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Comparative Evaluation Of Pd-L1 And Zeb-1 In Oral Epithelial Dysplasia And Oral Squamous Cell Carcinoma

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ABSTRACT

INTRODUCTION- Oral squamous cell carcinoma (OSCC) *remains a major global health issue, especially in regions with high tobacco and areca nut use. Due to the lack of reliable biomarkers, diagnosis often occurs at advanced stages, limiting survival outcomes. This study evaluates the diagnostic significance of Programmed cell death ligand (PDL-1) and Zinc finger E box (ZEB-1) expression in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC), in comparison to normal oral mucosa (NOM).*

AIM*: To Quantify and compare IHC expression of Programmed cell death ligand I (PDL-I) and "Zinc finger E box" (ZEB-I) on Normal Oral Mucsoa , OED and Oral squamous cell carcinoma (OSCC) .*

OBJECTIVES

1.To quantify IHC expression of PDL-I and ZEB-1 in normal oral mucosa obtained from healthy volunteers.(Group-I)
2.To quantify IHC expression of PDL-I and ZEB-I in oral epithelial dysplasia(Group II)
3.To quantify IHC expression of PDL-I and ZEB-I in OSCC (Group-III).
4.To compare the finding within and between the groups.

MATERIALS AND METHODOLOGY- *This retrospective case-control study analyzed 60 paraffin-embedded tissue samples—20 each from healthy normal oral mucosa (NOM), oral epithelial dysplasia (OED), and oral squamous cell carcinoma (OSCC). Immunohistochemical staining was performed using rabbit monoclonal antibodies for PD-L1 and ZEB-1. Expression levels were quantified via labeling index and histoscore, and statistical differences were assessed using the Kruskal–Wallis and Mann–Whitney tests.*

RESULTS: *OSCC samples exhibited significantly higher expression of PD-L1 and ZEB-1 compared to OED and NOM. Their elevated levels in OSCC highlight their potential as diagnostic biomarkers, with PD-L1 linked to immune checkpoint modulation and ZEB-1 indicating enhanced tumor invasiveness and metastatic behavior.*

CONCLUSION: *While PD-L1 and ZEB-1 are overexpressed in malignant tissues and show promise as diagnostic markers for OED and OSCC, their use should be complemented with additional biomarkers to enhance diagnostic accuracy and reliability.*

KEYWORDS: *Oral squamous cell carcinoma (OSCC), oral epithelial dysplasia (OED) ,PD-L1, ZEB-1.*

INTRODUCTION-

Oral squamous cell carcinoma (OSCC) is driven by complex oncogenic pathways, with biomarkers such as p53, Ki-67, and E-cadherin aiding in early risk prediction. Identifying high-risk dysplastic lesions through genetic and histological analysis enhances early management and patient outcomes.[(1)]

PD-L1, an immune checkpoint protein, promotes tumor immune evasion by binding to the PD-1 receptor on T cells. Its expression is influenced by cytokines (e.g., IFN-γ, IL-6), genetic alterations (PTEN loss, JAK/STAT activation), and hypoxia. Overexpression in OSCC is linked to aggressive behavior, metastasis, and poor prognosis. PD-1/PD-L1–targeted immunotherapy has shown promising results across malignancies. [2]
ZEB-1, a key regulator of epithelial–mesenchymal transition (EMT), influences cell adhesion, migration, and stemness. Factors like TGF-β signaling, miR-200, and epigenetic changes drive its expression. In OSCC, ZEB-1 correlates with poor differentiation, metastasis, and therapy resistance. [1]
Beyond OSCC, PD-L1 and ZEB-1 are implicated in lung, breast, colorectal, and hepatocellular cancers. PD-L1 informs immunotherapy decisions, while ZEB-1 contributes to chemoresistance and tumor aggressiveness. [3,4]
This study evaluates the expression of PD-L1 and ZEB-1 in oral epithelial dysplasia (OED) and OSCC, exploring their roles as potential prognostic and therapeutic biomarkers.

MATERIAL AND METHODOLOGY-

Study Design and Sample Details

This retrospective case-control study was conducted at the Department of Oral & Maxillofacial Pathology and Oral Microbiology,Teerthanker Mahaveer dental college and research centre (TMDCRC). Archival formalin-fixed, paraffin-embedded (FFPE) tissue sections were sourced from healthy individuals (NOM), patients with oral epithelial dysplasia (OED), and oral squamous cell carcinoma (OSCC), with n = 20 in each group. Sample size was calculated using GPower v3.1* at 95% confidence interval and 80% power.

Inclusion and Exclusion Criteria

- **Group I:** NOM samples from healthy volunteers without tobacco use, systemic illness, or recent antibiotics.
- **Group II:** Histopathologically confirmed OED specimens with ≥5 years follow-up and complete data.
- **Group III:** OSCC specimens with sufficient tissue and complete TNM staging and histopathology.

Exclusions included systemic malignancy (Group I), inadequate or transformed tissue blocks (Group II), and incomplete clinical data (Group III).
AIM : To Quantify and compare IHC expression of Programmed cell death ligand I (PDL-I) and “Zinc finger E box” (ZEB-I) on Normal Oral Mucsoa , O ED and Oral squamous cell carcinoma (OSCC) .

OBJECTIVES

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H&E Staining Protocol

Tissue sections (3 μm) were mounted on egg albumin-coated slides, dewaxed in xylene, and hydrated through descending grades of ethanol. Harris hematoxylin and 1% Eosin-Y were used for staining, followed by dehydration and mounting with DPX. Slides were graded histologically per WHO criteria.

Immunohistochemistry (IHC) for PD-L1 and ZEB-1

Slide Preparation

Frosted slides were treated with 2% APES in acetone following autoclaving and were stored dust-free. FFPE tissue sections were mounted using a flotation bath.

Staining Procedure

Slides underwent deparaffinization, antigen retrieval via Tris-EDTA buffer (pH 9) in a pressure cooker, and peroxidase blocking using 3% H_2O_2. Primary monoclonal antibodies (PD-L1 and ZEB-1) were applied for 60 minutes. Detection involved PolyExcel Target Binder, PolyHRP, and DAB chromogen. Counterstaining was done with Mayer’s hematoxylin
Positive and negative controls used breast carcinoma tissues—with omission of primary antibody for negatives

Interpretation
Cells exhibiting brown nuclear or cytoplasmic staining were deemed positive for PD-L1 or ZEB-1.

Quantification of Expression
- **Labeling Index Method -Rahman et al**[5]
  - Five random high-power fields per specimen were imaged.
  - 1000 cells counted; percentage of positive cells was calculated for each biomarker.
- **Histoscore Analysis (Hinsch et al.** [6]**Maruse et al**[7]
  - Semi-quantitative scoring used the formula: $$\text{H-score} = 1 \times (\% \text{cells 1+}) + 2 \times (\% \text{cells 2+}) + 3 \times (\% \text{cells 3+})$$
  - Separate scores were tabulated for epithelial and mesenchymal regions.

RESULTS AND OBSERVATION

A total of 60 paraffin-embedded tissue blocks were sectioned at 4 µm thickness, mounted on frosted slides, and stained with H&E to reconfirm diagnosis. Based on histopathological evaluation, samples were categorized into three groups:
- **Group I** (n = 20): Normal oral mucosa (NOM) from healthy volunteers
- **Group II** (n = 20): Oral epithelial dysplasia (OED)
- **Group III** (n = 20): Oral squamous cell carcinoma (OSCC)

Demographics:
Mean Age: Group I – 29.25 ± 3.09, Group II – 29.75 ± 3.99, Group III – 34.65 ± 5.18 (p = 0.187),**Gender**: No significant difference across groups (p = 0.817),**Biopsy Site**: Buccal mucosa predominated (66.67%); site distribution was significant (p = 0.001)

Histopathology & TNM Staging:
- **Group II**: Mild (5), Moderate (9), Severe dysplasia (6)
- **Group III**: Well (7), Moderate (8), Poor differentiation (5); TNM stages: Stage I (30%), II (45%), III (25%)

Biomarker Expression
PD-L1 – Labeling Index

Tissue	Group I	Group II	Group III
Epithelium	48.95 ± 12.38	60.55 ± 7.9	77.15 ± 5.37
Mesenchyme	33.25 ± 5.5	62.1 ± 7.17	79.4 ± 4.92

ZEB-1 – Labeling Index

Tissue	Group I	Group II	Group III
Epithelium	49.6 ± 10.03	61.85 ± 8.51	79.15 ± 6.43
Mesenchyme	32.45 ± 4.33	62.45 ± 8.74	81.6 ± 5.99

H-Scores
- **PD-L1 Epithelium**: Group I – 74.85, Group II – 136.1, Group III – 171
- **PD-L1 Mesenchyme**: Group I – 74.05, Group II – 152.35, Group III – 182.05
- **ZEB-1 Epithelium**: Group I – 65.6, Group II – 143.95, Group III – 188.6
- **ZEB-1 Mesenchyme**: Group I – 70.7, Group II & III – 193.8

Statistical Insights

- **Normality Test**: Shapiro–Wilk indicated non-normal distribution ($p < 0.05$); non-parametric tests applied.
- **Kruskal–Wallis Test**: Significant differences in PD-L1 & ZEB-1 expression across groups ($p = 0.001$)
- **Mann–Whitney U Test**:
  - Significant epithelial vs. mesenchymal differences in **Group I**
  - **PD-L1** showed significant variation between tissue types in **Group II and III**
  - **ZEB-1** showed no significant tissue-type differences
- **Intra-Group Comparison (Group II – OED)**:
  - PD-L1 significant in epithelial and mesenchymal labeling indices ($p < 0.05$)
  - ZEB-1 significant in epithelial metrics; mixed significance in mesenchyme
- **Intra-Group Comparison (Group III – OSCC)**:
  - ZEB-1 consistently significant across differentiation grades
  - PD-L1 variably significant

Correlation Analysis (Spearman)

- **Epithelial PD-L1 & ZEB-1**: Strong correlation ($r = 0.76$, $p = 0.001$)
- **Mesenchymal**: Moderate correlation ($r = 0.64$, $p = 0.002$)
- **Cross-tissue Associations**: Moderate significance between epithelial PD-L1 and mesenchymal ZEB-1 ($r = 0.73$, $p = 0.001$)

DISSCUSSION-

Oral cancer is a major public health concern in India, accounting for 30–40% of all cancers, with higher prevalence in males and peak incidence in the fifth to seventh decades. Key risk factors include tobacco use, betel quid chewing, alcohol, and HPV infection. Oral epithelial dysplasia (OED), characterized by cellular atypia and architectural disturbances, is a known precursor to OSCC. Progression risk from OED to OSCC increases with dysplasia severity, influenced by genetic, environmental, and molecular factors.

Epidemiological Profile Oral cancer primarily affects individuals over 40 years, with reported mean onset ages of 48.3 years in India and 56.6 years in Western populations in accordance with Sharma S, Jaber MA et al [8,9]. While most OED cases without malignant transformation occur in individuals over 50 according to Singh MP et al [10] , the current study revealed much earlier mean onset: **OSCC at 34.65 ± 5.18 years** and **OED at 29.75 ± 3.99 years**, likely due to regional lifestyle and cultural practices such as smokeless tobacco use which is in accordance to Ghatage B, et al. [11].

Gender and Anatomical Correlation Despite literature citing male predominance in oral cancer in study done by Greeshma RL, et al. and Lim JH, et al [12,13], which **is not in accordance to our study as no significant gender-age variation was** found **in our present study**. The **buccal mucosa** was the most frequently involved site, reflecting established associations with smokeless tobacco use in accordance with Adamski L, et al. [14].

Histological Trends and Staging OED grading (mild, moderate, severe) and OSCC differentiation (well, moderate, poor) demonstrated a **progressive histological spectrum**, consistent with prior findings Kujan O, Abdulla R, Jerjes W, et al. [15,16,17]. Most OSCC cases were detected at **Stage II (45%)**, underscoring potential screening benefits.

Biomarker Dynamics: PD-L1 & ZEB-1 PD-L1, associated with immune checkpoint regulation similar to Dong H, et al [18], and ZEB-1, linked to EMT similar to Joseph AP, et al. [19], showed **upregulated expression across disease stages**, particularly in mesenchymal PD-L1 and epithelial ZEB-1. Significant intergroup variation affirms their **correlation with disease progression** (NOM → OED → OSCC), corroborated by Lim et al. and Greeshma RL et al.[13,12].

Tissue-Specific Expression Patterns Differences in PD-L1 and ZEB-1 between **epithelium and mesenchyme** were marked in NOM, but less so in OED/OSCC, suggesting a shift in expression behavior as malignancy evolves as in accordance with Adamski L, et al. [14].PD-L1 showed significant compartmental variation; ZEB-1 was more uniform, yet **influenced by disease severity**, especially in epithelial components.

Correlation Analysis A strong epithelial correlation between PD-L1 and ZEB-1 was observed in labeling indices and H-scores. Cross-tissue associations were moderate, indicating that **epithelial–mesenchymal interactions** may influence biomarker behavior during transformation stages similar to Cui B et al , Bankhead P, et al [2,20].
Clinical Implications and Limitations The study highlights PD-L1 and ZEB-1 as potential **diagnostic and prognostic markers** for monitoring progression. However, **limitations** include small sample size, limited etiological data, and variable staining sensitivity. Future studies should employ **molecular platforms** (e.g., RNA-seq, AI-driven histopathology) to improve specificity and reproducibility.

CONCLUSION-

The study shows that **PD-L1** and **ZEB-1** levels increase as oral disease progresses from **OED** to **OSCC**. PD-L1 helps tumors hide from the immune system, while ZEB-1 makes them more likely to spread. Their combined rise suggests a connection between immune escape and cancer spread. These markers could help to predict disease progression and design targeted treatments. More research with larger sample sizes and advanced methods is needed to better understand their role and improve early diagnosis of oral cancer.

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