a useful predictive tool in O-SCC patients.[117]

Additionally emphasising LNR as an independent prognostic factor in O-SCC cases was mentioned by Ebrahimi and other authors. The findings of this study confirmed that LNR's ability to forecast patient outcomes, therefore underscoring its possibilities to improve prognostic evaluations and customize treatment plans among these patients.[94]

Moreover, Huang and other authors investigated the prognostic relevance of LNR in O-SCC methodically and meta-ally. The findings of this study revealed that LNR is an independent prognostic predictor, therefore supporting its use as a survival besides recurrence prediction marker in the study population.[118]

In O-SCC cases, these studies mentioned that LNM especially cervical lymph node involvement and LNR are fundamental prognostic determinants. Guiding surgical as well as adjuvant therapy plans, these measures provide vital insights into disease progression and patient survival.

**ECE**

A major predictive determinant in O-SCC cases is ECE in LNM. It is linked to poor clinical outcomes including greater recurrence rates besides decreased survival and also shows the aggressive character of the illness.

Rajappa et al. underlined how ECE affected survival in O-SCC and recurrence patterns. Emphasizing the necessity of ECE's assessment in lymph node assessments to guide treatment choices, the study's findings also indicated that it predicted poor prognosis among the patients.[119]

Lewis and other authors explored ECE as a poor prognostic factor in oropharyngeal SCC. Though the research focused on another site, it highlighted the broad prognostic relevance of ECE in O-SCC diagnosed patients.[120]

Myers and his colleagues investigated the role of ECE in a subgroup of O-SCC, SCC of the tongue, Reiterating its significance as a prognostic marker in the

therapy of O-SCC patients, they discovered that ECE was a strong predictor of treatment failure as well as inferior outcomes.[121]

Shaw et al. shed light on how ECE's prognostic consequences in O-SCC cases may be. They concluded that ECE significantly affects survival rates and is a consistent indicator of bad results. The findings of this study supports the need of adding ECE into staging and therapy preparation.[122]

Wreesmann et al. investigated how much extracapsular dissemination affected O-SCC prognosis amongst their study patients. They underlined its important function in guiding treatment choices as well as prognostic evaluations as they showed that higher ECE extent linked with lower survival outcomes.[123]

An important prognostic indicator reflecting the aggressiveness of the tumor and potential for recurrence in O-SCC cases is ECE. Correct evaluation and use of ECE into clinical settings may improve prognostic quality and guide treatment plans.

**Distant metastasis (DM)**

A major prognostic determinant in O-SCC patients, DM greatly affects patient survival as well as course of therapy among them. Usually indicating advanced illness of O-SCC, the presence of DM is connected with a bad prognosis amongst these patients.

For O-SCC cases, Hasegawa and other authors assessed the incidence and risk variables for DM. Their findings underlined that DM is a major determinant of survival outcomes; its presence is correlated with a significant drop in general survival rates. To improve patient outcomes, they underlined the need of early diagnosis and DM treatment.[124]

Kowalski et al. looked at prognostic elements for DM in O-SCC patients. Their studies found a number of risk indicators, including advanced T-stage besides

nodal involvement, which are rather closely related to the onset of DM. The research underlined the need of thorough staging as well as monitoring in order to reduce the hazards related with remote spread.[125]

Liao and other authors looked at risk variables for DM in O-SCC cases and also observed that survival in these patients is greatly influenced by DM. Their results indicated that individuals with DM had much reduced survival rates, therefore highlighting the important function of DM as a prognostic indicator. This research supports strong treatment strategies for those who have high risk of acquiring diabetes.[126]

DM in O-SCC is a major predictive factor lowering survival. Improving results for individuals with or at risk of diabetes mellitus depends critically on early detection and management.

**Broder's histological classification**

It is a traditional method used to grade O-SCC based on the degree of cellular differentiation. This system categorizes tumors into four grades, from well-differentiated (Grade I) to undifferentiated (Grade IV), based on the percentage of differentiated tumour cells. It's a valuable prognostic tool, helping predict tumour behaviour and potential outcomes.[127]

***Table 2: Broder's histological classification grades***

| Grading | Characteristics |
|---|---|
| Grade I | 0 – 25% undifferentiated cells |
| Grade II | 25 – 50% undifferentiated cells |
| Grade III | 50 – 75% undifferentiated cells |
| Grade IV | 75 – 100% undifferentiated cells |

**TNM staging system for oral and lip cancer- The AJCC (8th edition)**

The AJCC (8th edition) TNM staging system for oral cavity and lip cancer is a standardized approach used to evaluate the severity and extent of cancer, helping guide treatment and predict outcomes. It incorporates three key components: Tumor size (T), regional lymph Node involvement (N), and distant Metastasis (M). The simplicity of TNM staging makes it the most accepted and used system in clinical practice. In order to increase acceptance and compliance, by design the TNM staging system has to be kept simple and user-friendly. This staging system enables healthcare professionals to assess cancer's progression comprehensively, facilitating accurate prognosis and treatment planning. It also promotes clear communication among multidisciplinary teams, ensuring consistent patient care and research efforts.[128] For T stage, the maximum gross tumour thickness along with the microscopic DOI is taken

| **Primary tumour (pT)** | |
|---|---|
| **Tx** | Primary tumour cannot be assessed |
| **Tis** | Carcinoma in situ |
| **T1** | Tumour ≤ 2cm with DOI ≤ 5mm |
| **T2** | Tumour ≤ 2cm with DOI 5 - 10mm OR<br>Tumour 2 – 4cm with DOI ≤ 10mm |
| **T3** | Tumour 2 – 4cm with DOI > 10mm OR |

| | |
|---|---|
| **T4** | **T4a** (Moderately advanced disease):<br><br>Tumour > 4cm with DOI >10mm **OR**<br><br>Tumour invades adjacent structures only (e.g. through cortical bone of the mandible/maxilla or involves the maxillary sinus or skin of the face)<br><br>**T4b** (Very advanced local disease):<br><br>Tumour invades masticator space, pterygoid plates or skull base or encases the internal carotid artery |

- DOI – Depth of invasion, and not tumour thickness
- Superficial erosion of bone/tooth socket alone by a gingival primary is not sufficient to classify a tumour as T4

For N stage, it is the size, number and the extracapsular extension that determine the stage.

| **Pathological regional lymph nodes (pN)** | |
|---|---|
| **Nx** | Regional lymph nodes cannot be assessed |
| **N0** | No regional lymph node metastasis |

| | |
|---|---|
| **N1** | Metastasis in a single ipsilateral lymph node,<br><br>≤ 3cm in greatest dimension and ENE (-) |
| **N2** | **N2a**: Metastasis in either<br><br>Single ipsilateral lymph node that is ≤3cm and ENE (+) OR<br><br>Single ipsilateral lymph node that is 3 –6 cm and ENE (-)<br><br>**N2b**: Metastasis in multiple ipsilateral lymph node, ≤ 6cm in greatest dimension and ENE (-)<br><br>**N2c**: Metastasis in bilateral or contralateral lymph nodes, ≤6cm in greatest dimension and ENE (-) |
| **N3** | **N3a**: Metastasis in a lymph node that is ≥ 6cm in greatest dimension and ENE (-)<br><br>**N3b**: Metastasis in either<br><br>Single ipsilateral lymph node > 3cm and ENE (+) **OR**<br><br>Multiple ipsilateral, contralateral/bilateral lymph nodes with ENE (+) **OR**<br><br>Single contralateral lymph node of any size and ENE (+) |

| **Distant metastasis (M)** | |
|---|---|
| **M0** | No distant metastasis |
| **M1** | Distant metastasis |

- Metastasis found on imaging is considered cM1
- Biopsy proven metastasis is considered pM1

| **AJCC prognostic stage grouping for oral cavity** | |
|---|---|
| **Stage 0** | TisN0M0 |
| **Stage I** | T1N0M0 |
| **Stage II** | T2N0M0 |
| **Stage III** | T3N0M0<br>T1-3N1M0 |
| **Stage IVA** | T4aN0-1M0 |

| | |
|---|---|
| **Stage IV B** | Any T N3M0<br><br>T4b Any N M0 |
| **Stage IV C** | Any T Any N M1 |

## The patch field carcinoma model and field cancerization

They are key concepts in understanding O-SCC. The patch field carcinoma model suggests that an initial genetic alteration in a small patch of oral mucosa can expand into a larger field, leading to multiple primary tumours or recurrences.[129,130] Field cancerization, first described by Slaughter et al. in 1953, refers to the process where a region of tissue undergoes genetic alterations, predisposing it to cancer development even after initial tumour removal.[131] This concept underscores the risk of local recurrence and secondary primary tumours in O-SCC.[132,133] Understanding these models is crucial for developing strategies to prevent cancer progression and recurrence in patients with O-SCC.

**TME**

Cancer development as well as LNM in O-SCC are strongly influenced by the TME. Comprising many cell types: CAFs, immunological cells, endothelial cells, along with the ECM, all of which actively interact with tumour cells, this complex environment Alves et al. claim that O-SCC development via macrophage and cytokine activity is much influenced by the inflammatory condition within the TME.[134]

Alves et al. showed in their research that M2-type macrophages predominate over M1 in O-SCC, therefore generating a pro-inflammatory milieu. Cytokines such IL-6, IL-1β, and TNF-α help this condition to improve tumour cell motility

and invasion. Through the STAT3-phosphorylation route, IL-6 was especially observed to enhance the migratory characteristics of O-SCC cells, therefore influencing Rac1 activation and fostering metastases.

The essential component of the TME, CAFs are known to release elements that modify the ECM and support cancer development and invasion. Enhanced resistance to treatment and higher metastatic potential might result from the interaction between CAFs and tumours cells.

The TME in O-SCC is overall not just a passive framework but also an active player in cancer progression. Knowing these interactions helps one to identify possible therapeutic targets to throw off these processes and enhance the results of therapy for O-SCC patients.

**TSR**

An increasing prognostic factor in O-SCC patients, it indicates the interaction between malignant cells as well as the surrounding stromal tissue. Since TSR may forecast results in O-SCC patients, it is attracting interest. Fewer studies have linked a worse prognosis to a high TSR, which indicates a larger amount of stroma.

In O-SCC diagnosed patients, Niranjan and Sarathy conducted a pilot investigation stressing the predictive relevance of TSR. They discovered that TSR might be utilized as an additional prognostic tool and readily detected from standard histological slides. Their studies imply that lower survival rates and more tumor aggressiveness are correlated with a greater TSR.[135]

Building on this, Qiu et al. investigated how CAFs could affect the TME. They showed that CAFs help to increase TSR, which separately predicts poor disease-free and overall survival in O-SCC patients. This work emphasizes the importance of CAFs as therapeutic targets and their critical part in the development of tumors.[136]

Also looking examining TSR as a predictor of occult cervical LNM in early-stage O-SCC was Huang and their colleagues. The findings of this study showed that patients with a higher TSR had a notably higher risk of metastases in the diagnosed cases, suggesting that TSR could direct therapeutic choices on early-stage neck dissection among these patients.[137]

Dourado and his collegaues assessed TSR and T-B as prognostic elements for O-SCC more recently in their study. They verified that poorer results were associated with a high TSR as well as with higher T-B in the study's patients. The findings of this study helps to better stratify patients' risks and customize treatment plans by supporting the inclusion of TSR into regular pathology evaluations.[138]

In O-SCC, the TSR is a useful prognostic indicator that offers understanding of cancer biology and development. Including TSR into therapeutic activities helps to improve patient care techniques as well as raise prognostic accuracy.

## CAFs

In many different malignancies, including O-SCC, they are absolutely essential for the TME. Their distinct markers and purposes set these fibroblasts apart from normal fibroblasts, which greatly help to explain cancer spread and development.

A collection of particular markers that help to identify CAFs and differentiate them from normal fibroblasts define these cells. α-SMA is one of the most often utilized markers as it shows the myofibroblastic character of CAFs. α-SMA expression in CAFs is linked to their contraction capacity and participation in ECM remodeling.[9]

Another important marker is FAP, a serine protease expressed only by CAFs in the tumour stroma. FAP is linked to encouraging tumour development and invasion as well as to help to modify the TME.[139]

Furthermore, important indicators for CAFs are PDGFRs, especially PDGFRβ. Different signalling systems involving these receptors control angiogenesis, migration, and cell proliferation. Observed high expression levels of PDGFRβ in CAFs suggest their active involvement in tumour-stromal interactions and thus influence on the tumour's behavior.[139]

By means of many pathways, CAFs significantly affect the TME, hence impacting cancer growth and treatment resistance. Their capacity to alter the ECM ( by secreting MMPs marks one main patho-mechanism. By opening channels across the ECM, this remodeling promotes cancer cell invasion and metastases.

Moreover, CAFs help the TME to create an immunosuppressive environment. Crucially important for anti-tumour immunity, they produce cytokines and chemokines that may stop the invasion and operation of immune cells including T cells. This immune modulation shields the cancer from immune monitoring and helps it to thrive.[140]

By producing VEGF, improving the blood flow to the developing tumour mass and thus enabling nutrition and oxygen delivery, CAFs also encourage angiogenesis. They also participate in the EMT process, therefore augmenting the invasiveness of cancer cells[141] These many functions of CAFs underline their importance in the TME and point to them as possible targets for therapy to stop cancer development.

**Functions of CAFs**

CAFs greatly affect O-SCC growth and help in many facets of tumor biology. Their main purposes include ECM remodelling, which helps tumours spread and invasion of cells is made possible. CaFs break down ECM components by secreting MMPs, hence generating channels for cancer cell movement.[142]

Moreover, CAFs promote angiogenesis and lymphangiogenesis, essential processes for tumour growth and metastasis. They secrete pro-angiogenic factors such as vascular endothelial growth factor (VEGF), enhancing blood and lymphatic vessel formation, which supports tumour nutrient supply and facilitates metastatic spread.[143]

CAFs also play a role in immunosuppression within the TME. They produce cytokines and chemokines that can modulate immune cell infiltration and function, effectively creating an immunosuppressive niche that protects the tumour from immune surveillance.[144]

Additionally, CAFs contribute to the EMT, a process by which epithelial cells acquire mesenchymal, invasive characteristics. This transition is facilitated by CAF-derived factors like transforming growth factor-beta (TGF-β), which activate signalling pathways involved in EMT and enhance the metastatic potential of O-SCC cells.[22]

Furthermore, recent studies have shown that CAFs can influence BI in O-SCC. Elmusrati et al. reported that CAFs expressing myofibroblast markers can induce osteoclastogenesis, leading to bone resorption and invasion, a significant concern in advanced O-SCC cases.[145]

CAFs are integral to the TME in O-SCC, influencing various aspects of tumour progression and metastasis through their unique markers and multifaceted functions. Understanding these roles provides insights into potential therapeutic targets, offering avenues for interventions aimed at disrupting CAF-related pathways to hinder O-SCC progression.

**Sustained proliferation in O-SCC**

Sustained proliferation is a hallmark of O-SCC, contributing significantly to tumour growth and progression. This persistent cell division is driven by alterations in cell cycle regulation and signalling pathways. Key players include

cell cycle proteins like cyclins and cyclin-dependent kinases, which are often upregulated in O-SCC, facilitating uncontrolled cell division.[146]

Promoting cellular proliferation in O-SCC patients is linked to signalling routes including the Sonic hedgehog (Shh) pathway. Different O-SCC cell lines have shown excessive expression of elements of this pathway, underscoring its function in preserving proliferative signals in the tumor.[147]

Furthermore, the existence of proteins such as survivin, which stop planned cell death of injured cells, thus enabling continued cancer development by so inhibiting apoptosis. [148]

Knowing these processes helps one to identify possible targets for interfering with the proliferative ability of O-SCC cells.

**Angiogenesis in O-SCC**

An important mechanism in the growth along with spread of O-SCC is angiogenesis. This mechanism provides the required nutrition besides oxygen to the multiplying cancer cells, hence promoting tumour development as well as metastases. Many researchers have clarified the function of angiogenesis in O-SCC cases and also underlined its possible therapeutic focus.

Higher microvessels density (MVD) in O-SCC corresponds, according to Ascani et al., with more cancer aggressiveness and worse prognosis. Their research underlined how important angiogenesis is for enabling O-SCC to disseminate metastases to lymph nodes.[149] Marla et al. also investigated the complex link between angiogenesis and O-SCC, stressing the possibilities of antiangiogenic treatments in control of this cancer.[150]

Penfold and colleagues focused on the relationship in O-SCC between angiogenesis and LNM. Their results suggested that greater risk of lymph node

involvement is linked to increased angiogenic activity, thereby implying that angiogenesis contributes to the metastatic cascade of O-SCC.[151]

Moreover, Shivamallappa et al. performed an immunohistochemistry analysis to underline the part angiogenesis plays in O-SCC growth and metastases. Their studies revealed how angiogenic markers may be used as O-SCC patients' possible prognostic guide.[152]

Using immunohistochemical analysis, Moriyama et al. also looked at tumor angiogenesis in O-SCC and found that tumour development is much influenced by vascular density. Their research supports the therapeutic targeting of angiogenesis to maybe stop O-SCC spread and development.[153]

Ultimately, angiogenesis influences cancer development, invasion, and metastases and is thus fundamental to the pathophysiology of O-SCC. Knowing its processes and effects on the TME helps one to create appropriate therapy plans meant to stop O-SCC development.

**Invasion and metastasis in O-SCC**

Crucially important events in the course of O-SCC include invasion as well as metastases, which will greatly affect the patient prognosis along with treatment plans. Few researchers have looked at these processes, stressing the intricate interaction among the molecular besides cellular systems involved.

Yamamoto et al. looked at the mechanism of invasion in O-SCC and related it to LNM. Their clinical and histological study revealed that certain invasion patterns indicate metastatic potential, therefore suggesting that lymph node involvement is mostly dependent on the mechanism of invasion.[154]

Furthermore, under great research is the function of the ECM in O-SCC invasion as well as metastases. According to Kumar and Hema et al., the ECM components enhance the metastatic spread of cancer cells by facilitating tumour

cell motility besides invasion, therefore serving as a scaffold. The findings of this study highlight the need of ECM remodeling in O-SCC cases.[155]

Emphasizing the interplay between cancer cells and the surrounding tissue milieu, Kawashiri and his colleagues created a novel model for researching invasion as well as metastases in O-SCC cases. The findings of this study revealed the importance of tumour-stroma interactions in fostering metastatic behaviour and offered understanding of possible treatment targets.[156]

Furthermore, linked to O-SCC invasion and metastases is the hepatocyte growth factor (HGF/c-Met signaling pathway). Targeting the HGF/c-Met pathway suggests that it may help to reduce metastatic spread as Uchida et al. showed that the system increases the invasive ability of O-SCC cells.[157]

Finally, Khwaja et al. investigated in O-SCC the pattern of invasion as a predictive element for LNM. Their findings confirmed the predictive significance of invasion patterns in clinical evaluations as certain invasion patterns are linked to a greater probability of lymph node involvement.[158]

### IHC markers of CAF

Using particular markers to separate CAFs from the normal fibroblasts, IHC is a fundamental method that is utilized in identification of these structures.

α-SMA is one of the IHC markers for CAFs that is utilized somewhat extensively. This marker is suggestive of the myofibroblastic phenotype of CAFs, along with their function in ECM remodelling and tumour invasion facilitation.[9]

Another important marker is FAP, which is greatly expressed in CAFs but lacking from normal fibroblasts. FAP links to poor prognosis in O-SCC patients and is implicated in matrix degradation.[159]

Furthermore, noted in a subgroup of CAFs in O-SCC is CD68, usually a marker for macrophages. Linked to immunosuppressive conditions within malignancies, this marker influences regulatory T-cell invasion and less favourable results.[160] These markers not only enable the identification of CAFs but also provide light on their functional functions in O-SCC, therefore stressing their possible treatment targets.

**α-SMA expression in CAFs**

An important indication of CAFs' function in the O-SCC development is α-SMA expression in those types of cases. Particularly by their myofibroblastic nature, which is defined by the production of α-SMA, CAFs that are noticed to greatly affect the TME.

Patel et al. underlined that variations in α-SMA expression in CAFs reflect their genetic stability as well as functional diversity within the TME. This variation in expression implies that many CAF subtypes might have diverse functions in O-SCC development among the cases diagnosed.[161]

α-SMA-expressing CAFs were linked, according to El-Kammar et al., to poor prognosis in O-SCC as well as cancer development. Their research underlined how important these CAFs are in encouraging tumour invasion plus metastases, therefore making them possible treatment targets.[162]

In O-SCC, Lin et al., investigated the relationship between angiogenesis and α-SMA expression. They discovered that CAFs expressing α-SMA support angiogenesis, therefore enabling tumour development and maybe metastases.[142]

Parajuli et al. looked at how integrin α11 and α-SMA expression correlated in head and neck squamous cell carcinomas—including O-SCC. Their findings indicate a cooperative involvement in cancer growth as integrin α11, which is overexpressed in the tumour stroma, correlates favourably with α-SMA expression.[163]

Vered et al. investigated how CAFs can contribute to EMT in metastatic O-SCC and noted that α-SMA expression marks the activated fibroblastic phenotype. Their results show a correlation between aggressive tumour behaviour and metastases and α-SMA-positive CAFs.[164]

A major indicator of their O-SCC activity in CAFs, α-SMA expression affects angiogenesis, invasion, and metastases. This expression not only clarifies CAF heterogeneity but also offers possible paths for focused treatments in O-SCC control.

**Role of CAF in tumorigenesis and metastasis**

CAFs contribute to tumorigenesis by remodelling the ECM, which enhances the structural support for tumour growth and facilitates cancer cell invasion. Patel et al. discussed how CAFs secrete MMPs, enzymes that degrade ECM components, thereby enabling cancer cells to invade surrounding tissues.[161]

In the context of metastasis, CAFs secrete growth factors and cytokines that promote angiogenesis, the formation of new blood vessels, providing the tumour with nutrients and oxygen necessary for growth. Lin et al. highlighted the role of CAFs in enhancing angiogenesis in O-SCC, which correlates with increased metastatic potential.[142]

Moreover, CAFs influence the EMT, a process where epithelial cells acquire mesenchymal properties, enhancing their migratory and invasive capabilities. Vered et al. noted that CAFs induce EMT in O-SCC through paracrine signalling, thereby facilitating metastasis.[164]

The interaction between CAFs and immune cells within the TME is also crucial. CAFs can modulate immune responses, often creating an immunosuppressive environment that allows tumour cells to evade immune surveillance. Zhao et al. observed that CAFs promote the infiltration of regulatory T cells (Tregs), which contribute to an immunosuppressive milieu, facilitating tumour progression.[160]

## Future directives and clinical applications, including targeted therapy

Future directives in managing O-SCC focus significantly on targeting CAFs, given their crucial role in tumour progression and metastasis. Targeted therapy against CAFs aims to disrupt the supportive TME they create. Arebro et al. emphasize the potential of therapies directed at specific CAF subtypes, which could enhance treatment efficacy by mitigating their pro-tumorigenic activities.[6]

Moreover, the use of FAP as a marker for CAFs in CAR-T cell therapy shows promise in selectively targeting the tumour stroma, offering a novel approach to curb O-SCC progression.[165] Continued research into the signalling pathways of CAFs is crucial, as understanding these pathways could uncover new therapeutic targets and improve clinical outcomes.[166]

## CAF in O-SCC

In 2021 Zhang and associates investigated the function of lysyl oxidase (LOX) expressed by CAFs in O-SCC diagnosed patients. They came to the conclusion that LOX greatly expresses in CAFs and helps to provide more matrix rigidity. This change in ECM enhances tumour cell invasion as well as EMT by activating the focal adhesion kinase (FAK) phosphorylation pathway. The results imply that aiming at LOX could be a good approach for O-SCC treatment.[167]

Furthermore, very important in controlling immune responses inside the TME are CAFs. Zhao et al. underlined that CAFs help Tregs to be more permeable, thereby generating an immunosuppressive environment that lets cancer cells avoid immune monitoring. O-SCC development depends critically on this immunological regulation, which emphasizes even more the several function of CAFs.[160]

Furthermore, CAFs interact with cancer cells by secreting growth factors and cytokines that induce angiogenesis and tumour development, therefore acting

via paracrine signalling. According to Lin et al., CAFs improve angiogenesis in O-SCC and are in line with higher metastatic potential. Providing nutrition and oxygen to the developing cancer depends on this blood support.[142]

Another area of major interest is CAF heterogeneity. Patel and colleagues examined the variation in α-SMA expression among CAFs, which reflects their various functional roles in the TME. Development of specific treatments depends on an awareness of these variations.[161]

In summary, CAFs are integral to O-SCC pathogenesis, influencing tumour progression through ECM remodelling, immune modulation, and angiogenesis. Their complex interactions with cancer cells and the microenvironment make them attractive targets for therapeutic intervention, with ongoing research aimed at unravelling the molecular pathways involved in their diverse functions.

# MATERIALS AND METHODS

## STUDY DESIGN AND STUDY SETTING:

This laboratory-based analytical study was conducted in the Department of Pathology at Sri Devaraj Urs Medical College, Kolar, Karnataka, India. The surgical specimens were obtained from patients who had undergone surgical excision at the attached R.L. Jalappa Hospital and Research Centre.

## STUDY PERIOD AND DURATION:

The study was conducted over a 16-month period from September 2022 to December 2023. During this time, the diagnostic specimens from patients who underwent surgical resection for O-SCC were collected, processed, and analysed according to the study protocol. Their clinicodemographic details was collected from the archives of Pathology.

## SAMPLE SIZE CALCULATION

The sample size was calculated using Cochran's formula: $n = Z^2 \times p \times (1-p) / d^2$, where Z is the standard normal variate at 5% type I error (1.96), p is the expected proportion based on previous studies, and d is the absolute error or precision.

Sample size was estimated based on **88.2%** prevalence for alpha SMA in O-SCC as reported in the Kellermann et al study.[7] Considering an absolute error of **7%** at **95%** confidence interval, the estimated sample size for the study was **88** cases of O-SCC.[20]

Fujii et al. reported CAF prevalence in 60.5% of O-SCC cases in their study population of 60 patients.

## INCLUSION CRITERIA:

The study included histopathologically confirmed cases of O-SCC treated by composite resection with cervical lymph node dissection. Only primary O-SCC

cases with available paraffin-embedded tissue blocks and corresponding clinical and pathological data were included in the analysis.

**EXCLUSION CRITERIA:**

(1) Recurrent cases of oral carcinoma,

(2) Patients who received neoadjuvant chemotherapy for head and neck malignancy prior to surgical intervention, and

(3) Second primary cancers.

**SAMPLING METHOD:**

Consecutive sampling technique was employed to recruit subjects into the study. All histopathologically confirmed cases of O-SCC meeting the inclusion criteria during the study period were included until the required sample size was achieved. The sampling process ensured representation across various pathological grades, anatomical sites, and clinical stages of O-SCC.

**DATA COLLECTION PROCEDURE**

Paraffin blocks and slides of histo-pathologically diagnosed O-SCC cases were retrieved from the archives of the Department of Pathology. Patient demographic data including age, gender, and anatomical location of the tumour were recorded from clinical records. All hematoxylin and eosin (H&E) stained slides were reviewed by two pathologists independently to confirm the diagnosis and histopathological grade according to AJCC $8^{th}$ edition.

Immunohistochemical staining for α-SMA was performed on 4μm sections of formalin-fixed paraffin-embedded tissue samples. After deparaffinization and rehydration, antigen retrieval was performed using microwave heat-induced epitope retrieval. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. Sections were incubated with primary monoclonal antibody against α-SMA for 60 minutes at room temperature, followed by incubation with HRP-conjugated secondary antibody. Diaminobenzidine (DAB) was used

as the chromogen, and slides were counterstained with haematoxylin. Ductal carcinoma of the breast tissue served as positive control, while endothelial cells of blood vessels served as internal positive control.

The αSMA-stained (cytoplasmic stain) CAFs in the O-SCC islands were counted using 2007 Kellermann et al. scoring system[20] :

**Score 1** (−) (Negative): when no staining or if <1% of CAF stained with αSMA

**Score 2** (+) (Scanty): when more than 1% and <50% CAFs were stained with αSMA

**Score 3** (++) (Abundant): when more than 50% of CAFs were stained with αSMA

Considering the distribution pattern of CAF, the arrangement of positive-stained cells was classified into three groups:

**Focal**: Focal CAF with no special arrangement in different areas of tumour stroma

**Network**: Interwoven network arrangement of CAF in the tumour stroma

**Spindle:** Spindled arrangement of CAF in one to three rows in the periphery of the neoplastic

islands or the connective tissues.[20]

Additionally, TSR, WPOI, T-B, and TILs were assessed in each specimen.

**DATA ANALYSIS**

1. The gathered data were imported into Microsoft Excel and then examined by IBM. software for statistics SPSS 23.0.

2. Descriptive statistics were employed to characterize the study population, including measures of central tendency (mean, median) and dispersion (standard deviation) for continuous variables such as age and DOI.

3. Frequency distributions and percentages were calculated for categorical variables including gender, tumour lateralization, histological differentiation, anatomical site, and various histopathological parameters.

4. For analysing associations between categorical variables, Pearson's chi-square ($\chi^2$) test was utilized to assess statistical significance, with p-values less than 0.05 considered statistically significant. This approach was specifically applied to evaluate relationships between CAF scores and LNM, CAF architectural patterns and lymph node status, and CAF scores in relation to pTNM staging.

# RESULTS

88 cases of O-SCC were studied and the results are as follows:

***Table 3: Age distribution of samples***

| Age group | Frequency | Percent |
|---|---|---|
| 0-10 | - | |
| 11-20 | - | |
| 21-30 | 1 | 1.1 |
| 31-40 | 4 | 4.5 |
| 41-50 | 27 | 30.7 |
| 51-60 | 23 | 26.1 |
| 61-70 | 24 | 27.3 |
| 71-80 | 8 | 9.1 |
| 81-90 | 1 | 1.1 |
| Total | 88 | 100.0 |

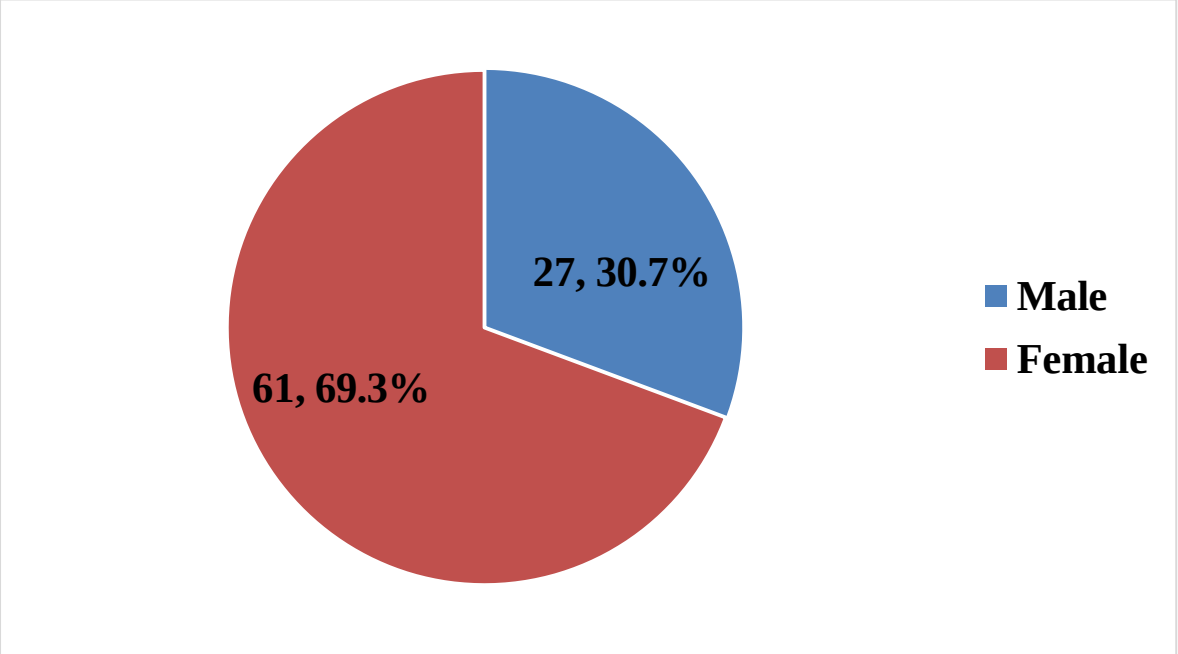
The patient population demonstrated a mean age of 56.97 years (median = 55 years), encompassing a considerable age spectrum ranging from 30 to 90 years. Most cases fell into the age range of 41-50 years (30.7%), followed by 61-70 years (27.3%) and 51-60 years (26.1%)

*Table 4: Gender Distribution*

| Gender | Frequency | Percent |
|---|---|---|
| Male | 27 | 30.7 |
| Female | 61 | 69.3 |
| Total | 88 | 100 |

Gender distribution demonstrated a significant female preponderance, with 61 female patients constituting more than two-thirds (69.3%) of the total study population, while male participants numbered 27, representing less than one-third (30.7%) of the aggregate sample size of 88 subjects.

*Figure 1: Gender Distribution*




M:E ratio= 0.44:1, implying for every 2 female cases there was 1 male O-SCC case.

***Table 5: Laterality of Lesions in O-SCC Patients***

| Side | Frequency | Percent |
|---|---|---|
| Left | 53 | 60.2 |
| Right | 33 | 37.5 |
| Central | 2 | 2.3 |
| Total | 88 | 100 |

Regarding the anatomical lateralization of lesions, a distinct predisposition toward unilateral involvement was observed, with left-sided lesions demonstrating marked predominance at 53 cases (60.2%), followed by right-sided lesions at 33 cases (37.5%). Central lesions represented a notably small proportion of the cohort with only 2 cases (2.3%) of the total 88 specimens examined.

*Figure 2: Laterality of Lesions in O-SCC Patients*

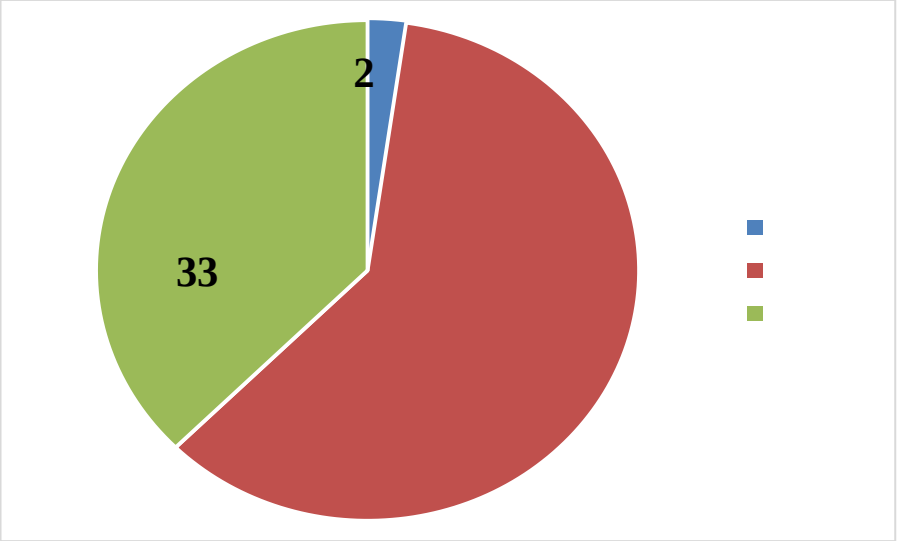



*Table 6: Anatomical Distribution of Primary O-SCC Lesions by Intraoral Subsite*

| Site of primary tumour | Frequency | Percent |
|---|---|---|
| Buccal mucosa | 44 | 50 |
| Lower gingiva buccal sulcus | 15 | 17 |
| Lateral border of tongue | 8 | 9.1 |
| Lower alveolus | 5 | 5.7 |
| Upper gingiva buccal sulcus | 4 | 4.5 |
| Retromolar trigone | 4 | 4.5 |
| Hard palate | 3 | 3.4 |
| Dorsum of tongue | 2 | 2.3 |

| | | |
|---|---|---|
| Floor of mouth | 1 | 1.1 |
| Lower lip | 1 | 1.1 |
| Upper alveolus | 1 | 1.1 |
| Total | 88 | 100 |

Buccal mucosa emerged as the predominant site of neoplastic origin, accounting for exactly 44 cases (50%) and thus constituting half of the entire sample population. The second most frequent anatomical location was the lower gingiva buccal sulcus with 15 cases (17%), followed by the lateral border of tongue with 8 cases (9.1%), demonstrating a considerably lower incidence compared to the buccal mucosa. Lower alveolus manifested 5 cases (5.7%), while both retromolar trigone and upper gingiva buccal sulcus each presented 4 cases (4.5%). Hard palate demonstrated modest representation with 3 cases (3.4%), and dorsum of tongue exhibited 2 cases (2.3%). The least frequently affected sites, each with solitary case representation of 1 (1.1%), included floor of mouth, lower lip, and upper alveolus.

***Table 7: Histopathological Differentiation Status of Primary O-SCC Specimens***

| **Degree of differentiation of primary tumour** | **Frequency** | **Percent** |
|---|---|---|
| Well Differentiated | 71 | 80.7 |
| Moderately differentiated | 14 | 15.9 |
| Poorly differentiated | 3 | 3.4 |
| Total | 88 | 100 |

Well-differentiated neoplasms constituted a majority with 71 cases (80.7%) of the total sample, demonstrating the predominance of tumours exhibiting minimal deviation from normal squamous epithelium and maintenance of characteristic cellular architecture. Moderately differentiated tumours represented a considerably smaller proportion with 14 cases (15.9%), reflecting intermediate histological alterations and partial loss of normal cellular organization. Poorly differentiated lesions were notably infrequent, comprising merely 3 cases (3.4%) of the aggregate sample of 88 specimens, indicating the relative rarity of highly anaplastic tumours with marked cellular pleomorphism and minimal resemblance to the tissue of origin

***Figure 3: Histopathological Differentiation Status of Primary O-SCC Specimens***

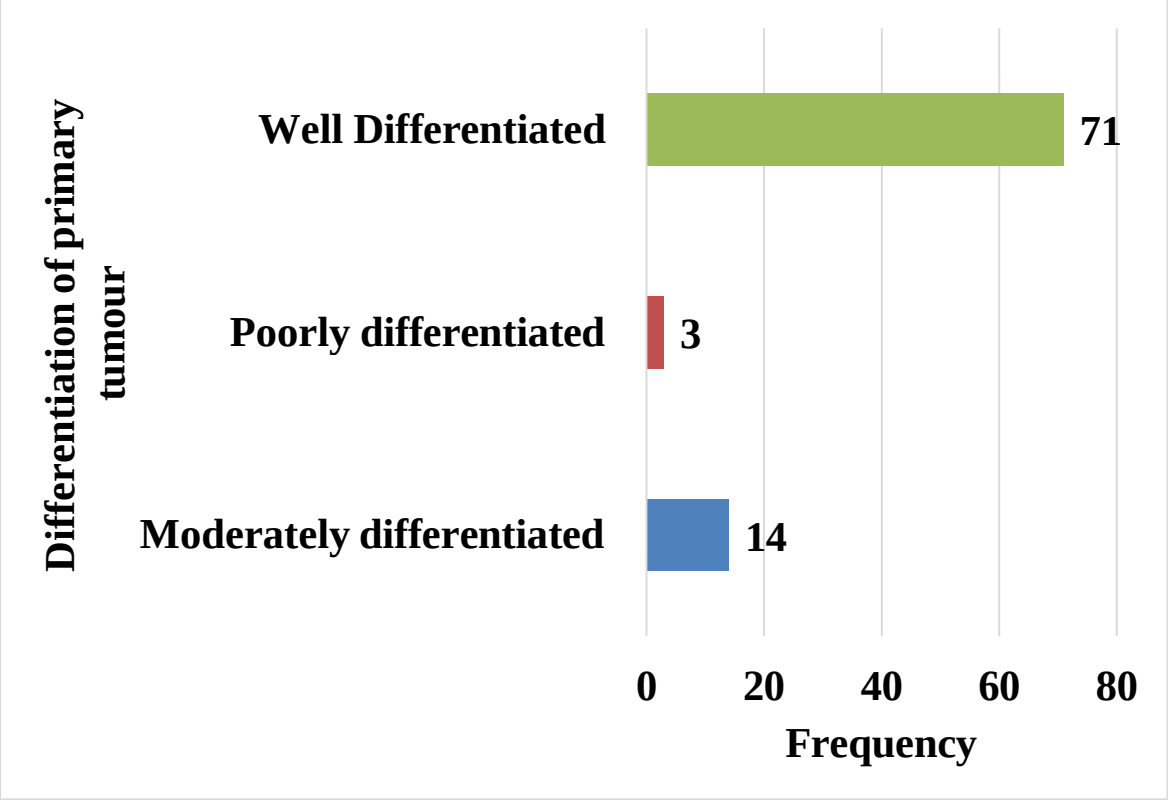


*Table 8: DOI of primary tumour*

| DOI (in mm) | Frequency | Percent |
|---|---|---|
| <= 5 mm | 21 | 23.9 |
| 5 – 10 mm | 33 | 37.5 |
| >10mm | 34 | 38.6 |
| Total | 88 | 100.0 |

Regarding tumour invasiveness, the DOI demonstrated substantial variability across the sample as evidenced by the range extending from 2 mm to 45 mm. DOI measured was found to be >10 mm in majority of cases (38.6%) demonstrating aggressive behaviour, followed by 5-10 mm in 37.5% and 23.9% had the DOI of <=5 mm.

*Table 9: Distribution and Frequency of Soft Tissue Involvement in O-SCC Specimens*

1. **Lymph vascular invasion (LVI)**

| LVI | Frequency | Percent |
|---|---|---|
| Present | 3 | 3.4 |
| Absent | 85 | 96.6 |
| Total | 88 | 100 |

2. **PNI**

| **PNI** | **Frequency** | **Percent** |
|---|---|---|
| Present | 13 | 14.8 |
| Absent | 75 | 85.2 |
| Total | 88 | 100 |

3. **Bone involvement**

| **Bone involvement** | **Frequency** | **Percent** |
|---|---|---|
| Present | 12 | 13.6 |
| Absent | 76 | 86.4 |
| Total | 88 | 100 |

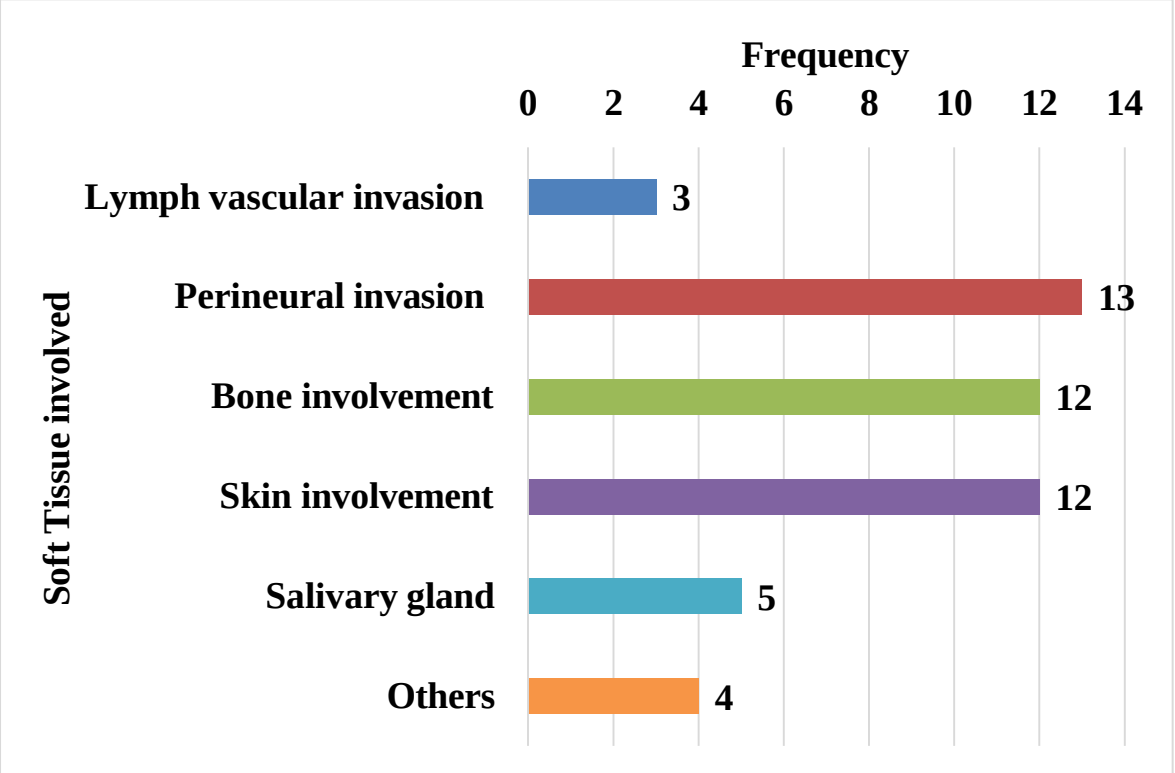
4. **Skin involvement**

| **Skin involvement** | **Frequency** | **Percent** |
|---|---|---|
| Present | 12 | 13.6 |
| Absent | 76 | 86.4 |
| Total | 88 | 100 |

5. **Any other tissue involved**

| Other tissue involvement | Frequency | Percent |
|---|---|---|
| Salivary gland | 5 | 5.8 |
| Infratemporal fossa clearance | 1 | 1.1 |
| Left turbinate | 1 | 1.1 |
| Muscle fibres of tongue | 1 | 1.1 |
| Skeletal muscle | 1 | 1.1 |

***Figure 4: Distribution and Frequency of Soft Tissue Involvement in O-SCC Specimens***




PNI demonstrated the highest prevalence with 13 cases (14.8%), constituting the predominant mode of neoplastic dissemination within the cohort. Both bone

involvement and skin involvement exhibited identical frequencies of 12 cases (13.6%) each, representing the second most prevailing infiltrative patterns and indicating substantial local aggressiveness of the tumours examined. Salivary gland infiltration was documented in 5 cases (5.8%), while other anatomical structures—specifically categorized as infratemporal fossa, left turbinate, muscle fibres of tongue, and skeletal muscle—collectively accounted for 4 cases. Lymph vascular invasion, a critical prognosticator for metastatic potential, demonstrated the lowest frequency with merely 3 cases (3.4%).

***Table 10: Lymph nodal metastasis***

| **Positive lymph nodes** | **Frequency** | **Percent** |
|---|---|---|
| Present | 47 | 53.4 |
| Absent | 41 | 46.6 |
| Total | 88 | 100.0 |

The lymph node involvement was observed in 53.4 % of cases ranging from, ranging from 1 to 24 in number; signifying aggressive disease.

*Table 11: Incidence of ECE in LNMs of O-SCC*

| ECE | Frequency | Percent |
|---|---|---|
| Present | 9 | 10.2 |
| Absent | 79 | 89.8 |
| Total | 88 | 100 |

ECE, a critical prognostic parameter indicative of trans capsular penetration of metastatic tumour cells beyond the confines of lymph node architecture, was conspicuously absent in 79 cases (89.8%) of the examined specimens. Conversely, the presence of ECE was documented in merely 9 cases (10.2%) of the aggregate sample of 88 subjects.

*Figure 5: Incidence of ECE in LNM of O-SCC*

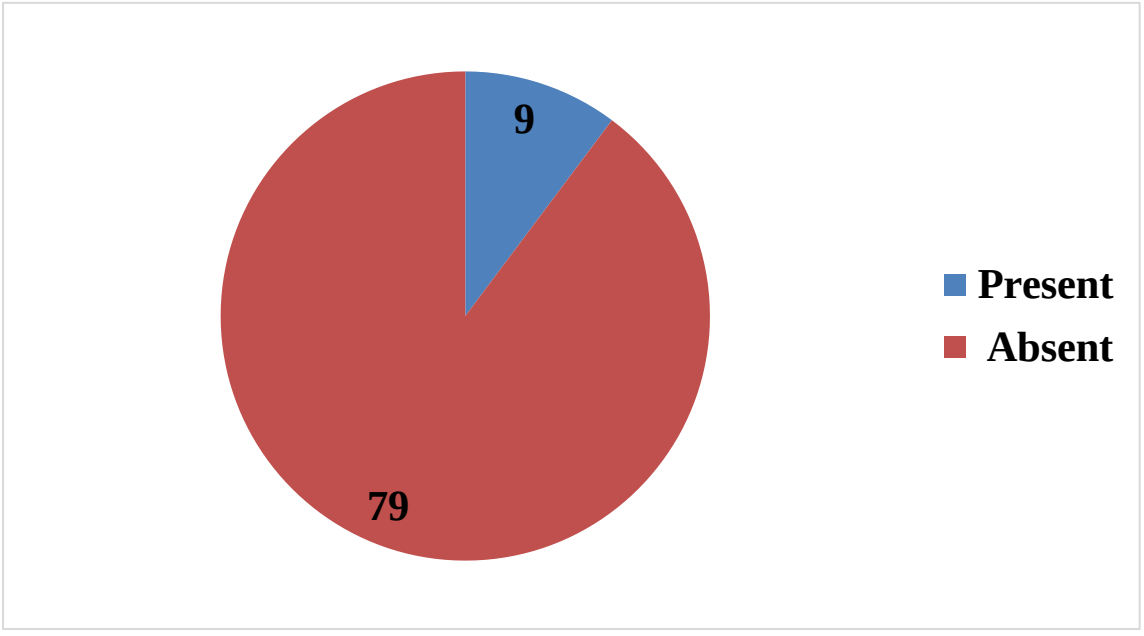


*Table 12: Distribution of O-SCC Cases Based on Pathological N Staging (pN)*

| pN staging | Frequency | Percent |
|---|---|---|
| 0 | 41 | 46.6 |
| 1 | 20 | 22.7 |
| 2a | 2 | 2.3 |
| 2b | 13 | 14.8 |
| 2c | 3 | 3.4 |
| 3a | 0 | 0.0 |
| 3b | 9 | 10.2 |
| Total | 88 | 100 |

The pathological lymph node status distribution reveals that the majority of cases, 41 (46.6%), demonstrate no LNM (pN0). Among cases with nodal involvement, pN1 category accounts for 20 (22.7%) specimens. Within the pN2 subcategories, pN2b predominates with 13 (14.8%) cases, followed by pN3b with 9 (10.2%), pN2c with 3 (3.4%), and pN2a with 2 (2.3%) cases.

*Figure 7: Distribution of O-SCC Cases Based on Pathological N Staging (pN)*

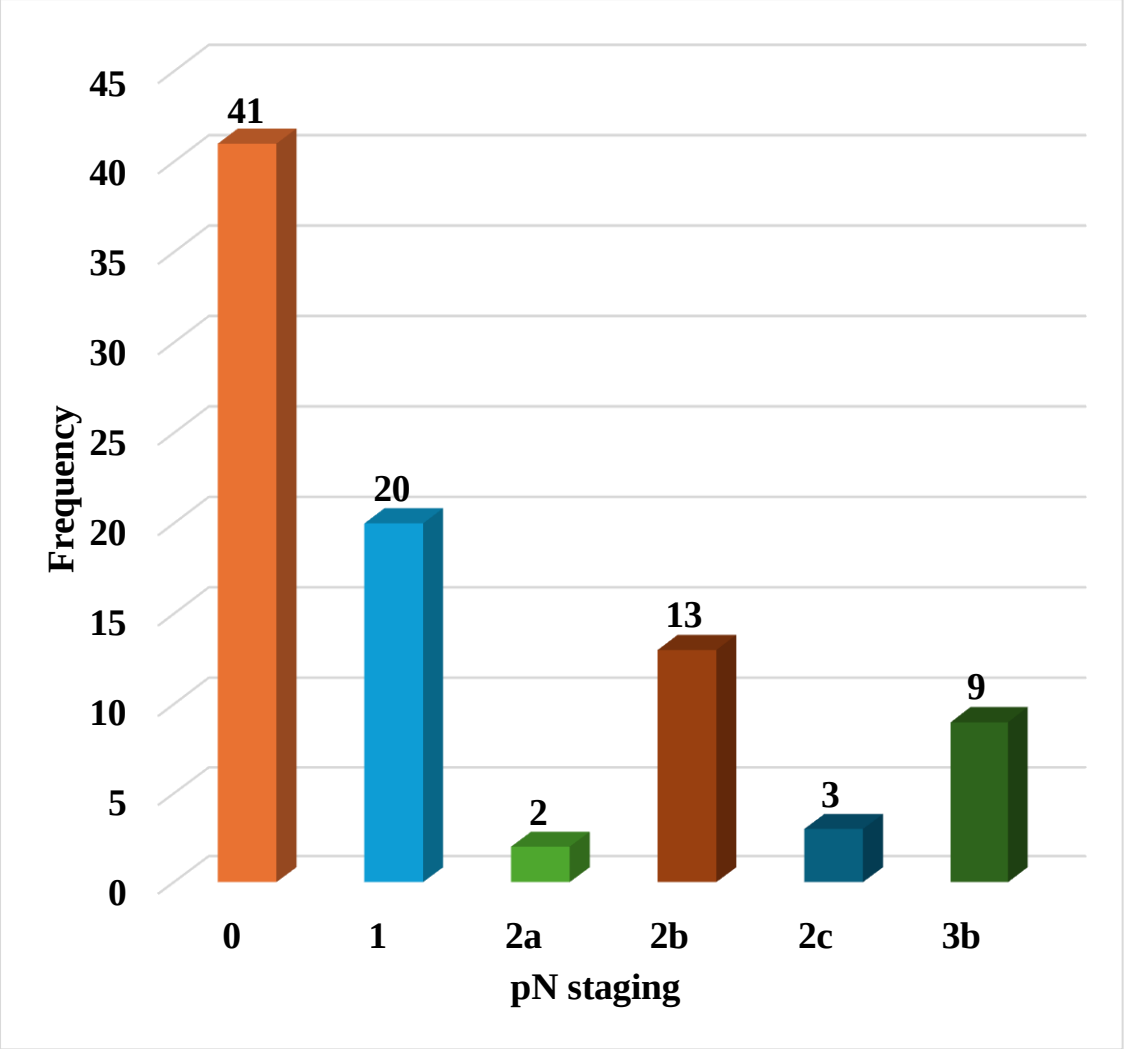



*Table 13: Distribution of O-SCC Cases Based on Pathological T Staging (pT)*

| pT staging | Frequency | Percent |
|---|---|---|
| 1 | 7 | 8 |
| 2 | 37 | 42 |
| 3 | 24 | 27.3 |
| 4a | 20 | 22.7 |
| 4b | 0 | 0.0 |
| Total | 88 | 100 |

The distribution of O-SCC cases according to pathological T staging demonstrates a predominant representation of stage 2 tumours, comprising 37 (42%) of the total sample. Stage 3 carcinomas constitute 24 (27.3%) cases, while stage 4a tumours account for 20 (22.7%) of the specimens. The least prevalent category is stage 1, with only 7 (8%) cases documented.

***Figure 6: Distribution of O-SCC Cases Based on Pathological T Staging (pT)***

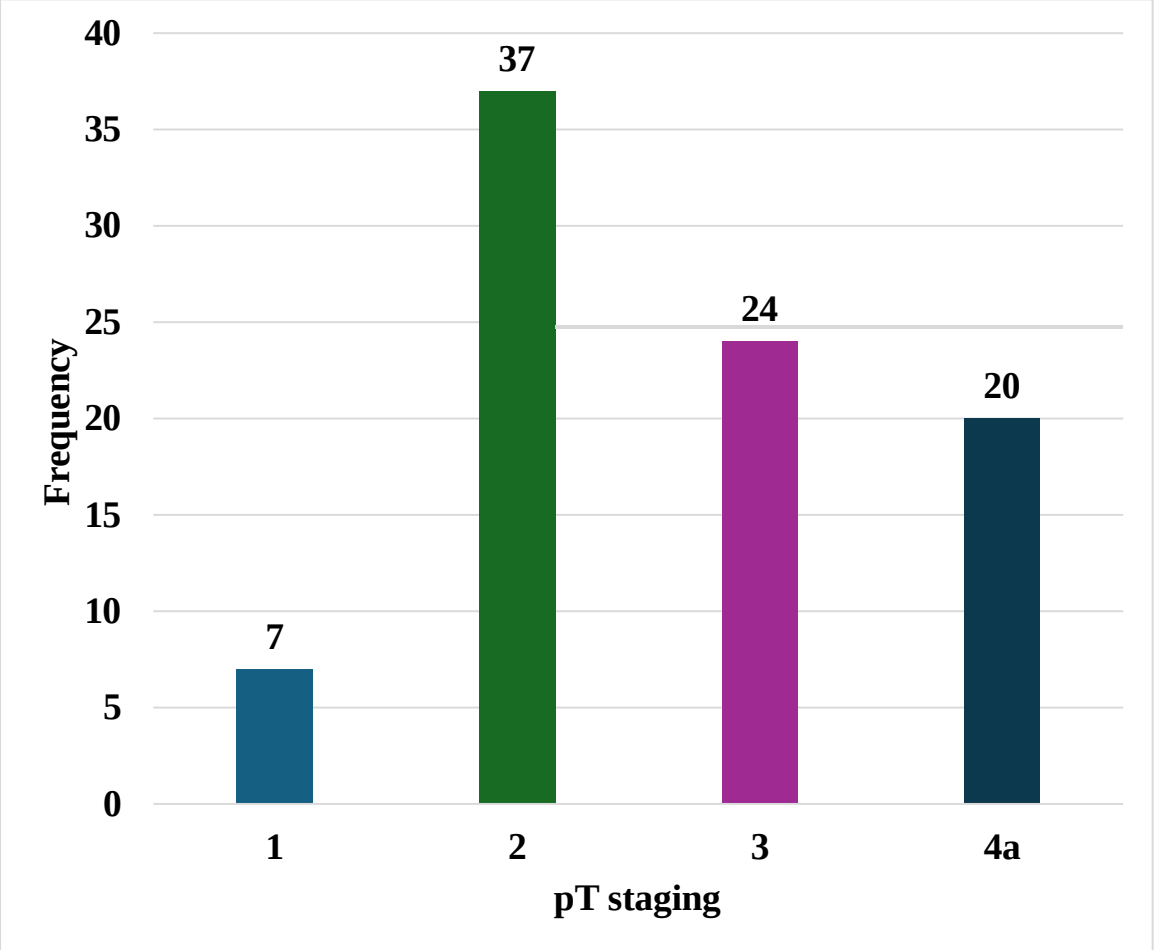


***Table 14: Distribution of O-SCC Cases According to TNM Staging Classification***

| TNM Stage | Frequency | Percent |
|---|---|---|
| I | 4 | 4.5 |
| II | 18 | 20.5 |
| III | 28 | 31.8 |
| IV A | 29 | 33 |
| IV B | 9 | 10.2 |
| Total | 88 | 100 |

The stratification of O-SCC specimens based on the comprehensive TNM staging system reveals a predominant representation of advanced disease categories. Stage IV A demonstrates the highest prevalence with 29 (33%) cases, followed closely by stage III with 28 (31.8%) cases. Stage II comprises 18 (20.5%) of the analysed specimens, while stage IV B represents 9 (10.2%) cases. The least prevalent category is stage I, accounting for merely 4 (4.5%) of the total sample.

*Figure 8: Distribution of O-SCC Cases According to TNM Staging Classification*

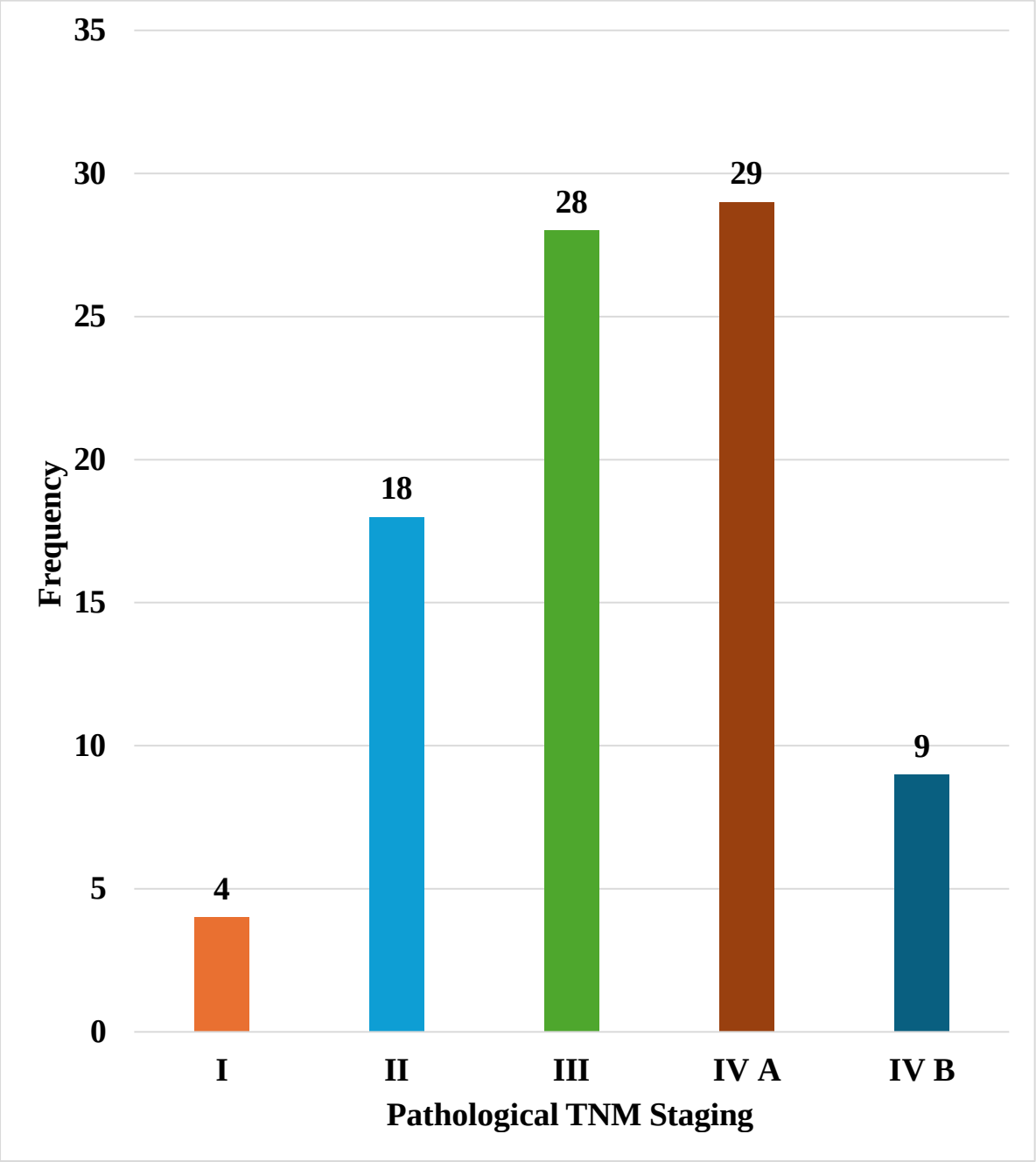


***Table 15: Distribution of CAF Scores in O-SCC Cases***

| CAF score | Frequency | Percent |
|---|---|---|
| 1 | 3 | 3.4 |
| 2 | 36 | 40.9 |
| 3 | 49 | 55.7 |
| Total | 88 | 100 |

The quantitative analysis of CAFs utilizing actin IHC demonstrates a notable predominance of high CAF expression within the TME. Score 3, indicating abundant CAF presence (>50% positively stained cells), was observed in 49 (55.7%) cases, representing the majority of the specimens. Score 2, denoting scanty CAF presence (1-50% positively stained cells), was identified in 36 (40.9%) cases. Conversely, score 1, signifying negative or minimal CAF presence (<1% positively stained cells), was observed in only 3 (3.4%) cases. This distribution across 88 (100%) specimens suggests a significant tendency toward elevated CAF presence in the tumour stroma of O-SCC, which may have profound implications for understanding the TME and its potential correlation with pathological staging and nodal metastasis.

*Figure 9: Distribution of CAF Scores in O-SCC Cases*

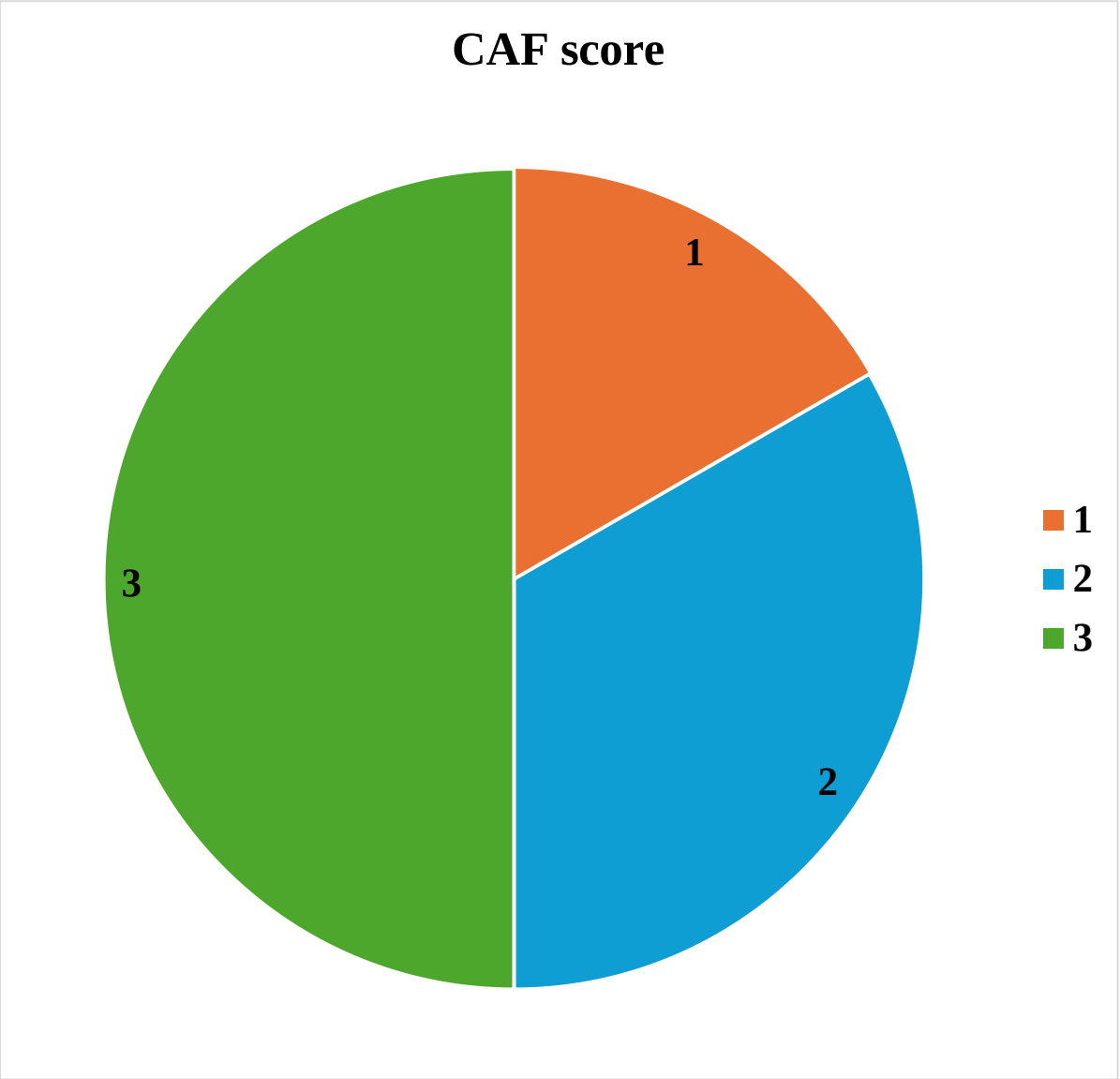


*Table 16: Distribution Patterns of CAFs in O-SCC*

| CAF distribution pattern | Frequency | Percent |
|---|---|---|
| Focal | 27 | 30.7 |
| Network | 34 | 38.6 |
| Spindle | 27 | 30.7 |
| Total | 88 | 100 |

The morphological characterization of CAFs within the TME reveals distinctive architectural arrangements with potential implications for tumour-stromal interactions. The network pattern, characterized by an interwoven arrangement of CAFs within the tumour stroma, demonstrates predominance with 34 (38.6%) cases. Both focal distribution, defined by non-specific arrangement of CAFs in discrete areas of tumour stroma, and spindle configuration, manifested as parallel arrangements in one to three rows at the periphery of neoplastic islands, exhibited identical prevalence with 27 (30.7%) cases each.

*Figure 10: Distribution Patterns of CAFs in O-SCC*

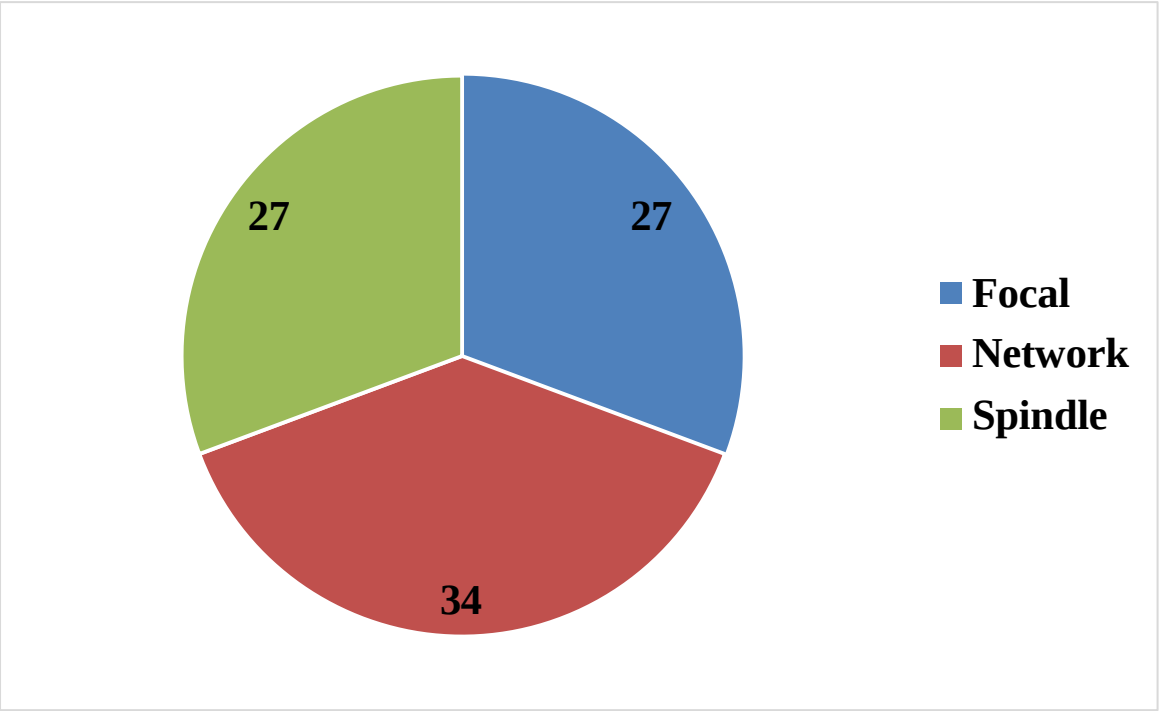



*Table 17: TSR Distribution in O-SCC Specimens*

| Tumour stroma ratio | Frequency | Percent |
|---|---|---|
| <50 | 59 | 67 |
| ≥50 | 29 | 33 |
| Total | 88 | 100 |

A substantial majority of specimens, specifically 59 (67%), exhibit a TSR of less than 50, indicating stromal predominance. Conversely, 29 (33%) cases present with a TSR equal to or exceeding 50, signifying tumour cell predominance.

*Figure 11: TSR Distribution in O-SCC Specimens*

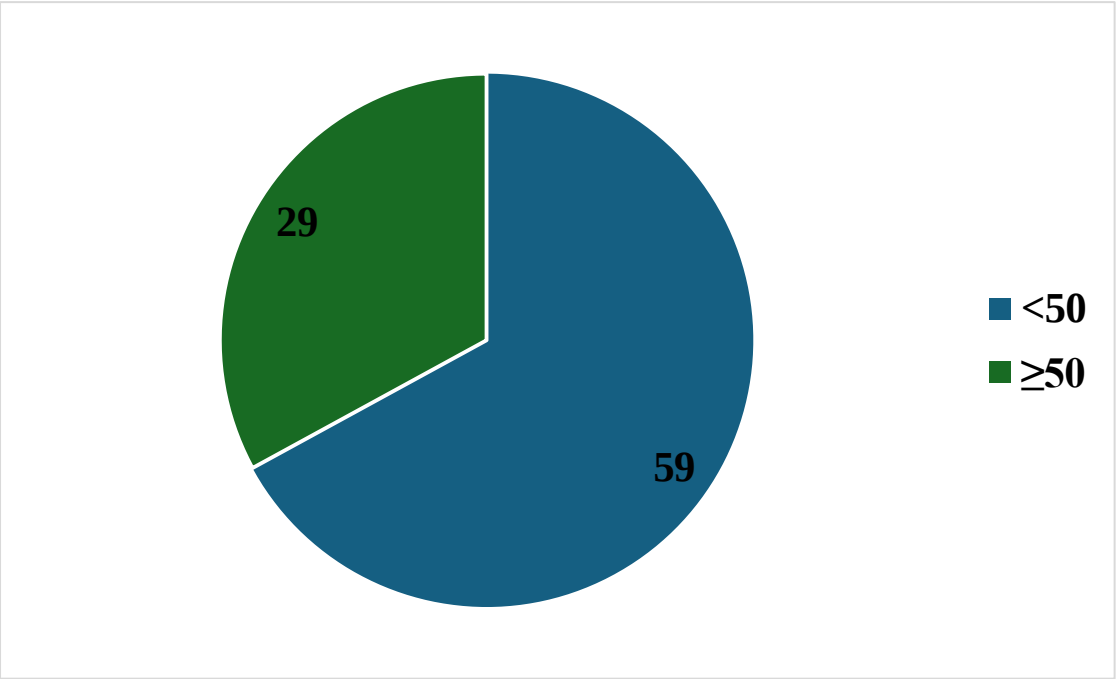



*Table 18: Distribution of WPOI types*

| WPOI Types | Frequency | Percent |
|---|---|---|
| 1 | - | |
| 2 | 6 | 6.8 |
| 3 | 20 | 22.7 |
| 4 | 36 | 40.9 |
| 5 | 26 | 29.5 |
| Total | 88 | 100 |

The histo-morphological assessment of invasive front characteristics, quantified through the WPOI types. Type 4, characterized by small groups or cords of infiltrating cells, constitutes the most prevalent category with 36 (40.9%) specimens. Type 5, representing the most aggressive phenotype with marked and widespread cellular dissociation, accounts for 26 (29.5%) cases. Type 3, typified by groups of infiltrating cells with >15 cells per island, is observed in 20(22.7%) specimens. Conversely, Type 2, defined by solid cords with broad pushing borders, demonstrates minimal representation with merely 6 (6.8%) cases.

***Table 19: Quantitative Analysis of T-B***

| **T-B** | **Frequency** | **Percent** |
|---|---|---|
| <5 | 40 | 45.5 |
| 5 to 10 | 35 | 39.8 |
| >10 | 13 | 14.8 |
| Total | 88 | 100 |

The quantitative evaluation of T-B, a histopathological parameter defined by the presence of isolated tumour cells or small clusters (<5 cells) at the invasive front, reveals a stratified distribution across the established categorical thresholds. The predominant category comprises specimens exhibiting fewer than 5 tumour buds per high-power field, accounting for 40 (45.5%) cases. An intermediate burden of T-B, characterized by 5 to 10 buds per high-power field, is observed in 35 (39.8%) specimens. High-intensity T-B, exceeding 10 buds per high-power field, represents the least prevalent category with 13 (14.8%) cases.

*Figure 12: Quantitative Analysis of T-B*

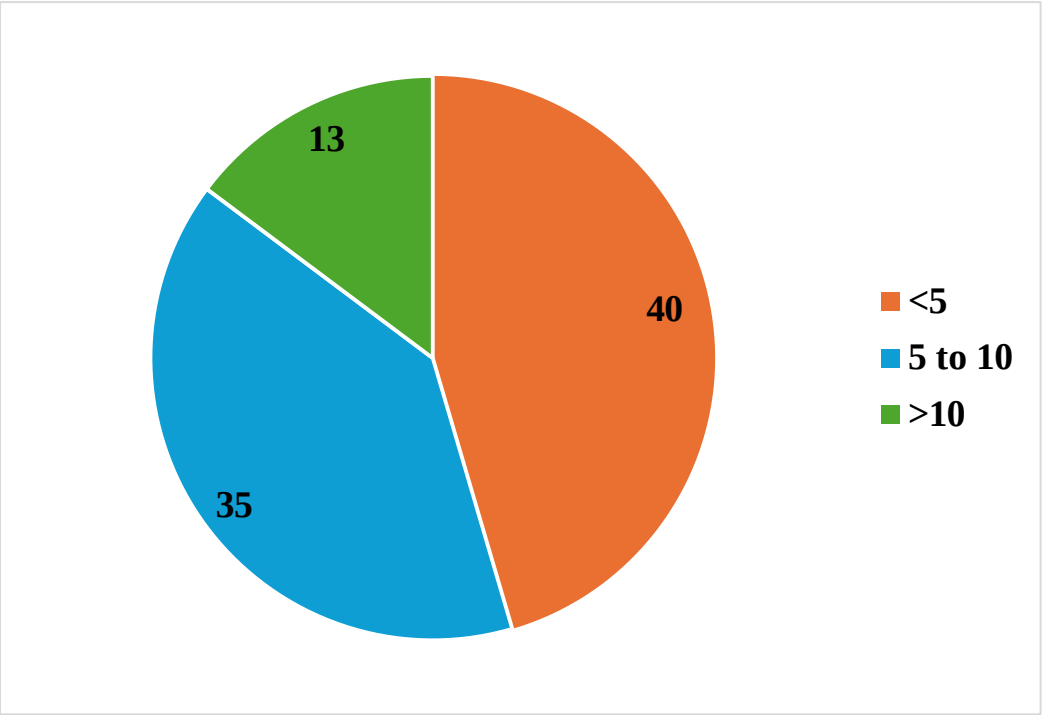



*Table 20: Quantitative Distribution of TILs*

| TILs (%) | Frequency | Percent |
|---|---|---|
| <20 | 33 | 37.5 |
| 20-80 | 51 | 58 |
| >80 | 4 | 4.5 |
| Total | 88 | 100 |

The immunological microenvironment assessment, quantified through tumour-infiltrating lymphocyte (TIL) enumeration, demonstrates a predominant intermediate immunological response. The majority of specimens, comprising 51 (58%), exhibit a moderate TIL density ranging from 20 to 80 lymphocytes per high-power field, suggesting an intermediate level of immunological

engagement. A substantial minority, 33 (37.5%) cases, demonstrates limited lymphocytic infiltration with fewer than 20 lymphocytes per high-power field, potentially indicative of immune evasion or suppression mechanisms. Notably, robust lymphocytic infiltration exceeding 80 lymphocytes per high-power field represents an exceptional finding, observed in merely 4 (4.5%) specimens.

***Figure 13: Quantitative Distribution of TILs***

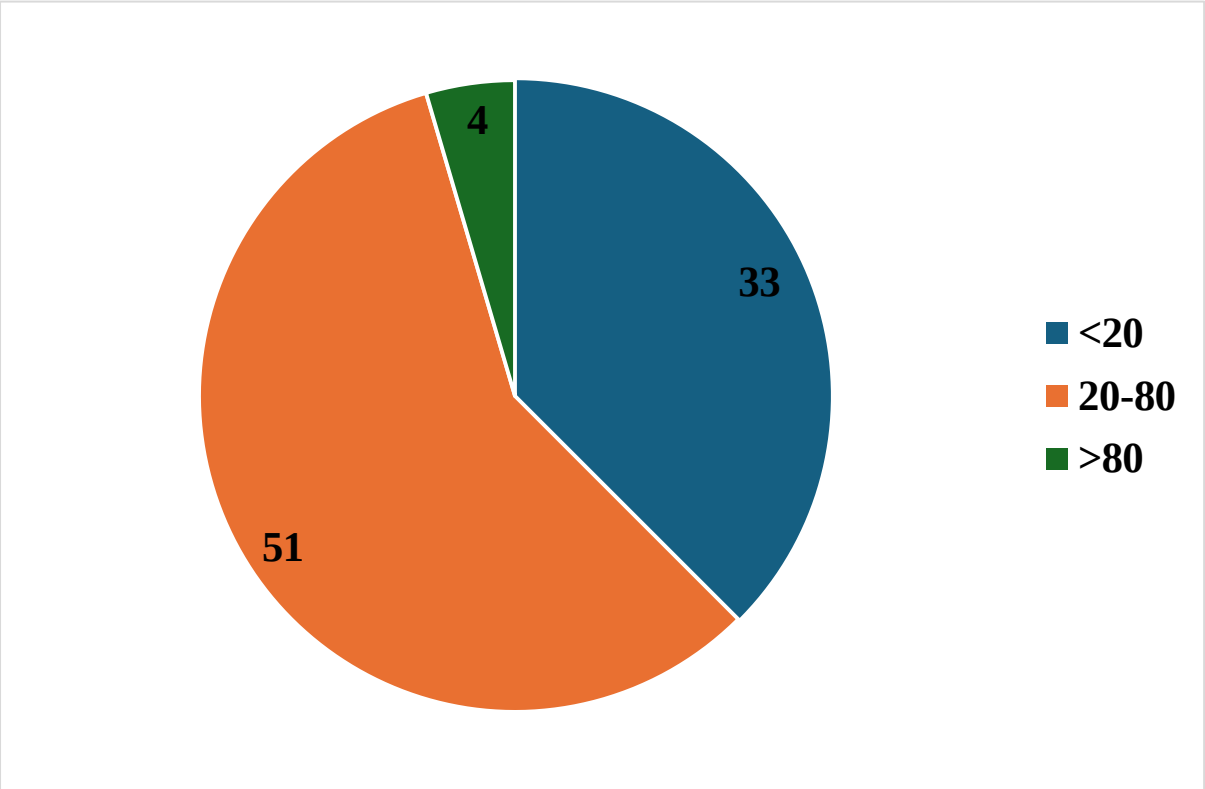



***Table 21: Association Between LNM and CAF Scores in O-SCC***

| LNM | CAF score | | |
|---|---|---|---|
| | 1 (n=3) | 2 (n=36) | 3 (n=49) |
| Yes | 1 (2.1%) | 20 (42.6%) | 26 (55.3%) |
| No | 2 (4.9%) | 16 (39%) | 23 (56.1%) |
| Chi-square value: 0.555 | | p-value: 0.758 | |

Among specimens with LNM, the preponderance exhibited elevated CAF scores, with 26 (55.3%) cases demonstrating abundant CAF presence (score 3) and 20 (42.6%) cases exhibiting intermediate CAF density (score 2). Minimal CAF expression (score 1) was observed in only 1 (2.1%) metastatic specimen. Similarly, in non-metastatic cases, high CAF expression predominated with 23 (56.1%) specimens classified as score 3, while 16 (39%) cases exhibited intermediate CAF density, and 2 (4.9%) cases demonstrated minimal CAF presence. Statistical analysis utilizing the chi-square test yielded a value of 0.555 with a corresponding p-value of 0.758, substantially exceeding the conventional threshold of statistical significance ($p<0.05$). This finding suggests that despite the apparent tendency toward elevated CAF expression across both metastatic and non-metastatic subgroups, the distribution patterns do not significantly differ between these categories, indicating that CAF score alone may not function as an independent predictive biomarker for lymph node metastatic potential in O-SCC.

***Table 22: Association Between LNM and CAF Distribution Patterns***

| LNM | CAF distribution pattern | | |
|---|---|---|---|
| | **Focal (n=27)** | **Network (n=34)** | **Spindle (n=27)** |
| Yes | 12 (25.5%) | 17 (36.2%) | 18 (38.3%) |
| No | 15 (36.6%) | 17 (41.5%) | 9 (22.0%) |
| Chi-square value: 2.938 | | p-value: 0.230 | |

The cross-tabulation analysis examining the relationship between lymph node metastatic status and CAF architectural organization reveals intriguing distributional variations that approach, but do not achieve, conventional thresholds of statistical significance. Within specimens exhibiting metastatic disease, the spindle configuration predominates with 18 (38.3%) cases, followed by the network arrangement with 17 (36.2%) cases, while the focal pattern demonstrates the lowest prevalence with 12 (25.5%) specimens. Conversely, among non-metastatic cases, both focal and network patterns demonstrate equivalent representation with 15 (36.6%) and 17 (41.5%) specimens respectively, whereas the spindle configuration exhibits substantially reduced prevalence with merely 9 (22.0%) cases. Statistical assessment utilizing Pearson's chi-square test yielded a value of 2.938 with a corresponding p-value of 0.230, exceeding the conventional threshold for statistical significance ($p<0.05$).

*Table 23: Relationship Between pTNM Staging and CAF Scores*

| pTNM stage | CAF scores | | |
|---|---|---|---|
| | **1 (n=3)** | **2 (n=36)** | **3 (n=49)** |
| I | 0 (0.0%) | 3 (75.0%) | 1 (25.0%) |
| II | 1 (5.6%) | 9 (50.0%) | 8 (44.4%) |
| III | 1 (3.6%) | 12 (42.9%) | 15 (53.6%) |
| IV A | 1 (3.4%) | 11 (37.9%) | 17 (58.6%) |
| IV B | 0 (0.0%) | 1 (11.1%) | 8 (88.9%) |
| Chi-square value: 7.165 | | p-value: 0.519 | |

The contingency analysis investigating the association between pTNM staging and quantitative assessment of CAF expression demonstrates a progressive incrementation in high-intensity CAF representation concomitant with advancing disease stage, although this trend fails to achieve statistical significance. In stage I disease, intermediate CAF expression (score 2) predominates with 3 (75.0%) specimens, while abundant CAF presence (score 3) represents 1 (25.0%) case, with complete absence of minimal CAF expression. Stage II carcinomas demonstrate relative equilibrium between intermediate and abundant CAF expression with 9 (50.0%) and 8 (44.4%) cases respectively, with minimal CAF presence (score 1) representing merely 1 (5.6%) specimen. In stage III disease, abundant CAF expression becomes predominant with 15 (53.6%) cases compared to 12 (42.9%) specimens with intermediate expression and 1 (3.6%) case with minimal CAF presence. This

pattern intensifies in stage IVA, where abundant CAF expression characterizes 17 (58.6%) specimens, with 11 (37.9%) cases demonstrating intermediate expression and 1 (3.4%) specimen exhibiting minimal CAF presence. Stage IVB demonstrates the most pronounced skew toward abundant CAF expression with 8 (88.9%) cases, compared to 1 (11.1%) specimen with intermediate expression and complete absence of minimal CAF presence. Statistical analysis utilizing chi-square methodology yielded a value of 7.165 with a corresponding p-value of 0.519, substantially exceeding conventional thresholds of statistical significance.

***Table 24: Association Between CAF Distribution Patterns and pTNM Staging***

| **pTNM stage** | **CAF distribution pattern** | | |
|---|---|---|---|
| | **Focal (n=27)** | **Network (n=34)** | **Spindle (n=27)** |
| I | 2 (50.0%) | 0 (0.0%) | 2 (50%) |
| II | 10 (55.6%) | 6 (33.3%) | 2 (11.1%) |
| III | 12 (42.9%) | 10 (35.7%) | 6 (21.4%) |
| IV A | 3 (10.3%) | 16 (55.2%) | 10 (34.5%) |
| IV B | 0 (0.0%) | 2 (22.2%) | 7 (77.8%) |
| Chi-square value: 26.716 | | p-value: 0.001 | |

The analysis of CAF distribution patterns in relation to pTNM staging demonstrates a statistically significant association ($\chi^2 = 26.716$, $p = 0.001$). In stage I disease, CAFs exhibited equal distribution between focal and spindle patterns, each accounting for 2 (50.0%) cases, with no cases (0.0%) displaying network patterns. Stage II predominantly featured focal CAF distribution in 10 (55.6%) cases, followed by network pattern in 6 (33.3%) cases, while spindle

arrangement was observed in only 2 (11.1%) cases. Among stage III tumors, focal pattern remained predominant in 12 (42.9%) cases, with network pattern in 10 (35.7%) cases and spindle arrangement in 6 (21.4%) cases. In contrast, stage IVA revealed a notable shift toward network pattern predominance, observed in 16 (55.2%) cases, with spindle arrangement in 10 (34.5%) cases and focal distribution in merely 3 (10.3%) cases. Stage IVB demonstrated the most striking pattern shift, with spindle arrangement occurring in 7 (77.8%) cases, network pattern in 2 (22.2%) cases, and complete absence of focal distribution (0.0%). This progressive transition from focal to network and ultimately to spindle CAF arrangements with advancing TNM stages suggests a correlation between CAF distribution patterns and tumour progression.

***Table 25: Association Between Tumour Stroma Ratio and CAF Score***

| **Tumour stroma ratio** | **CAF score** | | |
|---|---|---|---|
| | **1 (n=3)** | **2 (n=36)** | **3 (n=49)** |
| <50 | 0 (0.0%) | 15 (25.4%) | 44 (74.6%) |
| ≥50 | 3 (10.3%) | 21 (72.4%) | 5 (17.2%) |
| Chi-square value: 28.077 | | p-value: <0.0001 | |

The relationship between Tumour Stroma Ratio (TSR) and CAF score demonstrates a robust statistical association ($\chi^2 = 28.077$, $p < 0.0001$). Among specimens with TSR < 50, a striking predominance of high CAF scores was observed, with score 3 occurring in 44 (74.6%) cases, score 2 in 15 (25.4%) cases, and complete absence of score 1 (0.0%). Conversely, specimens with TSR ≥ 50 exhibited a markedly different distribution pattern, with score 2 predominating in 21 (72.4%) cases, followed by score 3 in only 5 (17.2%)

cases, and score 1 in 3 (10.3%) cases. These findings suggest an inverse relationship between TSR and CAF score, wherein stroma rich cases (TSR < 50) demonstrated higher CAF scores, potentially indicating that a more concentrated CAF population within the abundant stroma may exert an enhanced tumorigenic influence.

***Table 26: Association Between WPOI and T-B***

| **WPOI** | **T-B** | | |
|---|---|---|---|
| | **<5 (n=40)** | **>10 (n=13)** | **5 to 10 (n=35)** |
| 2 | 6 (100.0%) | 0 (0.0%) | 0 (0.0%) |
| 3 | 19 (95.0%) | 0 (0.0%) | 1 (5.0%) |
| 4 | 15 (41.7%) | 6 (16.7%) | 15 (41.7%) |
| 5 | 0 (0.0%) | 7 (26.9%) | 19 (73.1%) |
| Chi-square value: 48.937 | | p-value: <0.0001 | |

The analysis examining the relationship between WPOI and T-B demonstrates a robust statistical correlation ($\chi^2 = 48.937$, $p < 0.0001$), indicative of a non-random association between these histopathological parameters. In specimens exhibiting WPOI Type 2, characterized by relatively well-defined invasive tumour fronts, all cases 6 (100.0%) demonstrated minimal T-B (<5), with complete absence of moderate (5 to 10) or extensive (>10) budding (0.0%). Similarly, in WPOI Type 3 specimens, an overwhelming majority 19 (95.0%) exhibited minimal T-B (<5), with only 1 (5.0%) case displaying moderate budding (5 to 10), and none (0.0%) manifesting extensive budding (>10). A marked diversification in the pattern emerged with WPOI score 4, wherein equal proportions 15 (41.7%) demonstrated minimal (<5) and moderate (5 to 10) T-B, while 6 (16.7%) cases exhibited extensive budding (>10). The most

aggressive invasion pattern, WPOI Type 5, displayed a stark contrast to lower scores, with a predominance of moderate budding (5 to 10) in 19 (73.1%) cases, extensive budding (>10) in 7 (26.9%) cases, and complete absence of minimal budding 0 (0.0%). This progressive increase in T-B frequency and intensity with advancing WPOI scores suggests a synchronous evolution of invasive patterns and T-B phenomena, potentially reflecting coordinated molecular mechanisms underlying O-SCC progression.

***Table 27: Association Between Tumour Stroma Ratio (TSR) and CAF Score***

| **TSR** | **CAF score** | | |
|---|---|---|---|
| | **1 (n=3)** | **2 (n=36)** | **3 (n=49)** |
| <50 | 0 (0.0%) | 15 (25.4%) | 44 (74.6%) |
| ≥50 | 3 (10.3%) | 21 (72.4%) | 5 (17.2%) |
| Chi-square value: 28.077 | | p-value: <0.0001 | |

The relationship between TSR) and CAF score reveals a statistically significant association ($\chi^2 = 28.077$, $p < 0.0001$), suggesting a non-stochastic interrelationship between stromal proportion and fibroblastic activation. In specimens characterized by TSR < 50, indicating stroma rich, there was a complete absence of CAF score 1 (0.0%), with CAF score 2 observed in 15 (25.4%) cases, and a marked predominance of CAF score 3 in 44 (74.6%) cases. Conversely, specimens with TSR ≥ 50, representing stroma poor, exhibited a substantially different distribution pattern: CAF score 1 was observed in 3 (10.3%) cases, while CAF score 2 predominated in 21 (72.4%) cases, and CAF score 3 was present in only 5 (17.2%) cases. These findings suggest a potential relationship between the stromal volume and CAF activity, wherein tumours with abundant stromal content (TSR < 50) demonstrate heightened CAF

activation, potentially indicating quantitative as well as qualitative aspects of tumour-stromal interactions. The preponderance of high CAF scores in stroma-dominant specimens substantiate an enhanced myofibroblastic differentiation, potentially augmenting the invasive and metastatic capabilities of O-SCC through concentrated paracrine signalling pathways and ECM remodelling activities.

***Table 28: Association Between T-B and CAF Score***

| **T-B** | **CAF score** | | |
|---|---|---|---|
| | **1 (n=3)** | **2 (n=36)** | **3 (n=49)** |
| <5 | 1 (2.5%) | 23 (57.5%) | 16 (40.0%) |
| 5 to 10 | 2 (5.7%) | 7 (20.0%) | 26 (74.3%) |
| >10 | 0 (0.0%) | 6 (46.2%) | 7 (53.8%) |
| Chi-square value: 11.555 | | p-value: 0.021 | |

The correlation analysis between T-B intensity and CAF score demonstrates a statistically significant relationship ($\chi^2 = 11.555$, $p = 0.021$), indicative of a non-random association between these histopathological parameters. In specimens exhibiting minimal T-B (<5), a predominance of moderate CAF activity was observed, with CAF score 2 identified in 23 (57.5%) cases, while CAF score 3 was detected in 16 (40.0%) specimens. Notably, only 1 (2.5%) specimen with minimal budding exhibited low CAF activity (score 1). Specimens characterized by moderate T-B (5 to 10) displayed a markedly different distribution pattern, with a substantial predominance of high CAF activity (score 3) in 26 (74.3%) cases, whereas CAF score 2 was observed in 7 (20.0%) specimens, and CAF score 1 in merely 2 (5.7%) cases. In specimens manifesting extensive T-B (>10), a complete absence of CAF score 1 (0.0%) was noted, with CAF scores 2 and 3 identified in 6 (46.2%) and 7 (53.8%) cases, respectively. This progressive increase in CAF activation scores with escalating T-B intensity

suggests a synergistic relationship between these parameters, potentially reflective of coordinated epithelial-mesenchymal interactions facilitating invasive tumour behaviour. The reciprocal enhancement of stromal activation and epithelial disaggregation at the invasive front may constitute a unified biological phenomenon underlining aggressive O-SCC phenotypes.

***Table 29: Relationship Between T-B and LNM***

| **T-B** | **LNM** | |
|---|---|---|
| | **Yes (n=47)** | **No (n=41)** |
| <5 | 20 (50.0%) | 20 (50.0%) |
| 5 to 10 | 16 (45.7%) | 19 (54.3%) |
| >10 | 11 (84.6%) | 2 (15.4%) |
| Chi-square value: 6.107 | p-value: 0.047 | |

The analysis examining the association between T-B intensity and lymph node metastatic status reveals a statistically significant relationship ($\chi^2 = 6.107$, $p = 0.047$), suggesting that T-B may serve as a histopathological predictor of nodal involvement in O-SCC. In specimens exhibiting minimal T-B (<5), an equal distribution between metastatic and non-metastatic cases was observed, with 20 (50.0%) specimens in each category. Among specimens characterized by moderate T-B (5 to 10), a slight predominance of non-metastatic cases was noted, with 19 (54.3%) specimens demonstrating absence of nodal metastasis and 16 (45.7%) exhibiting nodal involvement. The most striking differential pattern emerged in specimens with extensive T-B (>10), wherein a substantial

predominance of metastatic cases was observed, with 11 (84.6%) specimens demonstrating lymph node involvement, compared to merely 2 (15.4%) cases without nodal metastasis. This progressive increase in the proportion of metastatic cases with escalating T-B intensity underscores the potential utility of T-B as a histopathological biomarker for nodal metastasis risk stratification. The marked predilection for lymph node involvement in specimens with extensive budding suggests that the disaggregation of epithelial cells at the invasive tumour front may facilitate LVI, thereby promoting metastatic dissemination via lymphatic channels. This observation corroborates previous investigations implicating T-B as a manifestation of partial EMT conducive to enhanced cellular motility and metastatic propensity.

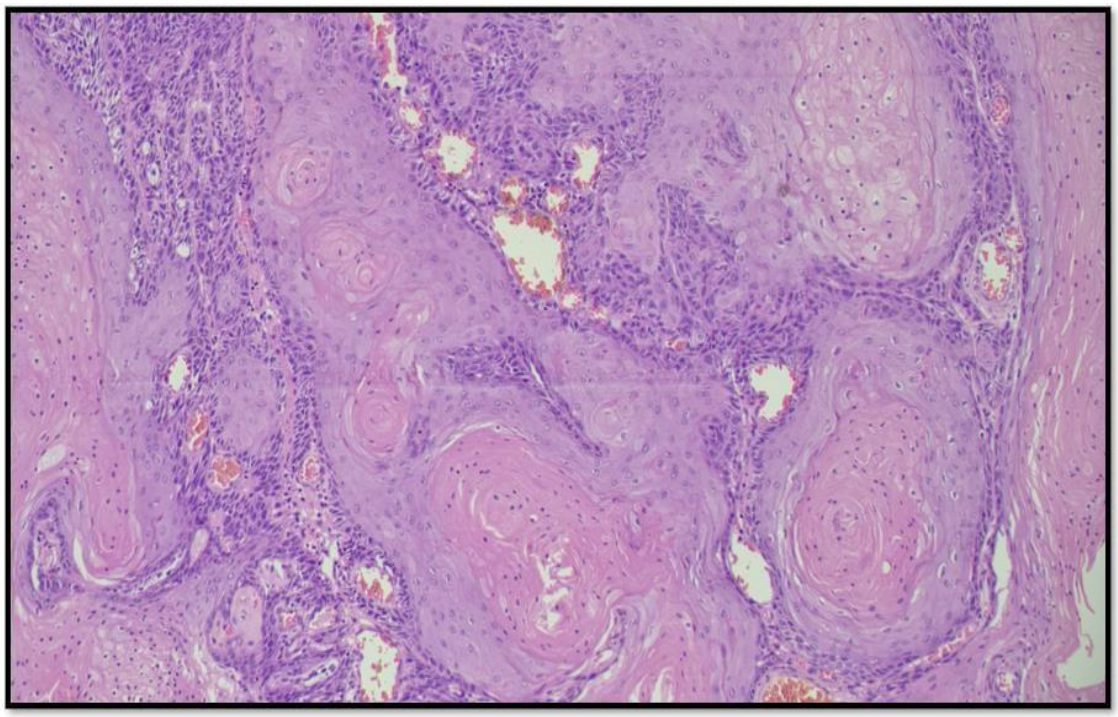


Figure 14: Microphotograph showing Well Differentiated OSCC (Original magnification, 200x)

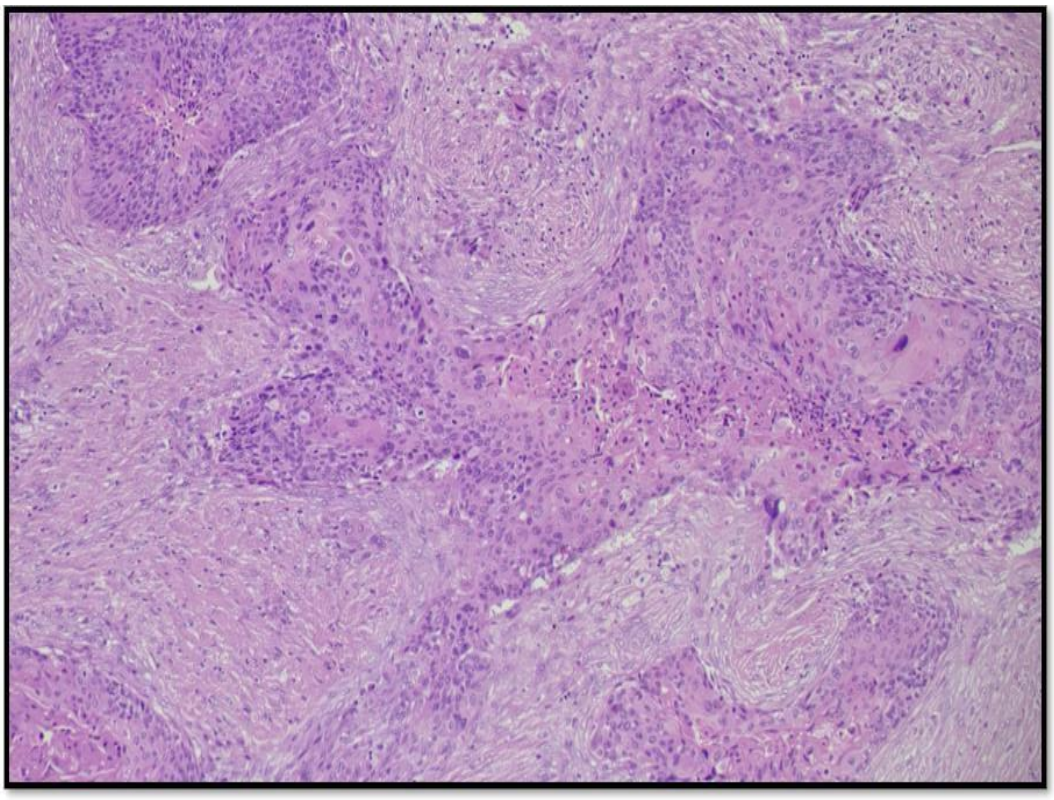


Figure 15: Microphotograph showing Moderately Differentiated OSCC (Original magnification, 100x)

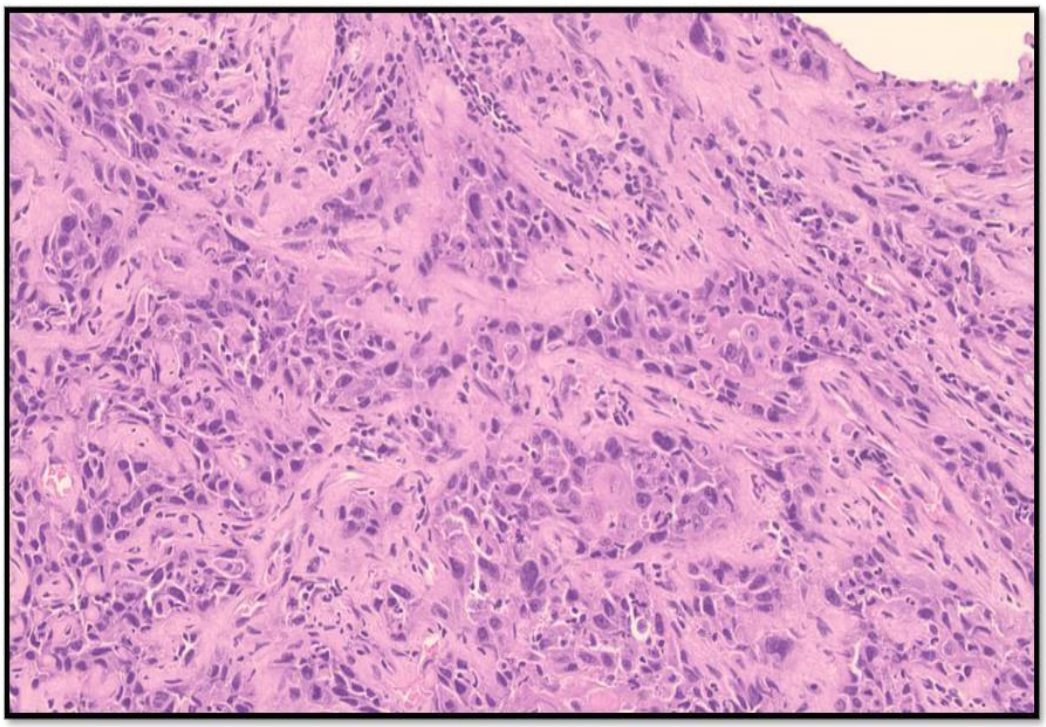


Figure 16: Microphotograph showing Poorly Differentiated OSCC (Original magnification, 400x)

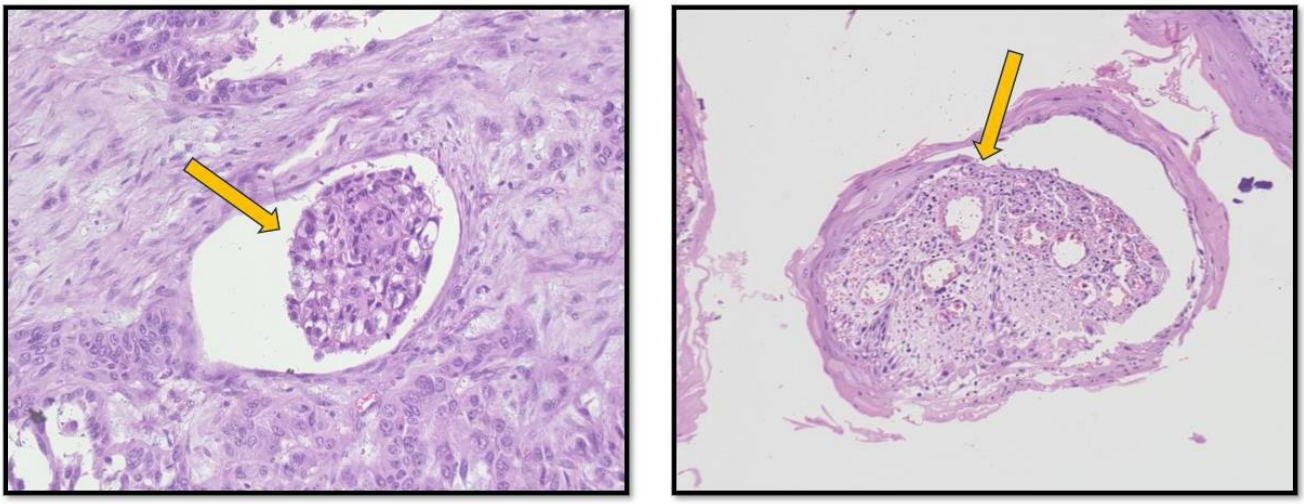


Figure 17: Microphotographs showing evidence of LVI (Original magnification, 400x)

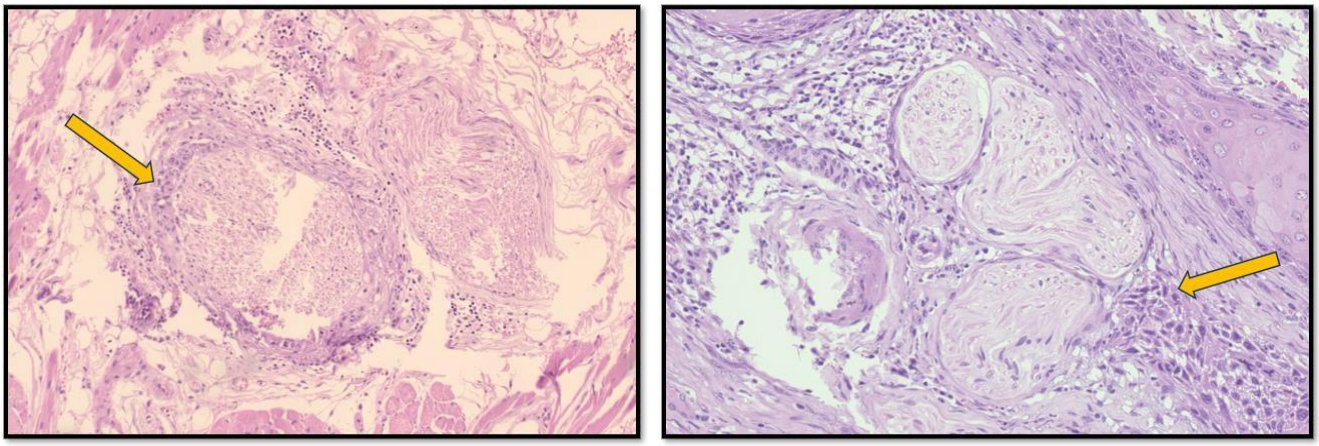


Figure 18: Microphotographs showing evidence of PNI (Original magnification, 200x)

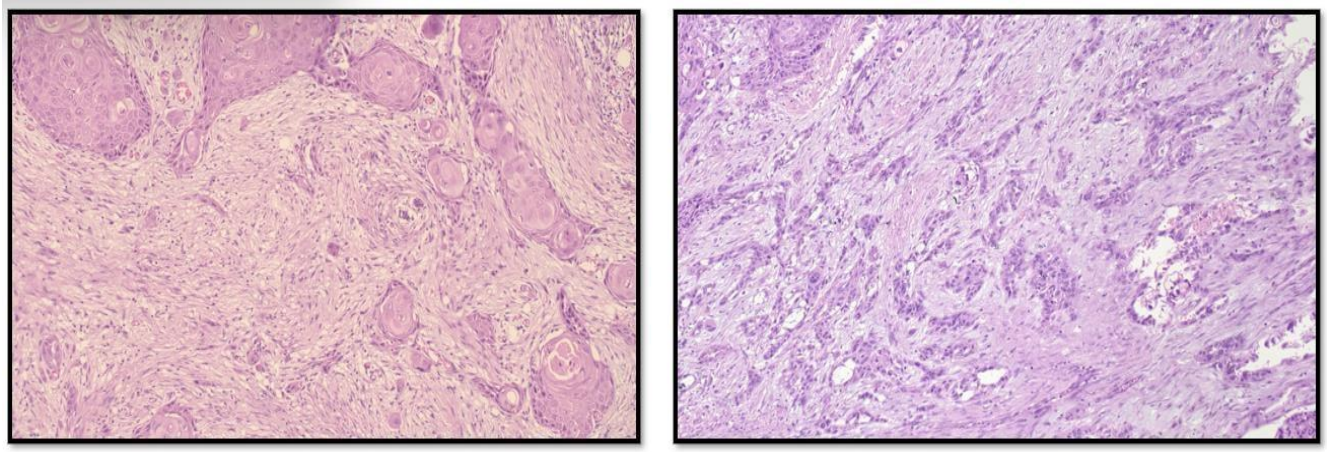


Figure 19: Microphotographs showing low TSR (stroma rich) (Original magnification, 100x)

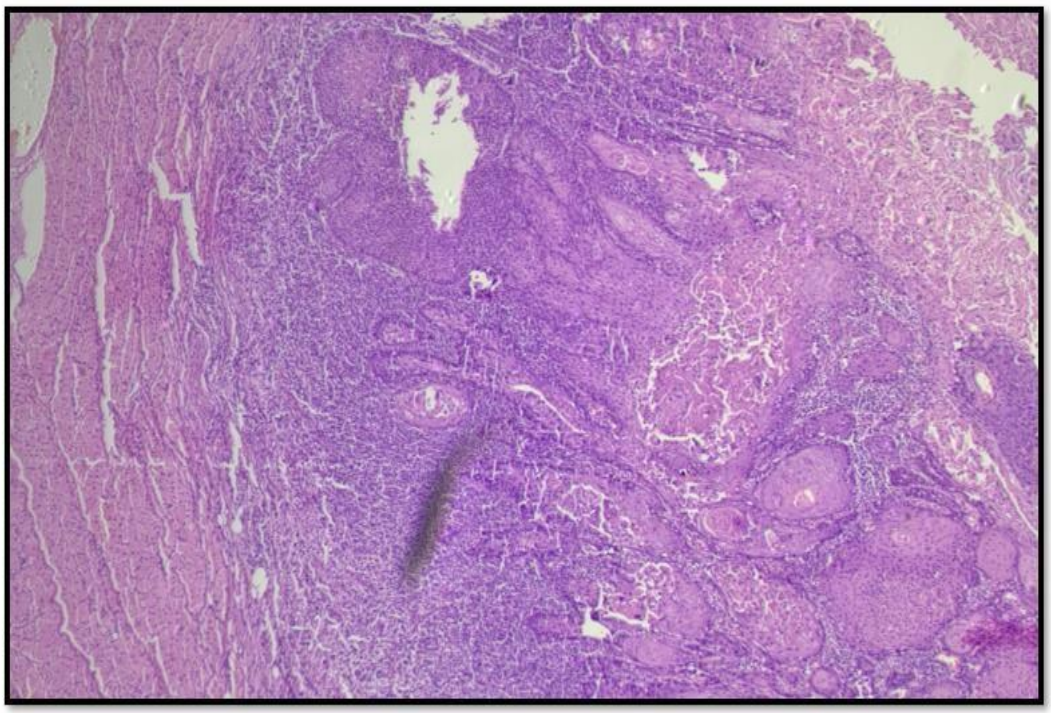


Figure 20: Microphotograph showing WPOI Type 3 (Original magnification, scanner view)

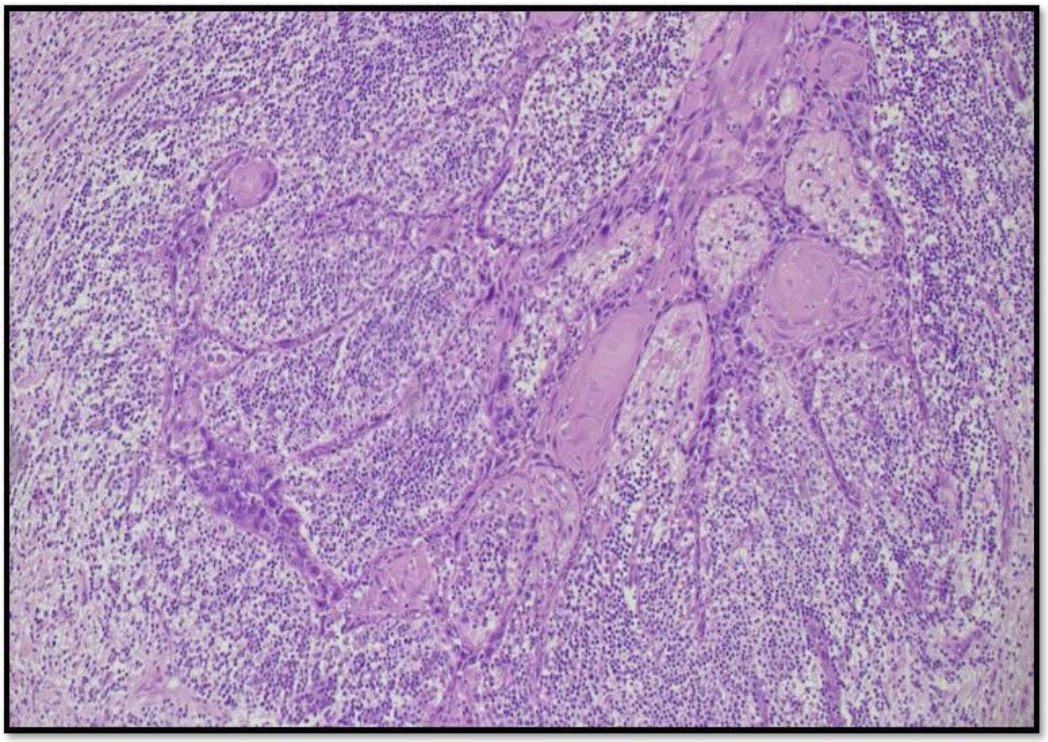


Figure 21: Microphotograph showing WPOI Type 4(Original magnification, scanner view)

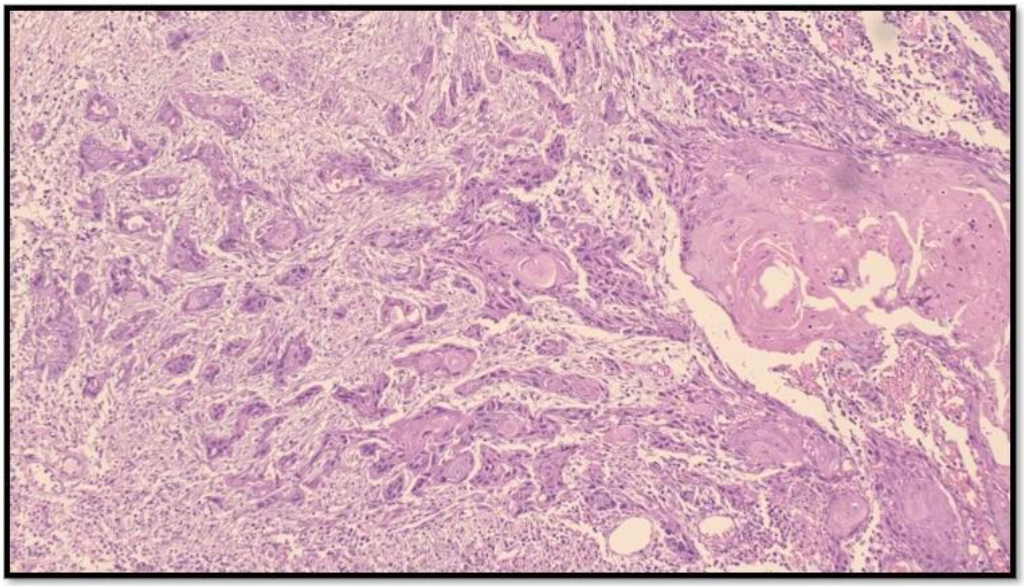


Figure 22: Microphotograph showing WPOI Type 5 (Original magnification, 100x)

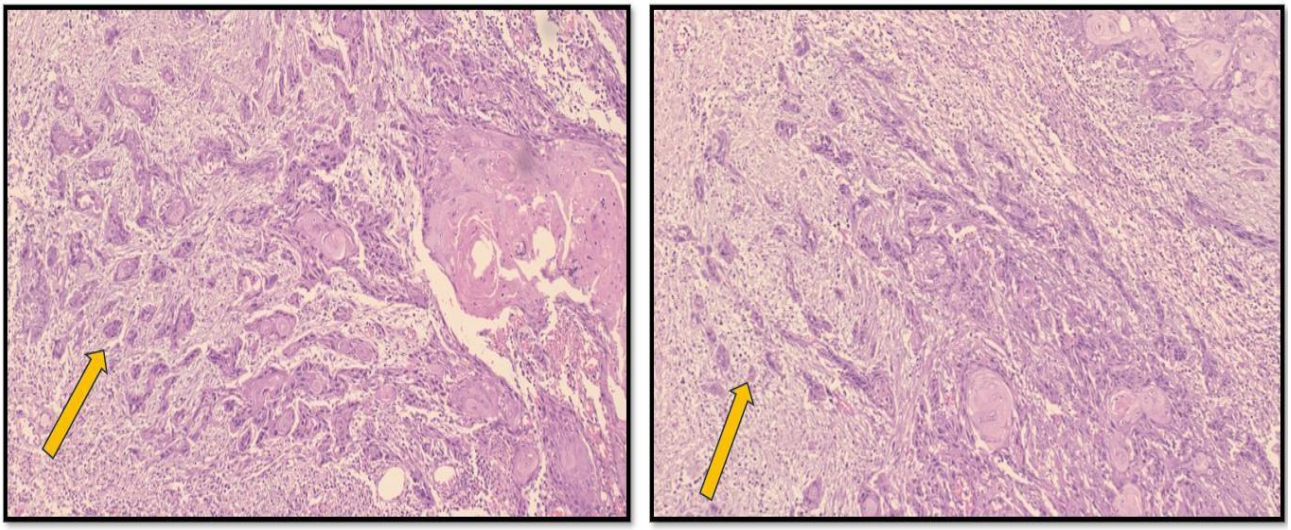


Figure 23: Microphotographs showing tumor buds (Original magnification, 100x)

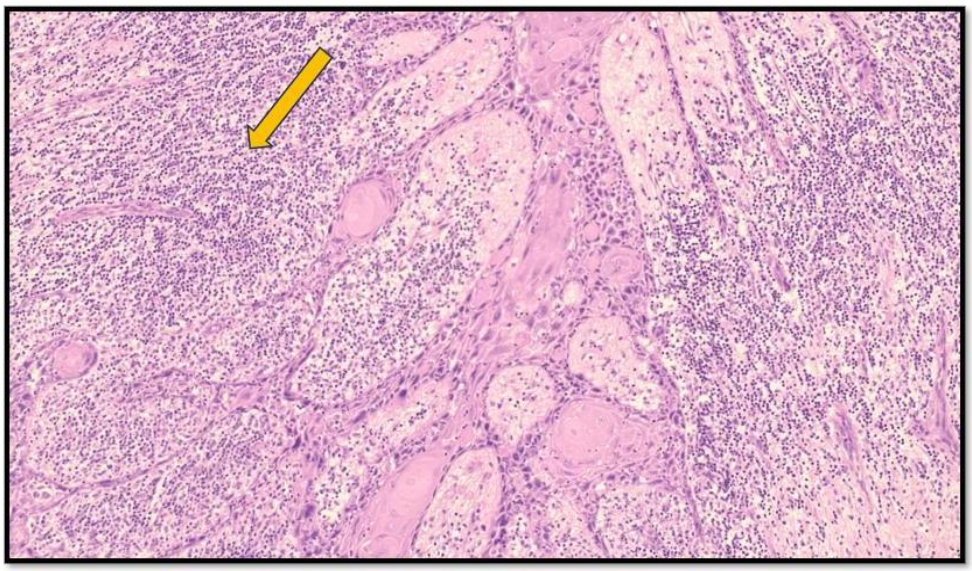


Figure 24: Microphotograph showing intratumoral moderate TILs (Original magnification, 100x)

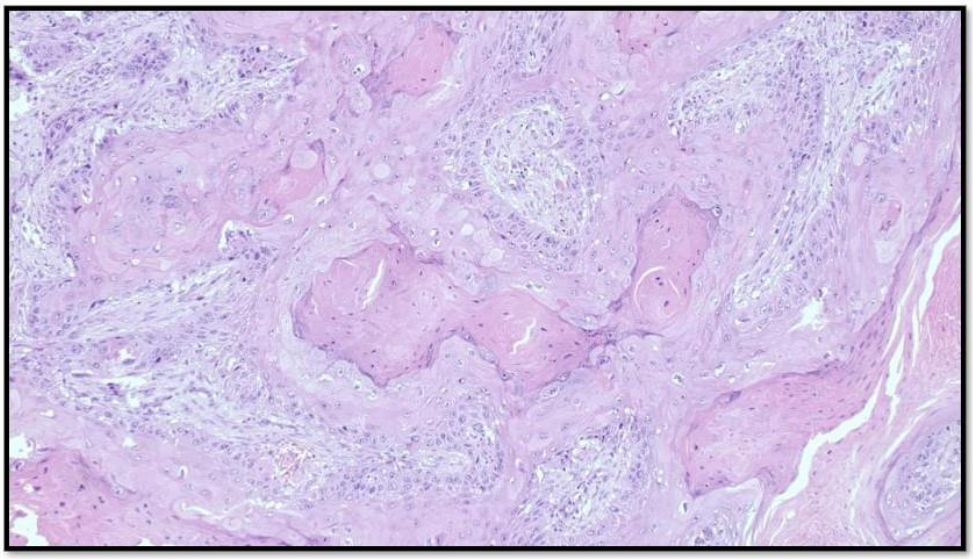


Figure 25 : Microphotograph showing tumor involvement in bone (Original magnification, 200x)

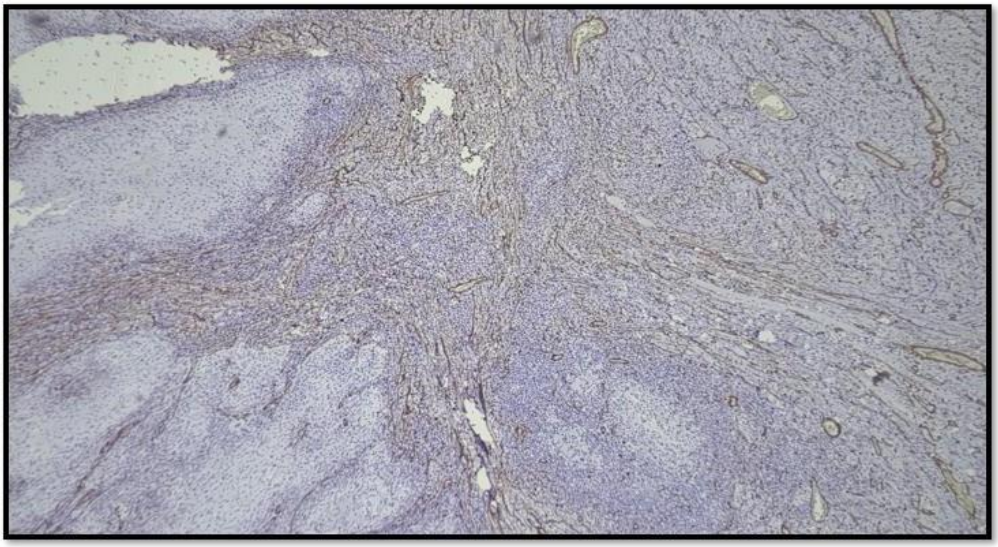


Figure 26: Microphotograph showing CAF score 2 (Original magnification, scanner view)

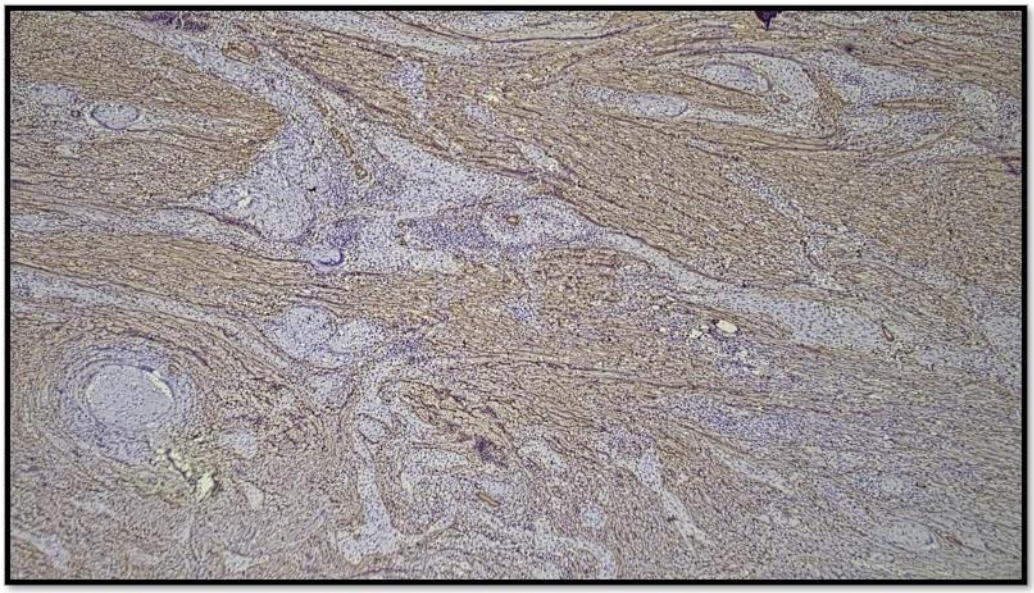


Figure 27: Microphotograph showing CAF score 3 (Original magnification, 200x)

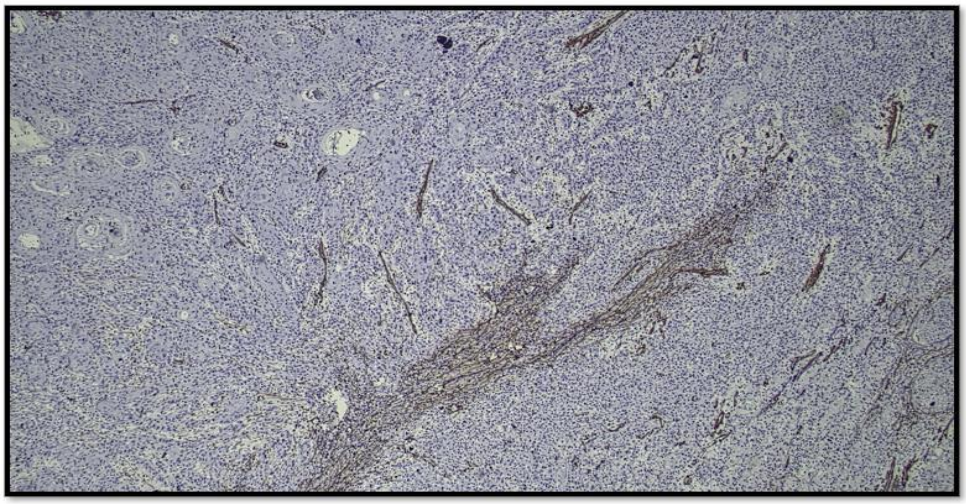


Figure 28: Microphotograph showing focal pattern of distribution of CAF (Original magnification, 100x)

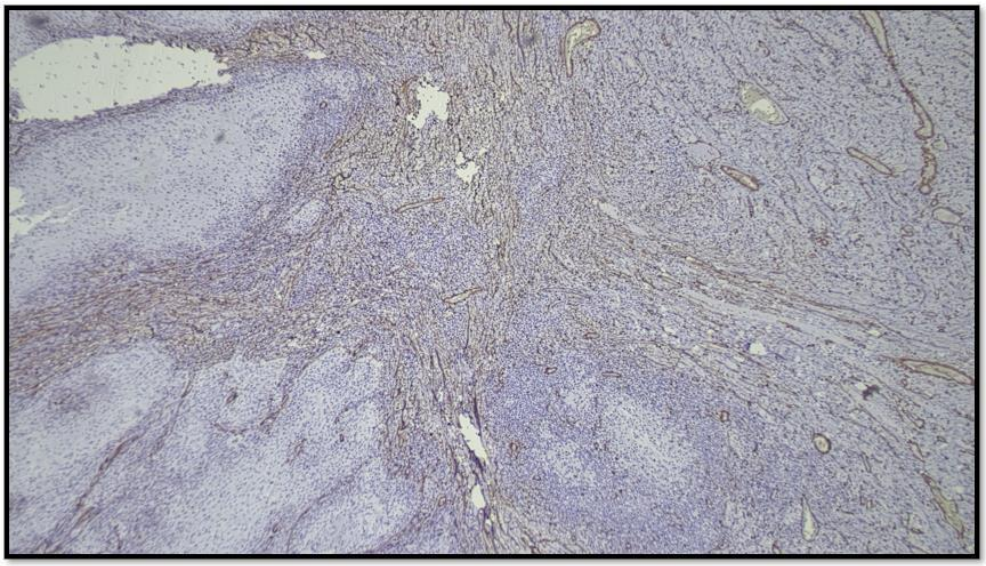


Figure 29: Microphotograph showing network pattern of distribution of CAF (Original magnification, x100)

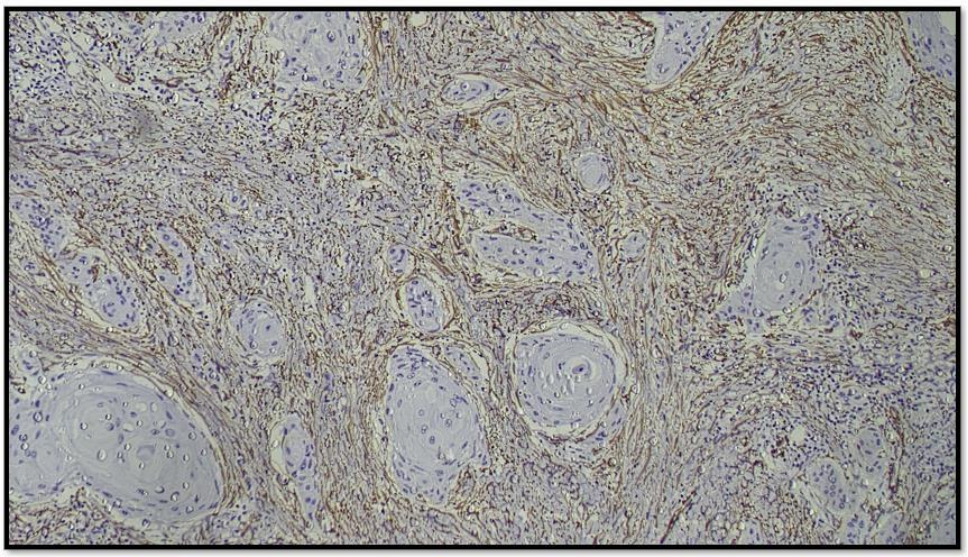


Figure 30: Microphotograph showing spindle pattern of distribution of CAF (Original magnification, x200)

# DISCUSSION

The female preponderance (69.3%) in our study contradicts global epidemiological patterns of O-SCC, which typically show male predominance. Bray et al. reported in GLOBOCAN 2022 estimates that oral cancer has higher incidence rates in males worldwide.[1] This gender disparity in our cohort may reflect region-specific risk factor exposure patterns, particularly related to cultural practices of tobacco use among females in the study population. Borse et al. noted similar gender variations in certain regions of India where tobacco chewing is common among women.[2]

The anatomical distribution of O-SCC in our cohort, with buccal mucosa representing 50% of cases, reflects regional variations in O-SCC predilection sites, potentially influenced by specific risk factors prevalent in the study population. This differs from Western populations, where tongue and floor of mouth are typically more common sites. Fatima et al. analysed 121 O-SCC cases from Pakistan and similarly found buccal mucosa as the predominant site (56.2%), attributing this to prevalent smokeless tobacco habits in the region.[15]

This comprehensive analysis of CAFs in O-SCC reveals several significant findings regarding CAF expression patterns, their morphological distribution, and associations with clinicopathological parameters, particularly LNM and pathologicl TNM staging.

This study demonstrates a predominance of high CAF expression (score 3) in 55.7% of O-SCC cases, with network pattern being the most prevalent architectural arrangement (38.6%), followed by focal and spindle patterns (30.7% each). When examining the relationship between CAF score and LNM, we found no statistically significant association (p=0.758), suggesting that the mere presence and quantity of CAFs may not directly predict nodal involvement. This finding contrasts with Fujii et al. who reported a significant correlation between CAF density and LNM in 108 O-SCC patients from Japan,

where higher CAF expression was associated with increased likelihood of nodal involvement ($p<0.001$).[4] The discrepancy could potentially be explained by differences in the TME heterogeneity, patient demographics, and even the methodological variations in CAF quantification.

While the CAF quantity (score) showed no significant association with LNM, CAF architectural pattern demonstrated a clear distributional variation approaching statistical significance ($p=0.230$). Specifically, among the metastatic specimens, spindle configuration predominated (38.3%), whereas non-metastatic cases showed preference for network (41.5%) and focal (36.6%) patterns. This suggests that the spatial organization and morphological characteristics of CAFs may have greater prognostic relevance than their absolute quantity. Luksic et al. also found that the arrangement pattern of myofibroblasts in 152 O-SCC patients in Croatia was more predictive of occult regional metastasis than their density alone ($p<0.001$), with spindle-shaped myofibroblasts at the invasive front correlating with higher metastatic potential.[21]

When analysing the relationship between CAF score and TNM staging, our results revealed a progressive increase in the proportion of cases with abundant CAF expression (score 3) from early to advanced disease stages, though not reaching statistical significance ($p=0.519$). This trend aligns with findings from Bello et al. who examined 80 mobile tongue cancer specimens and observed increased CAF presence in advanced stages.[11] However, the lack of statistical significance in our study contrasts with Li et al. who reported a strong significant association between CAF density and TNM stage in 178 tongue SCC patients in China ($p<0.001$).[17] This discrepancy may reflect differences in tumour site specificity, as our cohort included multiple anatomical subsites within the oral cavity other than tongue, potentially introducing heterogeneity in CAF expression patterns across various microenvironments.

Our analysis of the CAF distribution patterns in relation to pTNM staging demonstrated a strong statistically significant association (p=0.001). A distinct progression was observed from predominantly focal patterns in early stages to network patterns in stage IVA and spindle arrangements in stage IVB. This finding suggests an evolutionary shift in CAF architectural organization with disease progression, potentially reflecting dynamic alterations in tumour-stromal interactions during cancer advancement. Zhang et al similarly documented altered CAF morphology and distribution with increasing tumour stage in 124 O-SCC specimens from Chinese patients, with distinct arrangements correlating with invasive potential and metastatic behaviour ($p<0.01$).[167]

Another finding pertains to TSR and CAF score, revealing an inverse relationship ($p<0.0001$). Specimens with low TSR<50 (stroma rich) exhibited higher CAF scores, with 74.6% showing score 3, while specimens with high TSR (stroma poor) showed lower CAF scores, with 72.4% exhibiting score 2. This suggests that CAF functionality and activity may be enhanced in tumours with abundant stroma, indicating both quantitative and qualitative aspects of tumour-stromal interactions drive aggressive behaviour. Takabatake et al. reported similar observations in 60 O-SCC specimens from Japan, noting that CAF concentration rather than absolute number correlated with invasive potential ($p<0.05$).[168]

Our study demonstrated a significant correlation between T-B intensity and CAF score (p=0.021), with increasing T-B associated with higher CAF activity. In specimens with minimal budding (<5), moderate CAF activity (score 2) predominated (57.5%), while specimens with moderate budding (5-10) showed high CAF activity (score 3) in 74.3% of cases. This finding suggests a synergistic relationship between CAFs and T-B, potentially mediated through EMT mechanisms. Similar associations were reported by Ko et al. who found that CAF index significantly correlated with T-B in 112 O-SCC patients in

Taiwan ($p<0.001$), proposing that CAFs facilitate collective cell migration at the invasive front.[16]

Furthermore, T-B demonstrated a significant association with LNM in our study ($p=0.047$), with 84.6% of specimens exhibiting extensive budding (>10) showing nodal involvement. This progressive increase in metastatic cases with escalating T-B intensity underscores the potential utility of T-B as a histopathological biomarker for nodal metastasis risk stratification. Comparable findings were reported by Ramasubramanian et al. who analysed 122 O-SCC cases from South India and found that high-intensity T-B significantly predicted nodal metastasis ($p<0.001$).[14]

The analysis of WPOI and T-B revealed a strong correlation ($p<0.0001$), with more aggressive invasion patterns (WPOI Types 4 & 5) associated with higher T-B. In specimens with WPOI Type 5, 73.1% exhibited moderate budding and 26.9% showed extensive budding, with complete absence of minimal budding. This correlation suggests that the invasion pattern and T-B represent interrelated aspects of tumour progression, potentially driven by common molecular mechanisms. Khan et al. similarly found a significant association between pattern of invasion and other histopathological parameters in 53 O-SCC cases from India ($p<0.05$), supporting the concept that invasion patterns reflect fundamental biological properties of tumour aggressiveness.[23]

The morphological characterization of CAFs revealed distinctive architectural arrangements with potential implications for tumour-stromal interactions. The predominance of network pattern (38.6%) suggests an extensive interconnected CAF framework that may facilitate paracrine signalling and mechanical support for tumour progression. Kellermann et al. demonstrated in 83 O-SCC patients from Brazil that specific CAF arrangements correlated with clinical outcomes ($p=0.002$), with network-like patterns associated with poorer prognosis.[20] Dourado et al. observed various architectural patterns of CAFs in 30 O-SCC

specimens, noting that network arrangements predominated in more aggressive tumours ($p<0.05$).[9]

Our findings regarding TILs revealed a predominant intermediate immunological response, with 58% of specimens exhibiting moderate TIL density. This observation, coupled with the significant CAF presence, suggests complex immunomodulatory interactions within the TME. Takahashi et al. demonstrated that CAFs promote an immunosuppressive microenvironment through the induction of pro-tumoral macrophages in 60 O-SCC specimens from Japan ($p<0.01$), highlighting the role of CAFs in immune evasion.[8] Similarly, Sun et al. reviewed evidence that chronic inflammation influences O-SCC progression through various immunosuppressive mechanisms, many of which involve CAF-mediated pathways.[19]

Keeping in mind the right dexterity of hand which conveniently allows the placement of tobacco quid in the left lower quadrant of mouth accounts to the involvement of left side of buccal mucosa and lower alveolus.

The significant association between T-B and LNM ($p=0.047$) has substantial implications for clinical decision-making, particularly in early-stage O-SCC cases where occult nodal metastasis remains a critical challenge. Our finding that 84.6% of specimens with extensive T-B (>10) exhibited nodal involvement suggests that incorporating T-B assessment into routine histopathological evaluation could improve the accuracy of predicting lymph node status. This could guide more informed decisions regarding the necessity of elective neck dissection in clinically node-negative patients, potentially reducing both under-treatment and over-treatment. Particularly in resource-limited settings where advanced imaging modalities may be less accessible, T-B assessment represents a cost-effective approach to risk stratification.

The strong association between WPOI and T-B ($p<0.0001$) further reinforces the value of detailed histo-morphological assessment of the invasive front. The

complete absence of minimal budding in specimens with WPOI Type 5 highlights how these parameters could be evaluated in conjunction to better predict aggressive behaviour. Implementing standardized reporting of both WPOI and T-B could provide clinicians with more comprehensive risk assessment tools that better reflect the biological behaviour of individual tumours, potentially allowing for more personalized treatment planning.

The relationship between TSR and CAF score ($p<0.0001$) supports the conventional understanding of stromal contributions to tumour progression and suggests both qualitative as well as quantitative assessment of CAF functionality. This finding has implications for developing targeted therapeutic strategies aimed at modulating CAF activity and depleting stromal components. Potential approaches could include inhibiting specific CAF-derived factors or disrupting CAF-tumour cell communication pathways, which might prove more effective than strategies targeting gross stromal reduction.

The correlation between CAF score and T-B intensity ($p=0.021$) underscores the complex interplay between stromal components and epithelial tumour cells at the invasive front. This could guide the development of novel therapeutic strategies that simultaneously address multiple aspects of the TME.

The predominance of intermediate TIL density in our cohort (58% with moderate TIL density) provides insights into the immune microenvironment of O-SCC and its potential relationship with CAF activity. The co-existence of substantial CAF presence with moderate immune infiltration suggests immunomodulatory interactions that could influence response to immunotherapies. Assessment of both CAF characteristics and TIL density could potentially serve as predictive biomarkers for response to immune checkpoint inhibitors, which are increasingly being incorporated into O-SCC management protocols.

The findings of this study offer several clinically significant insights that could potentially transform the management approaches for O-SCC patients. The strong correlation between CAF architectural patterns and TNM staging (p=0.001) presents an opportunity for enhancing prognostic assessment beyond conventional parameters. The progression from focal patterns in early stages to network patterns in stage IVA and spindle arrangements in stage IVB suggests that CAF pattern evaluation could serve as a complementary histopathological marker for tumour aggressiveness. This approach could potentially identify patients at higher risk for disease progression who might benefit from more aggressive therapeutic strategies despite otherwise favourable conventional prognostic indicators.

Hence by combining all our findings, it can be suggested that routine immunohistochemical staining for α-SMA to identify CAFs, coupled with detailed assessment of their distribution patterns, could significantly enhance the prognostic information derived from conventional histopathological examination. This approach could be particularly valuable in settings where advanced molecular testing is limited, offering an accessible method to improve risk stratification.

Our observation regarding CAF morphological patterns and their association with metastatic potential suggests that detailed characterization of CAF architecture could enhance current histopathological assessments. The predominance of spindle configuration in node metastatic specimens (38.3%) compared to network pattern predominance in non-metastatic cases (41.5%) indicates that specific CAF arrangements may facilitate different aspects of tumour progression. Incorporating CAF pattern assessment into pathology reporting could provide additional information regarding metastatic risk, potentially influencing surveillance strategies and adjuvant therapy decisions.

Additionally, the significant correlations between CAF-related parameters and established prognostic factors (LNM, TNM staging, WPOI, T-B) suggest that

CAF assessment could be integrated into existing prognostic models to enhance their predictive accuracy. This could potentially lead to the development of composite scoring systems that incorporate both conventional clinicopathological parameters and detailed CAF characterization to better guide treatment decisions.

Given the accessibility and relatively low cost of IHC compared to molecular techniques, implementation of routine CAF assessment in O-SCC specimens represents a feasible approach to enhancing prognostic information in various clinical settings. Standardization of CAF evaluation methodologies would be essential to ensure consistency and reproducibility, potentially leading to the development of consensus guidelines for CAF assessment in O-SCC similar to those established for other histopathological parameters.

# CONCLUSION

The findings of this study show a strong association between pTNM staging as well as CAF morphological features, implying the important function of CAFs in O-SCC development. Especially, the spindle arrangement dominated in later stages of TNM staging system—mostly Stage IVB—signifying its interaction with hostile disease phenotypes. Thus, the correlation between TSR and CAF expression clarifies the qualitative features of stromal-tumour connections going beyond simple volumetric considerations in O-SCC cases. Moreover, the findings of this study reveal significant relationships between T-B with intensity, CAF scores, and T-B intensity with LNM, thereby clarifying possible mechanical routes behind metastatic spread.

# SUMMARY

This analytical study looked at the relationship in the hospital between CAFs, LNM, and pTNM staging in O-SCC cases diagnosed. This study looked at 88 verified histologically O-SCC patients from September 2022 to December 2023. These cases were from patients who had undergone surgical excision at R.L. Jalappa Hospital.

Using specimens categorized using the Kellermann et al. 2007 grading method based on percentage of α-SMA stained cells (Score 1: <1%, Score 2: 1-50%, Score 3: >50%), Alpha-Smooth Muscle Actin (α-SMA) IHC was used to identify CAFs (focal, network, spindle).

Comprising mostly female patients (69.3%) with a mean age of 56.97 years, the research population. The most common location of neoplastic origin (50%), buccal mucosa was followed by left-sided lesions predominating (60.2%). The bulk of cases—80.7%—were well-differentiated tumours histologically. Following a preponderance of high expression (Score 3) in 55.7% of instances, the quantitative analysis of CAFs indicated moderate expression (Score 2) in 40.9% of cases, with minimum expression (Score 1) detected in only 3.4% of specimens. In 38.6% of instances, CAFs showed a network structure with focal and spindle patterns equally represented at 30.7% each. Their morphological characterisation revealed With a TSR of less than 50 most cases (67%), stromal predominance was indicated.

Although CAF score and LNM (p=0.758) or CAF distribution pattern and lymph node status (p=0.230) showed no statistically significant correlation, CAF distribution patterns and pTNM staging (φ2=26.716, p=0.001) clearly showed a significant link. Early-stage disease (I-II) mostly showed localized CAF distribution; subsequent stages (III, IV) showed a change toward network and spindle patterns; stage IVB showed spindle organization in 77.8% of patients.

With 84.6% of specimens demonstrating considerable T-B associated with nodal involvement, further significant relationships were found between TSR and CAF score ($p<0.0001$), WPOI and T-B ($p=0.0001$), and T-B intensity and LNM ($p=0.047$).

These results imply that whereas CAF density by itself would not be able to forecast nodal metastases, architectural structure of CAFs greatly corresponds with disease stage and could thus affect tumour development. The contradictory negative association between stromal volume and CAF activity supports qualitative rather than quantitative factors of tumour-stromal interactions because cancers with less stromal content show increased CAF activation. Moreover, the strong correlation between T-B intensity and LNM emphasizes the possible use of T-B as a histopathological biomarkers for risk categorization of nodal metastases. These findings taken together show the intricate interaction between CAFs, TME, and disease development in O-SCC with consequences for prognosis and maybe therapeutic targeting of the tumour-stroma interface.

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# ANNEXURE-I

## PATIENT INFORMATION SHEET

**STUDY TITLE: A STUDY ON CANCER ASSOCIATED FIBROBLAST USING ALPHA SMOOTH MUSCLE ACTIN IMMUNOHISTOCHEMISTRY IN ORAL SQUAMOUS CELL CARCINOMA.**

**PLACE OF STUDY: Department of Pathology, Sri Devaraj URS Medical College, Kolar.**

The main aim of the study is to determine proportion and intensity of immunohistochemical expression of **ALPHA- SMOOTH MUSCLE ACTIN** in Oral squamous cell carcinoma (OSCC) and to evaluate its correlation with lymph node metastasis and pathological TNM staging. You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on histopathologically diagnosed cases of OSCC in the surgical excision specimens. The specimens will be collected from the department of pathology, SDUMC, Kolar. For this study no extra tissue will be collected from you. This study is approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

**PRINCIPAL INVESTIGATOR:**

**Name: Dr.AmbikaKunhikannan**

**Phone no: 8904877946**

# ANNEXURE-II

## INFORMED CONSENT FORM

**STUDY TITLE:** A STUDY ON CANCER-ASSOCIATED FIBROBLAST USING ALPHA SMOOTH MUSCLE ACTIN IMMUNOHISTOCHEMISTRY IN ORAL SQUAMOUS CELL CARCINOMA.

I, _____have read /have been read to me, the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

I have had my opportunity to ask my questions regarding various aspects of the study and my questions have been answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

(subject)

Place:

Name and signature / thumb impression : Date:

(Witness/Parent/ Guardian/ Husband)

Place:

## ರೋಗಿಯ ಮಾಹಿತಿ ಮತ್ತು ಸಮ್ಮತಿ ಪತ್ರ

**ಕ್ರಮ ಸಂಖ್ಯೆ :**

**ರೋಗಿಯ ಹೆಸರು :**

**ಮೊಬೈಲ್ ನಂಬರ್** :

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: ಓರಲ್ ಸ್ಕ್ವಾಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಆಲ್ಫಾ ಸ್ಟೂತ್ ಮಸಲ್ ಆಕ್ಟಿನ್ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಸ್ಟ್ರಿ ಬಳಸಿಕೊಂಡು ಕ್ಯಾನ್ಸರ್ ಸಂಬಂಧಿತ ಫೈಬ್ರೊಬ್ಲಾಸ್ಟ್‌ನ ಅಧ್ಯಯನ.

ಅಧ್ಯಯನ ತಾಣ: ಆರ್.ಎಲ್.ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ

ಈಕೆಳಗೆ ರುಜು ಮಾಡಿರುವ ನಾನು, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು, ಅಧ್ಯಯನ ನಡೆಸಲು ಮತ್ತು ಈ ಸಮ್ಮತಿ ನಮೂನೆಯ ಅಂಶಗಳಂತೆ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಬಹಿರಂಗಪಡಿಸುವ ಒಪ್ಪಿಗೆ ನೀಡಿರುತ್ತೇನೆ.

ನನಗೆ ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶ ಹಾಗು ಗೋಪ್ಯತೆಯ ವಿಚಾರವನ್ನು ನನ್ನ ಭಾಷೆಯಾದ ಕನ್ನಡದಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ.

ಈ ಅಧ್ಯಯನದ ಕುರಿತಾದ ನನ್ನ ಎಲ್ಲ ಪ್ರಶ್ನೆಗಳಿಗೂ ಸಮಾಧಾನಕರ ಉತ್ತರ ನನಗೆ ದೊರಕಿರುತ್ತದೆ. ಎಲ್ಲ ಮಾಹಿತಿಗಳು ಸಂಶೋಧಗೆಗಾಗಿಯೇ ಬಳಸಲಾಗುವುದು.

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ವಿಷಯದ ಗುರುತನ್ನು ಸಹ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ಈ ಅಧ್ಯಯನದಿಂದ ನನ್ನ ಜೀವಕ್ಕೆ ಯಾವುದೇ ಹಾನಿ ಇರುವುದಿಲ್ಲ ಮತ್ತು ಹೆಚ್ಚು ಅನುಕೂಲಕರವಾಗಿದೆ ಎಂದು ನನಗೆ ಅರ್ಥವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಾಯನಕ್ಕೆ ಬೇಕಾಗುವ ಎಲ್ಲಾ ಖಚು೯ವೆಚ್ಚವನ್ನು ಸಂಶೋದಕಿಯಾಗಿ ನಾನು ಭರಿಸುತ್ತೇನೆ, ಹಾಗೂ ಇದಕ್ಕಾಗಿ ನಿಮಗೆ ಯಾವುದೇ ರೀತಿಯ ಖಚು೯ ನಿಮಗೆ ಬರುವುದಿಲ್ಲ.

ನಾನು ಯಾವಾಗ ಬೇಕಾದರೂ ಈ ಅಧ್ಯಯನದಿಂದ ಹೊರನಡೆಯಬಹುದು ಮತ್ತು ನನಗೆ ಯಾವುದೇ ರೀತಿಯ ಅಧಿಕ ಖರ್ಚಾಗಿರುವುದಿಲ್ಲವೆಂದು ನಾನು ಒಪ್ಪಿಕೊಂಡಿರುತ್ತೇನೆ.

**ರೋಗಿಯ ಹೆಸರು ಮತ್ತು ರುಜು**/ಬೆರಳುಗುರುತು

**ಸಾಕ್ಷಿಗಳ ಹೆಸರು ಮತ್ತು ರುಜು**: **1.**
**2.**

ಪ್ರಮುಖ ಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ರುಜು: ಡಾ.ಅಂಬಿಕಾ ಕುಂಞಿಕಣ್ಣನ್

ಸಂಪರ್ಕ ಸಂಖ್ಯೆ: 8904877946

## ಒಪ್ಪಿಗೆ ಮಾಹಿತಿ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: ಓರಲ್ ಸ್ಕ್ವಾಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಆಲ್ಫಾ ಸ್ಮೂತ್ ಮಸಲ್ ಆಕ್ಟಿನ್ ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿ ಬಳಸಿಕೊಂಡು ಕ್ಯಾನ್ಸರ್ ಸಂಬಂಧಿತ ಫೈಬ್ರೊಬ್ಲಾಸ್ಟ್‌ನ ಅಧ್ಯಯನ.

ಅಧ್ಯಯನ ತಾಣ: ಆರ್.ಎಲ್.ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ

ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಅಧ್ಯಯನದ ಬಗ್ಗೆ ನೀವು ಯಾವುದೇ ಪ್ರಶ್ನೆಯನ್ನು ಕೇಳಬಹುದು. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪಿಕೊಂಡರೆ, ನಾವು ನಿಮ್ಮಿಂದ (ಮಾಹಿತಿ ಪ್ರಕಾರ) ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಸಂಗ್ರಹಿಸಿದ ಈ ಮಾಹಿತಿಯನ್ನು ಪ್ರೌಢಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿಯಿತಿಯನ್ನೂ ಗೌಪ್ಯವಾಗಾರಿಸಲಾಗುವುದು ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗ ಪಡಿಸಲಾಗುವುದಿಲ್ಲಾ.ವಿಷಯದ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಪರಿಶೀಲಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ನೀವು ಸಂಸ್ಥೆಯ ಎಥಿಕ್ಸ್ ಮಿತಿಯ ಸದಸ್ಯರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತಿರಿ . ಈ ಅಧ್ಯಯನಕ್ಕೆ ಒಪ್ಪಿಗೆ ನೀಡಲು ಯಾವುದೇ ಕಡ್ಡಾಯವಿಲ್ಲ.

ನೀವು ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ ನೀವು ನೀವು ಪಡೆಯುವ ಕಾಳಜಿ ಈ ಬದಾಲಾಗುವುದಿಲ್ಲ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಕೊಳ್ಳುವುದಾದರೆ ಮಾತ್ರ ಹೆಬ್ಬೆರಳು ಅನಿಸಿಕೆಗೆ ನೀವು ಸಿಹಿ ನೀಡಬೇಕಾಗಿದ.

ಮತ್ತಷ್ಟು ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಅಧ್ಯಯನ ಶೋಧಕವನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಆಗುವ ಎಲ್ಲಾ ಖರ್ಚನ್ನು ನಾನೆ ಸೇರಿಸುತ್ತೇನೆ

ಪ್ರಮುಖ ಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ರುಜು: ಡಾ.ಅಂಬಿಕಾ ಕುಂಞಿಕಣ್ಣನ್

ಸಂಪರ್ಕ ಸಂಖ್ಯೆ: 8904877946

# ANNEXURE-III

## PATIENT PROFORMA

**STUDY TITLE: A STUDY ON CANCER ASSOCIATED FIBROBLAST USING ALPHA SMOOTH MUSCLE ACTIN IMMUNOHISTOCHEMISTRY IN ORAL SQUAMOUS CELL CARCINOMA.**

Name: Hospital Number:

**Chief complaints:**

**History of presenting illness:**

**Significant Past history:**

**Personal history:**

**Local examination:**

**Type of Surgery:**

**Biopsy Number:**

**Histopathological examination of the resected specimen:**

- **Type of specimen:** Excisional biopsy
- **Procedure (select all that apply):** Excision / Glossectomy / Buccal mucosal resection / Mandibulectomy / Maxillectomy / Palatectomy with neck lymph node dissection/ Other
- **Clinical TNM stage**: T N M
- **Previous radiotherapy** : No / Not known
- **Previous chemotherapy**: No / Not known

**Primary tumour**

- **Site :**
- **Side :** Right / Left / Central
- **Tumour size :** x x (mm)

**Histological data:**

- **Histological type :** Squamous cell carcinoma
- **Maximum depth of invasion:** (mm)
- **Distance from invasive tumour to nearest mucosal margin:** (mm)
- **Lymphovascular invasion:** Yes / No
- **Perineural invasion:** Yes / No
- **Bone/cartilage invasion:** Yes / No
- **Skin involvement:** Yes / No
- **Any other:** Yes / No

**Lymph node status:**

- **Total number of lymph nodes retrieved :**
- **Number of positive nodes :**
- **Extra-capsular extension:** Present / Not identified

**pTNM Staging :**

**CAF score using alpha-SMA IHC:**

- **Distribution pattern:** focal/network/spindle
- **Proportion of staining –**
- **Immunohistochemical score -**

**Other parameters analysed:**

- **TSR (%):**
- **WPOI type:**
- **Tumor budding:**
- **TILs:**

| S.no | | Biopsy | Age | Gender | Hospital | Site | Side | Cl. TNM | Tumor size | DOI | Tumor | Nearest mucosal | LVI | PNI | Bone | Skin | Any other | Total | Positive | ECE | pT | pN | TNM | CAF | CAF | TSR | WPOI | Tumor | TILs @body |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Year | no | | | no | | | staging | (mm) | (mm) | grade | margin (mm) | + / - | '+ / - | '+ / - | '+ / - | '+ / - | Lns | Lns | '+ / - | stage | stage | Stage | score | pattern | (%) | | budding | & IF |
| 1 | 2023 | 549 | 41 | F | 196434 | Buccal mucosa | Right | cT4aN1M0 | 55x20x22 | 12 | Well-differentiated | 10(posterior) | Absent | Present | Absent | Absent | Salivary gland | 16 | 0 | Absent | 3 | 0 | III | 3 | Focal | <50 | 4 | 5 to 10 | <20 |
| 2 | 2023 | 452 | 75 | F | 194577 | Hard palate | Left | | 35x20x8 | 6 | Well-differentiated | 9(medial) | Absent | Absent | Absent | Absent | | 44 | 2 | Absent | 2 | 2b | IVA | 2 | Network | 50 | 4 | <5 | <20 |
| 3 | 2023 | 402 | 54 | F | 192098 | Buccal mucosa | Left | | 20x18x16 | 12 | Well-differentiated | 5(posterior) | Absent | Absent | Absent | Absent | | 29 | 0 | Absent | 3 | 0 | III | 3 | Spindle | <50 | 5 | >10 | 20-80 |
| 4 | 2023 | 373 | 75 | F | 191678 | Buccal mucosa | Right | | 25x20x12 | 8 | Well-differentiated | 8(medial) | Absent | Absent | Absent | Absent | | 12 | 0 | Absent | 2 | 0 | II | 3 | Network | <50 | 4 | 5 to 10 | <20 |
| 5 | 2023 | 308 | 73 | F | 89536 | Buccal mucosa | Left | cT4aN2bM0 | 21x18x8 | 6 | Well-differentiated | 8(posterior) | Absent | Absent | Absent | Absent | | 32 | 2 | Absent | 2 | 2b | IVA | 2 | Network | <50 | 5 | >10 | <20 |
| 6 | 2023 | 292 | 66 | M | 182736 | Buccal mucosa | Left | T2N1M0 | 28x25x10 | 9 | Well-differentiated | 2(anterior) | Absent | Absent | Absent | Absent | | 29 | 0 | Absent | 2 | 0 | II | 2 | Spindle | >50 | 3 | <5 | 20-80 |
| 7 | 2023 | 245 | 62 | F | 726902 | Hard palate | Left | | 30x28x8 | 6 | Well-differentiated | 6(anterior) | Absent | Absent | Absent | Absent | | 44 | 4 | Absent | 2 | 1 | III | 3 | Network | <50 | 4 | >10 | <20 |
| 8 | 2023 | 203 | 34 | F | 181490 | Buccal mucosa | Left | | 22x18x6 | 4 | Well-differentiated | 5(superior) | Absent | Present | Absent | Absent | | 28 | 1 | Absent | 2 | 1 | III | 2 | Focal | <50 | 4 | >10 | <20 |
| 9 | 2023 | 481 | 45 | M | 180512 | Lateral border of tongue | Left | | 10x22x8 | 6 | Well-differentiated | 5(posterior) | Absent | Absent | Absent | Absent | | 20 | 0 | Absent | 2 | 0 | II | 3 | Network | <50 | 3 | <5 | <20 |
| 10 | 2023 | 60 | 75 | M | 58070 | Lower gingivo buccal sulcus | Left | | 35x15x5 | 6 | Well-differentiated | 7(anterior) | Absent | Present | Absent | Absent | | 22 | 0 | Absent | 2 | 0 | II | 3 | Focal | <50 | 3 | 5 to 10 | <20 |
| 11 | 2023 | 49 | 69 | M | 178771 | Lateral border of tongue | Left | | 35x20x16 | 15 | Well-differentiated | 5(medial) | Absent | Absent | Absent | Absent | | 33 | 1 | Absent | 3 | 1 | III | 3 | Focal | <50 | 3 | <5 | 20-80 |
| 12 | 2022 | 3508 | 55 | M | 166089 | Buccal mucosa | Right | cT4aN1M0 | 43x27x16 | 11 | Well-differentiated | 5(superior) | Absent | Absent | Absent | Absent | | 30 | 0 | Absent | 3 | 0 | III | 3 | Network | >50 | 3 | <5 | <20 |
| 13 | 2022 | 3497 | 50 | F | 173257 | Buccal mucosa | Left | CT3N0 | 35x20x7 | 4 | Well-differentiated | 10(anterior) | Absent | Absent | Absent | Absent | | 21 | 0 | Absent | 2 | 0 | II | 3 | Network | 50 | 4 | 5 to 10 | <20 |
| 14 | 2022 | 3478 | 54 | F | 163284 | Lower gingivo buccal sulcus | Left | | 40x30x25 | 18 | Well-differentiated | 12(superior) | Absent | Present | Absent | Absent | | 15 | 1 | Absent | 3 | 1 | III | 3 | Spindle | <50 | 4 | 5 to 10 | <20 |
| 15 | 2023 | 1131 | 53 | M | 212475 | Buccal mucosa | Right | | 61x55x30 | 6 | Well-differentiated | 5(superior) | Absent | Present | Absent | Absent | Salivary gland | 19 | 1 | Absent | 3 | 1 | III | 2 | Spindle | 50 | 4 | 5 to 10 | <20 |
| 16 | 2022 | 3414 | 42 | M | 118423 | Lateral border of tongue | Right | | 31x20x12 | 11 | Well-differentiated | 5(anterior) | Absent | Absent | Absent | Absent | | 17 | 0 | Absent | 3 | 0 | III | 2 | Network | >50 | 4 | >10 | <20 |
| 17 | 2022 | 2438 | 55 | F | 130754 | Buccal mucosa | Left | | 35x38x12 | 6 | Well-differentiated | 9(posterior) | Absent | Absent | Absent | Absent | | 52 | 5 | Present | 2 | 3b | IV B | 3 | Spindle | <50 | 4 | 5 to 10 | 20-80 |
| 18 | 2022 | 3282 | 49 | M | 154030 | Lower gingivo buccal sulcus | Right | | 30x10x10 | 10 | Well-differentiated | 6(medial) | Absent | Absent | Absent | Absent | | 43 | 4 | Absent | 3 | 2b | IV A | 3 | Spindle | <50 | 5 | 5 to 10 | 20-80 |
| 19 | 2022 | 3295 | 36 | M | 157400 | Lateral border of tongue | Right | | 15x15x5 | 3 | Moderately differentiated | 6(lateral) | Absent | Absent | Absent | Absent | | 45 | 0 | Absent | 1 | 0 | I | 3 | Spindle | 50 | 5 | 5 | 20-80 |
| 20 | 2022 | 3256 | 68 | M | 163076 | Lower gingivo buccal sulcus | Left | | 40x25x15 | 14 | Well-differentiated | 10(anterior) | Absent | Absent | Absent | Absent | | 74 | 1 | Absent | 3 | 2a | IV A | 3 | Network | <50 | 3 | <5 | 20-80 |
| 21 | 2022 | 3257 | 40 | F | 163578 | Buccal mucosa | Left | | 35x15x10 | 5 | Well-differentiated | 6(superior) | Absent | Present | Absent | Absent | | 62 | 1 | Absent | 2 | 1 | III | 3 | Focal | >50 | 4 | <5 | 20-80 |
| 22 | 2022 | 3245 | 73 | F | 156561 | Lower lip | Right | T2N0M0 | 23x12x4 | 4 | Moderately differentiated | 8(posterior) | Absent | Present | Absent | Absent | | 29 | 0 | Absent | 2 | 0 | II | 2 | Network | >50 | 5 | 5 to 10 | 20-80 |
| 23 | 2022 | 3216 | 60 | F | 153301 | Dorsum of tongue | Right | | 25x15x6 | 6 | Well-differentiated | 10(lateral) | Absent | Absent | Absent | Absent | | 21 | 1 | Absent | 2 | 1 | III | 2 | Spindle | >50 | 4 | <5 | 20-80 |
| 24 | 2022 | 3191 | 54 | F | 154922 | Buccal mucosa | Left | | 20x20x9 | 6 | Well-differentiated | 6(superior) | Absent | Absent | Absent | Absent | | 47 | 1 | Absent | 2 | 1 | III | 2 | Focal | >50 | 4 | <5 | <20 |
| 25 | 2022 | 3192 | 60 | F | 153914 | Lower gingivo buccal sulcus | Left | | 35x13x9 | 6 | Well-differentiated | 10(posterior) | Absent | Absent | Present | Absent | | 23 | 1 | Absent | 2 | 1 | III | 2 | Focal | <50 | 5 | >10 | 20-80 |
| 26 | 2022 | 3193 | 49 | F | 15399 | Buccal mucosa | Left | | 40x40x15 | 12 | Well-differentiated | 6(posterior) | Absent | Absent | Absent | Present | | 21 | 4 | Absent | 4a | 2c | IVA | 3 | Network | <50 | 3 | <5 | <20 |
| 27 | 2022 | 3149 | 70 | F | 157151 | Floor of mouth | Central | | 60x50x30 | 5 | Moderately differentiated | 12(posterior) | Present | Absent | Present | Absent | | 73 | 7 | Absent | 4a | 2c | IVA | 3 | Spindle | <50 | 5 | 5 to 10 | <20 |
| 28 | 2022 | 3080 | 55 | M | 162477 | Upper gingivo buccal sulcus | Left | | 30x19x10 | 7 | Moderately differentiated | 5(posterior) | Absent | Absent | Absent | Absent | | 30 | 1 | Absent | 2 | 1 | III | 2 | Network | >50 | 3 | <5 | <20 |
| 29 | 2022 | 3174 | 70 | F | 158962 | Buccal mucosa | Left | T4aN2bM0 | 22x15x6 | 7 | Well-differentiated | 6(superior) | Absent | Absent | Absent | Absent | | 40 | 1 | Absent | 2 | 1 | III | 2 | Focal | <50 | 4 | <5 | <20 |
| 30 | 2022 | 3017 | 65 | M | 160313 | Buccal mucosa | Left | T3N0M0 | 40x27x12 | 7 | Well-differentiated | 9(anterior) | Absent | Present | Absent | Absent | Salivary gland | 42 | 0 | Absent | 2 | 0 | II | 2 | Focal | >50 | 3 | <5 | 20-80 |
| 31 | 2022 | 2988 | 30 | M | 157658 | Lateral border of tongue | Right | | 45x24x15 | 14 | Moderately differentiated | 5(anterior) | Absent | Present | Absent | Absent | | 29 | 3 | Present | 3 | 3b | IV B | 3 | Spindle | <50 | 5 | 5 to 10 | 20-80 |
| 32 | 2022 | 2923 | 58 | M | 156238 | Lower gingivo buccal sulcus | Left | | 27x27x12 | 12 | Well-differentiated | 9(posterior) | Absent | Absent | Present | Absent | | 28 | 0 | Absent | 4a | 0 | IV A | 3 | Spindle | <50 | 3 | <5 | <20 |
| 33 | 2022 | 2906 | 42 | F | 152124 | Buccal mucosa | Left | T4aN2bM0 | 45x44x35 | 12 | Well-differentiated | 2(posterior) | Absent | Absent | Absent | Present | | 31 | 1 | Absent | 4a | 1 | IVA | 3 | Network & spindle | <50 | 4 | >10 | 20-80 |
| 34 | 2022 | 2908 | 65 | F | 155815 | Lower retromolar trigone | Right | | 24x9x11 | 8 | Moderately differentiated | 5(anterior) | Absent | Absent | Absent | Absent | | 46 | 0 | Absent | 2 | 0 | II | 2 | Spindle | >50 | 4 | <5 | >80 |
| 35 | 2022 | 2824 | 50 | M | 151535 | Lateral border of tongue | Left | | 65x60x45 | 45 | Well-differentiated | 15(left lateral) | Absent | Absent | Absent | Absent | Muscle fibres of tongue | 72 | 5 | Absent | 4a | 2c | IV A | 2 | Network | <50 | 5 | >5 | <20 |
| 36 | 2022 | 2831 | 62 | F | 150687 | Lower gingivobuccal sulcus | Left | | 26x25 x25 | 11 | Well-differentiated | anterior involved | Absent | Absent | Absent | Absent | | 28 | 0 | Absent | 3 | 0 | III | 3 | Spindle | <50 | 5 | >5 | 20-80 |
| 37 | 2022 | 2812 | 50 | F | 153606 | Upper gingivo buccal sulcus | Left | | 28x30x12 | 12 | Moderately differentiated | 8(medial/palatal) | Absent | Absent | Absent | Absent | | 51 | 6 | Present | 3 | 3b | IV B | 3 | Network | <50 | 5 | 5 to 10 | >80 |
| 38 | 2022 | 2754 | 66 | F | 149001 | Buccal mucosa | Left | T2N1M0 | 30x15x10 | 6 | Poorly differentiated | 8(posterior) | Absent | Absent | Absent | Absent | | 26 | 2 | Absent | 2 | 2b | IVA | 2 | Network | >50 | 2 | <5 | >80 |
| 39 | 2022 | 1382 | 53 | M | 79119 | Buccal mucosa | Right | T4aN2cM0 | 50x40x25 | 25 | Poorly differentiated | 3(anterior) | Present | Present | Present | Present | Salivary gland | 19 | 5 | Present | 4a | 3b | IV B | 2 | Spindle | >50 | 5 | >10 | <20 |
| 40 | 2022 | 2596 | 50 | F | 143140 | Buccal mucosa | Right | | 44x28x17 | 12 | Well-differentiated | 6(lateral) | Absent | Absent | Absent | Absent | | 53 | 3 | Absent | 3 | 2b | IV A | 3 | Network | <50 | 5 | 5 to 10 | 20-80 |
| 41 | 2022 | 2549 | 70 | F | 139714 | Lower alveolus | Left | | 20x15x32 | 32 | Well-differentiated | 9(medial) | Absent | Present | Present | Present | Skeletal muscle | 21 | 0 | Absent | 4a | 0 | IVA | 3 | Spindle | <50 | 5 | 5 to 10 | <20 |
| 42 | 2022 | 2560 | 65 | F | 139456 | Buccal mucosa | Left | | 25x24x8 | 8 | Well-differentiated | superior involved | Absent | Absent | Absent | Absent | | 24 | 0 | Absent | 2 | 0 | II | 3 | Network | <50 | 4 | 5 to 10 | 20-80 |
| 43 | 2022 | 2561 | 46 | F | 140382 | Buccal mucosa | Left | | 50x20x6 | 2 | Well Differentiated | 5(superior) | Absent | Absent | Absent | Absent | | 27 | 0 | Absent | 3 | 0 | III | 1 | Focal | >50 | 3 | <5 | 20-80 |
| 44 | 2022 | 2466 | 70 | F | 129915 | Buccal mucosa | Left | | 35x20x15 | 12 | Well-differentiated | 10(posterior) | Absent | Absent | Absent | Present | Salivary gland | 24 | 1 | Present | 4a | 3b | IV B | 3 | Spindle | <50 | 4 | 5 to 10 | <20 |

| S.no | | Biopsy | Age | Gender | Hospital | Site | Side | Cl. TNM | Tumor size | DOI | Tumor | Nearest mucosal | LVI | PNI | Bone | Skin | Any other | Total | Positive | ECE | pT | pN | TNM | CAF | CAF | TSR | WPOI | Tumor | TILs @body |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Year | no | | | no | | | staging | (mm) | (mm) | grade | margin (mm) | + / - | '+ / - | '+ / - | '+ / - | '+ / - | Lns | Lns | '+ / - | stage | stage | Stage | score | pattern | (%) | | budding | & IF |
| 45 | 2022 | 2505 | 62 | F | 141370 | Buccal mucosa | Right | | 53x31x26 | 8 | Well Differentiated | 4(superior) | Absent | Absent | Absent | Present | | 25 | 6 | Absent | 4a | 2b | IV A | 3 | Network | <50 | 4 | >10 | <20 |
| 46 | 2022 | 2459 | 52 | F | 136515 | Buccal mucosa | Left | | 24x13x3 | 3 | Well Differentiated | 6(superior) | Absent | Absent | Absent | Absent | | 25 | 0 | Absent | 2 | 0 | II | 3 | Focal | <50 | 3 | <5 | 20-80 |
| 47 | 2022 | 1328 | 65 | F | 84236 | Buccal mucosa | Right | | 15x10x5 | 4 | Moderately differentiated | 10(posterior) | Absent | Absent | Absent | Absent | | 14 | 0 | Absent | 1 | 0 | I | 2 | Focal | <50 | 3 | <5 | 20-80 |
| 48 | 2022 | 2458 | 45 | F | 138981 | Buccal mucosa | Left | | 21x15x2 | 2 | Well Differentiated | 9(superior) | Absent | Absent | Absent | Absent | | 41 | 0 | Absent | 2 | 0 | II | 2 | Focal | <50 | 4 | <5 | 20-80 |
| 49 | 2022 | 2322 | 45 | F | 126801 | Buccal mucosa | Left | | 25x25x8 | 4 | Well Differentiated | 5(superior) | Absent | Absent | Absent | Absent | | 40 | 0 | Absent | 2 | 0 | II | 2 | Focal | >50 | 3 | <5 | 20-80 |
| 50 | 2022 | 2259 | 53 | F | 131250 | Lower gingivo buccal sulcus and alveolus | Left | | 15x7x5 | 6 | Well Differentiated | 8(superior) | Absent | Absent | Present | Absent | neovascularization | 42 | 22 | Present | 4a | 3b | IV B | 3 | Spindle | <50 | 4 | >10 | 20-80 |
| 51 | 2022 | 2260 | 60 | F | 93531 | Buccal mucosa | Right | | 20x15x9 | 10 | Well Differentiated | 4(posterior) | Absent | Absent | Absent | Present | | 37 | 0 | Absent | 4a | 0 | IV A | 2 | Network | <50 | 5 | 5 to 10 | 20-80 |
| 52 | 2022 | 2227 | 50 | M | 115561 | Lower gingivo buccal sulcus | Right | | 20x16x15 | 11 | Well Differentiated | 6(posterior) | Absent | Absent | Absent | Absent | | 35 | 2 | Absent | 3 | 2b | IV A | 3 | Spindle | <50 | 4 | <5 | 20-80 |
| 53 | 2022 | 2155 | 69 | F | 127387 | Buccal mucosa | Right | | 60x 35 x 10 | 6 | Moderately-differentiated | 3(posterior) | Absent | Absent | Present | Absent | Infratemporal fossa clearance | 51 | 1 | Absent | 4a | 1 | IV A | 3 | Spindle | <50 | 5 | >10 | <20 |
| 54 | 2022 | 2097 | 46 | M | 119614 | Retromolar trigone | Left | | 15x14x12 | 12 | Well-differentiated | 4(posterior) | Absent | Absent | Absent | Absent | | 51 | 0 | Absent | 3 | 0 | III | 3 | Network | <50 | 4 | 5 to 10 | 20-80 |
| 55 | 2022 | 2112 | 36 | F | 127022 | Buccal mucosa | Right | | 22x6x12 | 3 | Well-differentiated | 14(medial) | Absent | Absent | Absent | Absent | | 12 | 0 | Absent | 1 | 0 | I | 2 | Focal | <50 | 2 | <5 | >80 |
| 56 | 2022 | 2077 | 44 | F | 126563 | Buccal mucosa | Left | | 27x18x12 | 8 | Well-differentiated | 5(medial) | Absent | Absent | Absent | Absent | | 29 | 1 | Absent | 2 | 1 | III | 2 | Network | >50 | 4 | <5 | 20-80 |
| 57 | 2022 | 2051 | 52 | M | 124908 | Buccal mucosa | Left | | 25x14x10 | 11 | Well-differentiated | 10(superior) | Absent | Absent | Absent | Absent | | 17 | 0 | Absent | 3 | 0 | III | 3 | Network | <50 | 5 | 5 to 10 | 20-80 |
| 58 | 2022 | 1989 | 70 | M | 99508 | Hard palate | Left | | 35x20x25 | 12 | Moderately-differentiated | 23(anterior) | Absent | Absent | Absent | Absent | Left turbinate | 35 | 0 | Absent | 1 | 0 | I | 2 | Spindle | >50 | 5 | 5 to 10 | 20-80 |
| 59 | 2022 | 1822 | 50 | F | 105437 | Retromolar trigone | Left | | 45x29x10 | 8 | Well-differentiated | 4(posterior) | Absent | Absent | Absent | Absent | | 49 | 3 | Absent | 3 | 2b | IVA | 3 | Spindle | <50 | 3 | <5 | 20-80 |
| 60 | 2022 | 1876 | 65 | F | 108151 | Lower alveolus | Right | | 25x20x20 | 8 | Well-differentiated | 5(medial) | Absent | Absent | Absent | Absent | | 56 | 3 | Absent | 2 | 2b | IVA | 2 | Focal | <50 | 3 | <5 | 20-80 |
| 61 | 2023 | 1474 | 90 | F | 221367 | Buccal mucosa | Left | | 60x58x39 | 22 | Moderately differentiated | 6(anterior) | Present | Present | Present | Present | | 37 | 3 | Absent | 4a | 2b | IV A | 2 | Spindle | >50 | 5 | > 10 | 20-80 |
| 62 | 2022 | 1710 | 69 | M | 111405 | Lower gingivo buccal sulcus | Right | cT3N2bM0 | 5x3x3 | 2 | Well-differentiated | 10(posterior) | Absent | Absent | Absent | Absent | | 29 | 6 | Absent | 1 | 2b | IV A | 1 | Focal | >50 | 5 | 5 to 10 | <20 |
| 63 | 2022 | 1715 | 71 | F | 78443 | Lower gingivo buccal sulcus | Right | T4N1M0 | 14x14x7 | 2 | Well-differentiated | 5(superior) | Absent | Absent | Absent | Absent | | 14 | 1 | Absent | 1 | 1 | III | 2 | Focal | <50 | 2 | <5 | 20-80 |
| 64 | 2022 | 1731 | 64 | F | 110936 | Buccal mucosa | Left | T4N0M0 | 23x37x8 | 8 | Well-differentiated | 11(superior) | Absent | Absent | Absent | Absent | | 86 | 0 | Absent | 2 | 0 | II | 2 | Focal | <50 | 4 | <5 | 20-80 |
| 65 | 2022 | 1709 | 58 | F | 104971 | Lower alveolus | Right | | 17x11x6 | 5 | Well-differentiated | 5(medial) | Absent | Absent | Absent | Absent | | 38 | 0 | Absent | 2 | 0 | II | 1 | Focal | >50 | 5 | 5 to 10 | 20-80 |
| 66 | 2022 | 1695 | 50 | F | 110052 | Upper alveolus | Right | T4N1M0 | 25x23x7 | 8 | Well-differentiated | 4(anterior) | Absent | Absent | Absent | Absent | | 31 | 2 | Absent | 2 | 2b | IVA | 2 | Network | <50 | 4 | <5 | 20-80 |
| 67 | 2022 | 1683 | 57 | M | 105855 | Lower gingivo buccal sulcus | Left | | 50x45x23 | 15 | Well-differentiated | 6(posterior) | Absent | Absent | Absent | Present | | 41 | 1 | Absent | 4a | 1 | IVA | 3 | Spindle | <50 | 4 | 5 to 10 | <20 |
| 68 | 2022 | 1599 | 42 | F | 99070 | Buccal mucosa | Right | | 43x33x18 | 15 | Well-differentiated | 7(posterior) | Absent | Absent | Present | Absent | | 38 | 0 | Absent | 4a | 0 | IVA | 2 | Network | >50 | 4 | <5 | 20-80 |
| 69 | 2022 | 1552 | 58 | F | 96599 | Buccal mucosa | Left | | 25x12x20 | 11 | Well-differentiated | 5(posterior) | Absent | Absent | Present | Absent | | 39 | 0 | Absent | 4a | 0 | IVA | 2 | Network | >50 | 3 | <5 | 20-80 |
| 70 | 2022 | 1545 | 75 | F | 97832 | Lower alveolus | Left | | 45x30x29 | 25 | Well-differentiated | 10(medial) | Absent | Absent | Absent | Present | | 42 | 0 | Absent | 4a | 0 | IVA | 3 | Network | <50 | 4 | 5 to 10 | <20 |
| 71 | 2022 | 1512 | 65 | F | 93598 | Upper gingivo buccal sulcus | Left | | 38x25x10 | 5 | Poorly-differentiated | 5(posterior, medial) | Absent | Absent | Absent | Absent | | 27 | 0 | Absent | 2 | 0 | II | 2 | Focal | >50 | 3 | <5 | 20-80 |
| 72 | 2022 | 1535 | 67 | F | 95638 | Buccal mucosa | Left | | 18x10x8 | 5 | Well-differentiated | 4(posterior) | Absent | Absent | Absent | Absent | | 22 | 1 | Absent | 1 | 1 | III | 2 | Focal | <50 | 3 | <5 | 20-80 |
| 73 | 2022 | 1491 | 46 | F | 96528 | Dorsum of tongue | Central | T3N3bM0 | 21x20x7 | 4 | Moderately-differentiated | 5(deep resected margin) | Absent | Absent | Absent | Absent | | 28 | 3 | Present | 2 | 3b | IV B | 3 | Network | <50 | 5 | >10 | 20-80 |
| 74 | 2022 | 1418 | 49 | F | 91889 | Lower gingivo buccal sulcus | Left | | 28x11x11 | 7 | Well-differentiated | 9(medial) | Absent | Absent | Absent | Absent | | 29 | 0 | Absent | 2 | 0 | II | 3 | Network | <50 | 4 | 5 to 10 | 20-80 |
| 75 | 2022 | 1353 | 47 | F | 84903 | Buccal mucosa | Left | | 26x15x10 | 7 | Well-differentiated | 6(posterior) | Absent | Absent | Absent | Absent | | 26 | 1 | Absent | 2 | 1 | III | 3 | Focal | <50 | 2 | <5 | 20-80 |
| 76 | 2022 | 1181 | 50 | F | 84208 | Buccal mucosa | Right | T4aN1M0 | 30x20x25 | 13 | Well-differentiated | 5(posterior) | Absent | Absent | Absent | Absent | | 26 | 0 | Absent | 3 | 0 | III | 3 | Network | <50 | 5 | 5 to 10 | 20-80 |
| 77 | 2022 | 1294 | 45 | F | 79727 | Lateral border of tongue | Left | T3N1M0 | 35x20x15 | 8 | Well-differentiated | 5(posterior) | Absent | Absent | Absent | Absent | | 17 | 2 | Absent | 2 | 2b | IV A | 2 | Focal | >50 | 5 | 5 to 10 | <20 |
| 78 | 2022 | 1354 | 75 | F | 89776 | Retromolar trigone | Left | T3N1M0 | 23x15x15 | 15 | Well-differentiated | 7(posterior) | Absent | Absent | Absent | Absent | | 33 | 0 | Absent | 3 | 0 | III | 3 | Focal | >50 | 2 | <5 | 20-80 |
| 79 | 2022 | 1326 | 69 | F | 81730 | Lower gingivo buccal sulcus | Right | T4aN2bM0 | 35x15x25 | 3 | Well-differentiated | 8(anterior) | Absent | Present | Absent | Absent | | 30 | 7 | Present | 2 | 3b | IV B | 3 | Spindle | <50 | 4 | <5 | 20-80 |
| 80 | 2023 | 790 | 52 | M | 201296 | Lateral border of tongue | Right | | 22x8x9 | 3 | Moderately-differentiated | 7(ant+lat) | Absent | Absent | Absent | Absent | | 33 | 0 | Absent | 2 | 0 | II | 2 | Focal | >50 | 3 | <5 | 20-80 |
| 81 | 2023 | 943 | 50 | M | 203434 | Buccal mucosa | Right | | 49x31x40 | 23 | Well-differentiated | 6(posterior) | Absent | Absent | Absent | Absent | | 46 | 0 | Absent | 3 | 0 | III | 3 | Network | <50 | 2 | <5 | 20-80 |
| 82 | 2023 | 1088 | 55 | F | 204093 | Buccal mucosa | Right | | 90x65x53 | 18 | Well-differentiated | 8(posterior) | Absent | Absent | Absent | Present | | 28 | 24 | Present | 4a | 3b | IV B | 3 | Spindle | <50 | 4 | <5 | <20 |
| 83 | 2023 | 1496 | 60 | F | 223737 | Buccal mucosa | Left | T3N0M0 | 30x20x10 | 10 | Well-differentiated | 7(posterior) | Absent | Absent | Absent | Absent | | 27 | 0 | Absent | 2 | 0 | II | 3 | Focal | <50 | 4 | <5 | 20-80 |
| 84 | 2023 | 736 | 48 | F | 192513 | Buccal mucosa | Right | T4N1M0 | 50x30x30 | 12 | Well-differentiated | 10(superior) | Absent | Absent | Absent | Absent | | 18 | 1 | Absent | 3 | 1 | III | 2 | Network | <50 | 4 | 5 to 10 | 20-80 |
| 85 | 2023 | 737 | 58 | M | 199373 | Upper gingivo buccal sulcus | Left | T3N0M0 | 60x30x14 | 2 | Moderately-differentiated | 6(lateral) | Absent | Absent | Absent | Absent | | 20 | 0 | Absent | 3 | 0 | III | 3 | Spindle | <50 | 4 | 5 to 10 | <20 |
| 86 | 2023 | 710 | 50 | F | 195659 | Buccal mucosa | Right | T4aN1M0 | 65x30x25 | 12 | Well-differentiated | 6(superolateral) | Absent | Absent | Present | Absent | | 57 | 1 | Absent | 4a | 1 | IV A | 3 | Network | <50 | 5 | 5 to 10 | <20 |
| 87 | 2023 | 712 | 70 | M | 189714 | Lower alveolus | Left | T4N0M0 | 40x40x20 | 12 | Well-differentiated | 8(superior) | Absent | Absent | Absent | Absent | | 30 | 2 | Absent | 3 | 2a | IV A | 3 | Spindle | <50 | 5 | 5 to 10 | <20 |
| 88 | 2023 | 2407 | 55 | M | 253131 | Lower gingivo buccal sulcus | Right | T4aN2bM0 | 70x40x30 | 10 | Well-differentiated | 5(inferior) | Absent | Absent | Present | Present | Ulcerated skin covered by granulation tissue | 30 | 0 | Absent | 4a | 0 | IV A | 3 | Network | <50 | 4 | 5 to 10 | 20-80 |