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"Comparative Evaluation Of Immunohistochemical Expression Of Stoml1 And Stoml2 Proteins In Oral Squamous Cell Carcinoma"

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Abstract

INTRODUCTION-*Oral Squamous Cell Carcinoma (OSCC) remains a major global health concern, especially in regions with high tobacco and areca nut use. Despite progress in early detection and therapy, survival rates remain poor due to late diagnoses and the lack of reliable biomarkers. This study evaluates Stomatin-Like Proteins STOML1 and STOML2 in OSCC and normal oral mucosa (NOM), assessing their diagnostic and prognostic relevance.*

AIM-*To identify, quantify and compare IHC expression of Stomatin like protein-1(STOML1) and Stomatin like protein-2(STOML2) in Oral Squamous Cell Carcinoma and Normal Oral Mucosa obtained from healthy volunteers*

OBJECTIVES-*To quantify IHC expression of Stomatin like protein-1 (STOML-1) and Stomatin like protein-2 (STOML -2) IHC expression in tissue sections of Normal Oral Mucosa obtained from healthy volunteers (GROUP 1),To quantify IHC expression of Stomatin like protein-1 (STOML-1) and Stomatin like protein-2 (STOML -2) IHC expression in tissue sections of Oral Squamous Cell Carcinoma (GROUP 2),To compare and co-relate the findings within and between the groups.*

MATERIALS AND METHODOLOGY-*This retrospective case-control study analyzed 54 formalin-fixed paraffin-embedded (FFPE) tissue samples–27 OSCC cases and 27 normal oral mucosa (NOM) specimens from healthy individuals. Immunohistochemical staining was conducted using rabbit polyclonal antibodies specific to STOML1 and STOML2. Expression levels were quantified via histoscore analysis.*

RESULTS:*STOML1 and STOML2 showed elevated expression in OSCC tissues relative to normal oral mucosa (NOM). STOML1 expression was primarily observed in well-differentiated and early-stage OSCC, whereas STOML2 expression was more frequently associated with moderately differentiated tumors. ROC analysis indicated high sensitivity but low specificity for both markers in OSCC diagnosis.*

CONCLUSION:*STOML1 shows potential as a diagnostic biomarker for OSCC due to its marked overexpression in malignant tissue, though limited specificity highlights the need for complementary markers. Future research should explore the molecular pathways regulating STOML1 and STOML2 to advance targeted therapeutic strategies.*

KEYWORDS:*Oral Squamous Cell Carcinoma (OSCC), STOML1, STOML2*

INTRODUCTION-

Oral Squamous Cell Carcinoma (OSCC) constitutes the most prevalent form of oral cancer and remains a significant global health burden due to late-stage diagnosis, substantial mortality, and variable epidemiology [1]. It commonly affects anatomical sites such as the lips, tongue, buccal mucosa, floor of the mouth, and oropharynx. The incidence is notably high in South Asian countries due to tobacco and betel nut use, while HPV-associated cases are increasing in Western populations.

A key precursor to OSCC is oral epithelial dysplasia (OED), a potentially malignant disorder marked by architectural and cytological alterations [2]. The likelihood of malignant transformation rises with higher grades of dysplasia. Although histopathological evaluation is the diagnostic gold standard, biomarkers such as p53, Ki-67, and epigenetic modifiers are emerging as predictive adjuncts [3].

Stomatin-like proteins STOML1 and STOML2 have garnered interest for their roles in oncogenesis across multiple tumor types. STOML1, a lipid raft-associated protein, modulates ion channels, intracellular calcium homeostasis, and oncogenic signaling pathways including PI3K/AKT and MAPK [4]. Its overexpression is linked to proliferation, invasion, and chemoresistance in breast, colorectal, ovarian, lung, and glioblastoma tumors[5.6]. STOML2, associated with mitochondrial function, influences energy metabolism, oxidative stress response, and epithelial-mesenchymal transition (EMT) through HIF-1α and NF-κB pathways [7]. Its aberrant expression has been correlated with poor prognosis and enhanced metastatic potential in gastric, liver, pancreatic, and head and neck cancers [7]

Emerging data suggest STOML1 and STOML2 may serve not only as prognostic indicators but also as early markers of tumor initiation, with implications in ovarian, pancreatic, and colorectal cancers [8]. Their overexpression under hypoxic conditions and role in tumor adaptation underscore their potential in early cancer detection and therapeutic targeting.

Thus, this study aims to evaluate the expression of STOML1 and STOML2 in OSCC and normal oral mucosa (NOM), to assess their prognostic relevance and diagnostic utility.

MATERIAL AND METHODOLOGY-

A retrospective case-control study was conducted at the Department of Oral & Maxillofacial Pathology and Oral Microbiology, Teerthanker Mahaveer Dental College and Research Centre, Moradabad. Archival FFPE tissue specimens from 54 individuals—27 OSCC patients and 27 healthy volunteers—were analyzed. Normal oral mucosa (NOM) samples were obtained during third molar extractions, with strict exclusion criteria for systemic illness or malignancy. OSCC samples were histopathologically confirmed primary cases with complete clinical data (age, gender, site, TNM staging, and histological grade). Hematoxylin& eosin staining reconfirmed diagnoses.

Sample size was calculated using G*Power software based on an institutional OSCC incidence of 5%, desired precision of 15%, 95% confidence level, and 80% power, yielding 27 subjects per group. Convenient sampling was employed.Ethical approval was obtained from the Institutional Review Board of TMDCRC.

Aim-

To identify, quantify and compare IHC expression of Stomatin like protein-1(STOML1) and Stomatin like protein-2(STOML2) in Oral Squamous Cell Carcinoma and Normal Oral Mucosa.

Objectives-

1. To quantify IHC expression of Stomatin like protein-1 (STOML-1) and Stomatin like protein-2 (STOML -2) IHC expression in tissue sections of Normal Oral Mucosa obtained from healthy volunteers (GROUP 1)
2. To quantify IHC expression of Stomatin like protein-1 (STOML-1) and Stomatin like protein-2 (STOML -2) IHC expression in tissue sections of Oral Squamous Cell Carcinoma (GROUP 2)
3. To compare and co-relate the findings within and between the groups.

Inclusion criteria

- Group I (NOM): FFPE specimens from healthy volunteers (third molar removal)
- Group II (OSCC): Histopathologically confirmed primary OSCC cases with complete clinical data (age, gender, lesion site, TNM stage, histological grade).

Exclusion criteria

- Group I (NOM): Specimens with systemic medication history, prior malignancies, oral pathology, or severe inflammation on H&E staining.
- Group II (OSCC): Specimens with inadequate tissue volume, oral cavity metastases, or recurrent OSCC.

Histopathological and Immunohistochemical Procedures

Hematoxylin and Eosin (H&E) Staining

Four-micrometer-thick tissue sections were obtained from archival FFPE blocks using a semi-automatic microtome and mounted onto egg albumin-coated slides. Routine H&E staining was performed, followed by dehydration, xylene clearing, and DPX mounting. Slides were examined microscopically and OSCC cases were graded per WHO criteria.

Immunohistochemistry (IHC)-Slide Preparation: Frosted slides were treated with acid, acetone, and coated with 2% APES after autoclaving. **Sectioning & Mounting:** 4 µm FFPE sections were transferred onto APES-coated slides. **Staining Protocol:** Incubation at 37 °C (overnight) and 60 °C (1 hr)**,**Deparaffinization and hydration via graded isopropanol**,** Antigen retrieval in Tris-EDTA buffer (pH 9) using pressure cooker**,** Blocking endogenous peroxidase with 3% H_2O_2**,** Primary antibody incubation (rabbit polyclonal STOML1 or STOML2) for 60 min**,** Sequential application of Poly Excel Target Binder, HRP, and DAB chromogen**,** Counterstaining with Mayer's hematoxylin, dehydration, and DPX mounting**,** Positive and negative brain tissue controls were included with each batch.

Expression Detection and Quantification-STOML1 Positivity: Brown staining in nucleus, membrane, and cytoplasm**, STOML2 Positivity:** Brown staining in membrane and cytoplasm**, Quantification:** Expression intensity assessed per Maruse et al.; semi-quantitative H-score computed per Hinsch et al.: 18 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)

RESULTS AND OBSERVATION -A total of 54 paraffin-embedded tissue blocks were analyzed, comprising Group 1 (n=27, normal oral mucosa from healthy volunteers) and Group 2 (n=27, oral squamous cell carcinoma). Sections of 4 µm thickness were prepared and stained with H&E for diagnostic confirmation. All tissue samples were subjected to immunohistochemical staining utilizing rabbit polyclonal antibodies specific to STOML1 and STOML2. Clinical and demographic data were retrieved from departmental records. Data normality was assessed using the Kolmogorov-Smirnov test and found to be non-normal. Accordingly, the Mann–Whitney U test was applied for continuous variables, and the Chi-square test for categorical variables.

Demographics and Clinical Features- Mean age:Group I (NOM): 60.00 ± 8.47 years **,**Group II (OSCC): 56.56 ± 6.43 years

- Gender distribution:Group I: 46.7% male, 54.2% female,Group II: 53.3% male, 45.8% female
- OSCC differentiation:Well-differentiated: 66.7%,Moderately differentiated: 33.3%
- Clinical staging:Stage I: 40.8%; Stage II: 33.3%; Stage III: 25.9%
- Affected anatomical sites:Buccal mucosa (48.2%), tongue (25.9%), gingiva (25.9%)

STOML1 and STOML2 Expression in OSCC

Parameter	STOML1	STOML2
Expression Sites	Membrane, cytoplasm, nucleus	Membrane, cytoplasm
Overexpression in OSCC	Yes	Yes (but not statistically significant)
Mean Histoscore (NOM)	97.41 ± 48.72	77.78 ± 63.81
Mean Histoscore (OSCC)	132.59 ± 67.86	97.41 ± 69.04
Mann–Whitney p-value	0.121 (Not significant)	0.343 (Not significant)
Chi-Square Test	$\chi^2 = 15.79$, $p = 0.001$ (Significant)	$\chi^2 = 2.49$, $p = 0.47$ (Not significant)
Expression Category (OSCC)	Low (44%), Intermediate (37%), High (19%)	Low (67%), Intermediate (15%), High (15%)
Highest Histoscore by Grade	Well-differentiated: 135.86 ± 48.62	Moderately differentiated: 107.78 ± 81.21
Highest Histoscore by Stage	Stage I: 146.43 ± 61.97	Stage I: 106.36 ± 73.36
Highest Histoscore by Site	Buccal mucosa: 144.62 ± 48.75	Gingiva: 101 ± 68.07
Highest Histoscore by Age Group	>55 yrs: 135 ± 76.05	>55 yrs: 105 ± 77.64
Youden Index (Cutoff Score)	0.047 (H-score 150)	0.185 (H-score 50)
Logistic Regression (OR, *z*-score)	OR = 1.003, $z = 0.763$	OR = 0.999, $z = 0.166$
Sensitivity	100%	96.3%
Specificity	14.8%	14.8%

Parameter	STOML1	STOML2
AUC (ROC)	0.624	0.576
Overall Diagnostic Utility	Moderate (high sensitivity, low specificity)	Limited (high sensitivity, low specificity)

Interpretation
STOML1 shows moderate diagnostic relevance with significant histopathological associations, while STOML2 demonstrates limited utility based on statistical performance. Both markers exhibit high sensitivity but poor specificity, underscoring the need for combined biomarker panels to improve diagnostic accuracy for OSCC.

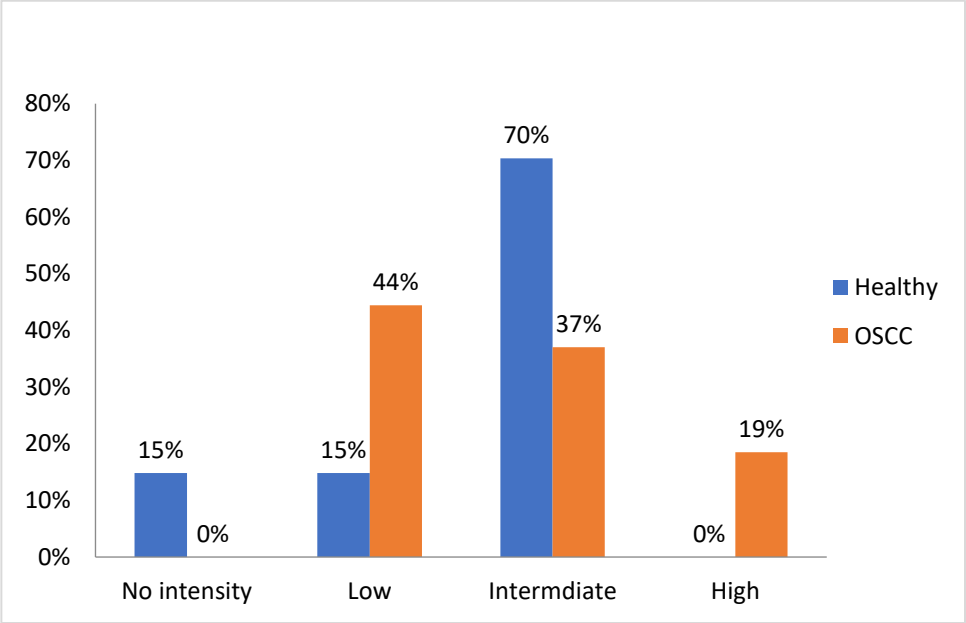
DISSCUSSION-
This study presents key molecular and clinicopathological distinctions between oral squamous cell carcinoma (OSCC) and healthy mucosa, with elevated expression of STOML1 and STOML2 observed in OSCC tissues—supporting their utility as potential diagnostic markers .
Epithelial dysplasia, a precursor to OSCC, is marked by stratification anomalies, suprabasal mitoses, and pleomorphism, yet histological grading shows interobserver variability, weakening predictive accuracy. Standardized diagnostic criteria and longitudinal validation are pivotal for improving prognostic accuracy and therapeutic outcomes in OSCC.
Accounting for approximately 90% of oral malignancies, OSCC predominantly involves the tongue, buccal mucosa, and floor of the mouth. In India, it ranks among the top three most prevalent cancers, with its incidence strongly linked to the use of tobacco, betel quid, alcohol consumption, and HPV infection. Regional studies highlight increased risk in underserved populations and rising incidence in younger demographics which is in contrast with the study given by Coelho KR. et al[9],Gupta B et al [10],Sankaranarayanan R et al[11],Ray JG et al[12].
Transformation from oral potentially malignant disorders (OPMDs) to OSCC may occur even without visible dysplasia. Goodson'sML et al[13] findings indicate that histologically non-dysplastic lesions—including those with lichenoid inflammation—may still progress to malignancy, emphasizing the need for proactive monitoring
Early-stage detection significantly improves prognosis, with stages I–II showing higher survival rates. Disease surveillance systems, clinical screening, and salivary metabolomic tools may enhance diagnostic efficiency and reduce OSCC mortality as similar toSingh R et al &Chen GS et al[14,15].
Age remains a critical factor in OSCC onset. While >95% of cases occur after age 40, variations exist. According to GS Chen et al[16]Indian populations show a mean onset of ~48 years ,Taiwanese at ~52 years, and Western populations at ~56 years, potentially driven by regional lifestyle and genetic factors.The findings of this study reaffirm the male predominance in OSCC incidence, aligning with the observations reported by Singh et al. [14] and Chen et al. [15], and according to Abdulla R et al [17]likely linked to elevated tobacco use among men. Contrary to global trends where advanced-stage diagnoses prevail Jerjes W et al [18], the present cohort showed early-stage predominance (stage I), with 66.7% of tumors well-differentiated, contrasting with prior studies favoring moderately differentiated OSCC Riveria H et al. , Ghatage M et al[19,20].
Buccal mucosa was the most frequently affected site (48.2%), aligning with Singh et al. [14], while regional contrasts exist (tongue predominance in Venezuela)similar to study Mairhofer M et al[21], possibly due to smokeless tobacco exposure. These findings highlight the importance of anatomical-site-focused screening and public health interventions.
At the molecular level, elevated expression of STOML1 and STOML2—members of the stomatin-like protein family—was detected in OSCC tissue specimens. STOML1, involved in sterol transport and membrane dynamics, showed higher histoscores in well-differentiated, early-stage, and buccal mucosa lesions, potentially linking it to malignancy initiation in contrast with the study done by Wang WX et al.[22]. Prognostic significance of STOML2 in gallbladder cancer. STOML2, localized to mitochondria and implicated in proliferation and epithelial-mesenchymal transition in several cancers [68–76], was more abundant in moderately differentiated and gingival lesions, suggesting aggressiveness but lacking significant correlation with stage or site similar to Arkhipova KA et al.[23], Hu G et al.[24], Zhu W et al.[25] , Wang D et al.[26].

CONCLUSION-

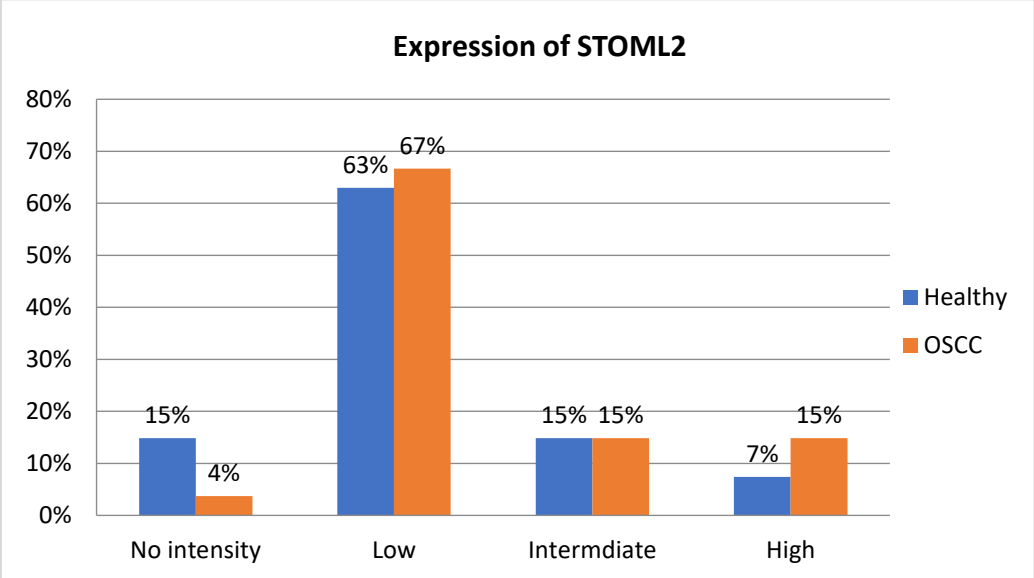
Both markers STOML1 and STOML2 exhibited good sensitivity but poor specificity (0.148), limiting standalone diagnostic use. Their biological roles—in membrane organization, ion channel modulation, and mitochondrial stress signaling warrant further evaluation. Larger studies integrating additional biomarkers are essential to clarify their clinical relevance.

LEGENDS

GRAPHS



Graph 1- Depicting STOML1 expression in group I & II.



Graph2:Depicting STOML2 expression in group I & II.

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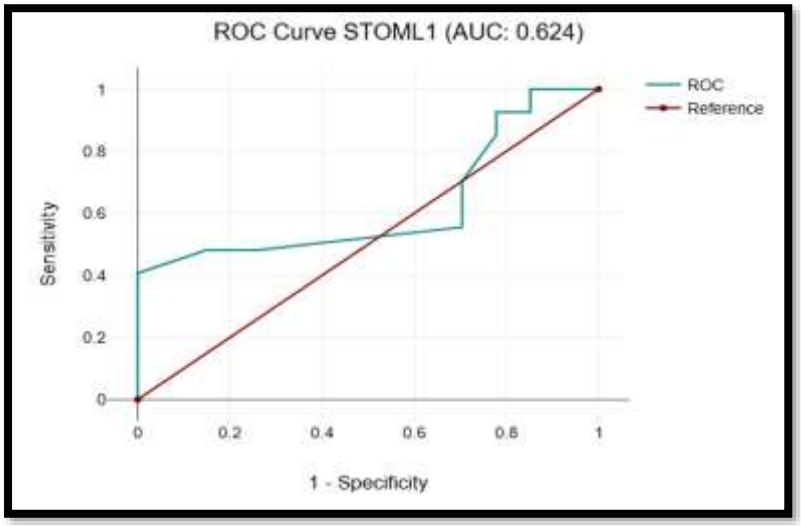
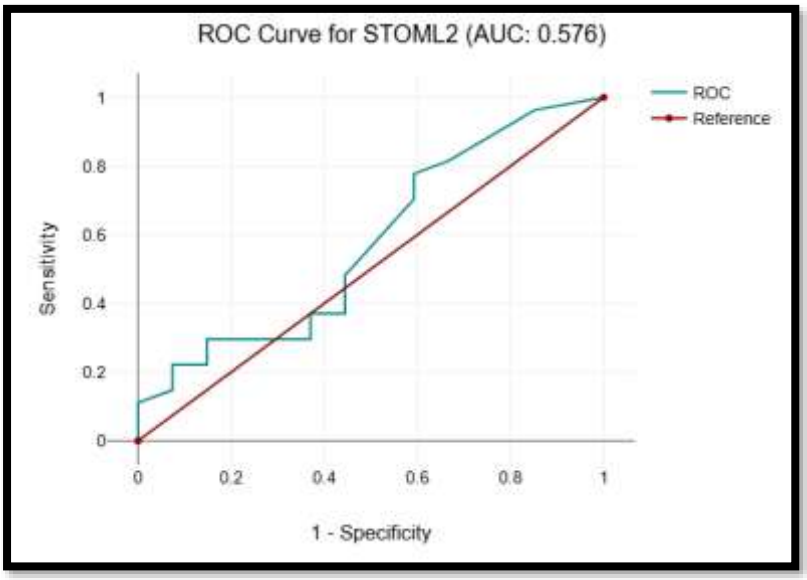
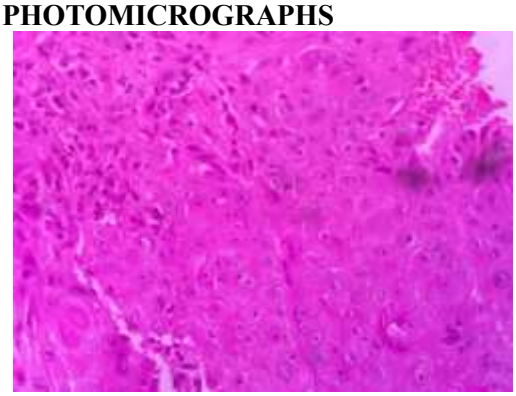


Graph 3- ROC curve for STOML1

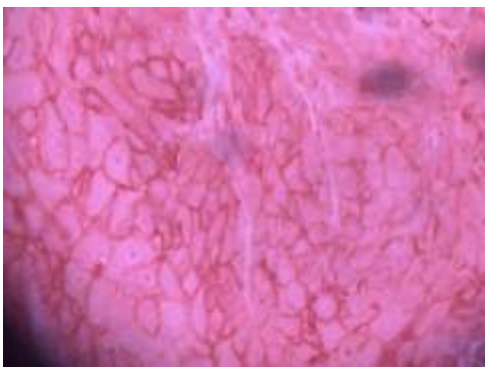


Graph 4- ROC Curve for STOML2

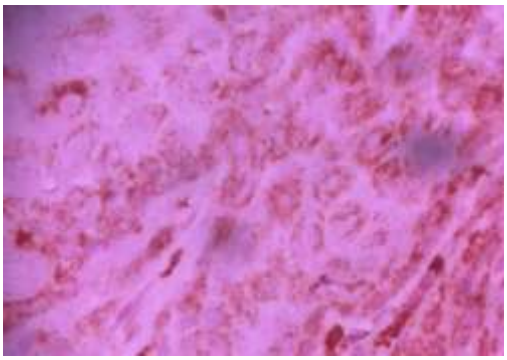
PHOTOMICROGRAPHS



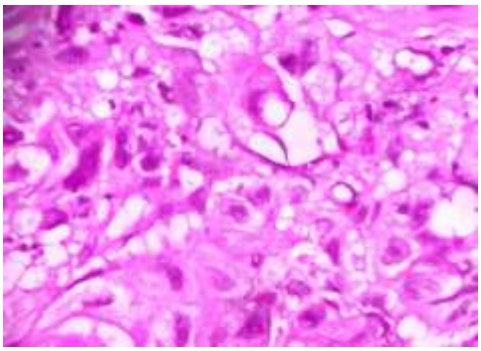
PHOTOMICROGRAPH 1: H&E stained well differentiated oral squamous carcinoma under 40x magnification



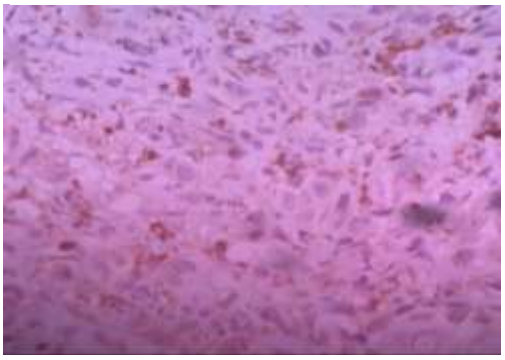
PHOTOMICROGRAPH 2: STOML1 IHC stained well differentiated oral squamous carcinoma under 40x magnification



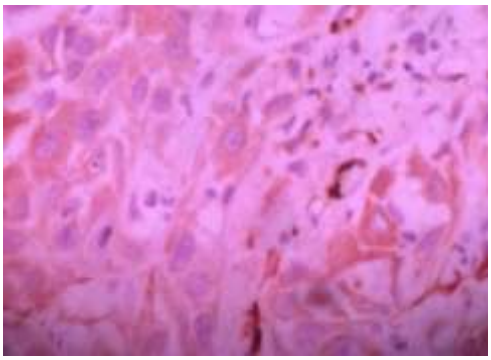
PHOTOMICROGRAPH 3: STOML2 IHC stained well differentiated oral squamous carcinoma under 40x



PHOTOMICROGRAPH 4: H&E stained moderately differentiated oral squamous carcinoma under 40x magnification



PHOTOMICROGRAPH 5: STOML1 IHC stained moderately differentiated oral squamous carcinoma under 40x magnification



PHOTOMICROGRAPH 6: STOML2 IHC stained moderately differentiated oral squamous carcinoma under 40x magnification

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