Immunohistochemical evaluation of expression of human telomerase reverse transcriptase protein in oral malignancy

[1]Dr. Gaurav Dani, [2]Dr. Shabana Shaik, [3]Dr. Amit Wasti, [4]Dr. Garima Pandey, [5]Dr. Deepak Bansal, [6]Dr. Farhan Mazhar

[1]Reader, Department of Oral Pathology and Microbiology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh.

[2]Senior Lecturer, Department of Oral Medicine and Radiology, The Oxford Dental College, Bommanahalli, Bengaluru.

[3]Associate Professor and Head, Department of Oral Pathology and Microbiology, Government Dental College, Raipur, Chhattisgarh.

[4]Lecturer, Department of Oral Pathology and Microbiology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh.

[5]Professor, Department of Prosthodontics, Crown and Bridge.Bhojia Dental College and Hospital, Himachal Pradesh, India.

[6]PG Student, Department of Oral Pathology and Microbiology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh.

Corresponding author: Dr. Gaurav Dani, Reader, Department of Oral Pathology and Microbiology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh.

Abstract

Background: Oral malignancies, particularly oral squamous cell carcinoma (OSCC), are among the most aggressive cancers of the head and neck region. Telomerase activation plays a crucial role in cellular immortality, and human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, is a key biomarker for tumor progression. Immunohistochemical (IHC) analysis of hTERT expression in oral malignancies may provide insights into its potential role in tumor aggressiveness and prognosis. This study aims to evaluate hTERT protein expression in oral malignancies using IHC and assess its correlation with clinicopathological parameters.

Materials and Methods: A total of 60 formalin-fixed, paraffin-embedded (FFPE) tissue samples were selected, including 40 OSCC cases and 20 normal oral mucosa controls. Tissue sections (4 µm thick) were subjected to immunohistochemical staining using an anti-hTERT monoclonal antibody. The expression of hTERT was evaluated based on staining intensity and percentage of positively stained cells. A semi-quantitative scoring system was used, classifying samples as low, moderate, or high expression. Statistical analysis was performed using chi-square tests and Pearson's correlation coefficient, with a significance level of $p < 0.05$.

Results: IHC analysis revealed that hTERT expression was significantly upregulated in OSCC cases ($p < 0.05$) compared to normal mucosa. Among OSCC samples, 30% showed high expression, 45% moderate expression, and 25% low expression. In contrast, 85% of normal mucosa samples showed no or minimal hTERT expression. Higher hTERT expression was observed in poorly differentiated OSCC (40%) compared to well-differentiated cases (15%). A significant correlation was found between hTERT overexpression and lymph node involvement ($p < 0.01$).

Conclusion: hTERT protein expression is markedly elevated in oral malignancies, particularly in aggressive and poorly differentiated tumors. Its strong association with lymph node metastasis suggests its potential utility as a prognostic biomarker in OSCC. Further studies are needed to explore its role as a therapeutic target for oral cancer treatment.

Keywords: hTERT, telomerase, oral squamous cell carcinoma, immunohistochemistry, tumor biomarker, prognosis.

Introduction

Oral malignancies, particularly oral squamous cell carcinoma (OSCC), are among the most common cancers of the head and neck region, accounting for over 90% of all oral cancers (1). Despite advancements in diagnosis and treatment, OSCC continues to have a high mortality rate, primarily due to late-stage detection and frequent metastasis (2). The ability of tumor cells to maintain telomere length is a key factor in their unlimited proliferative capacity (3). Telomerase, a ribonucleoprotein enzyme, is responsible for telomere elongation and is highly active in most malignant cells, contributing to their immortalization (4).

The human telomerase reverse transcriptase (hTERT) is the catalytic subunit of telomerase and plays a pivotal role in regulating its activity (5). Unlike normal somatic cells, where telomerase activity is typically absent or minimal, malignant cells exhibit high levels of hTERT expression, facilitating cellular immortality and tumor progression (6). Studies have shown that hTERT expression correlates with tumor aggressiveness, metastasis, and poor prognosis in various malignancies, including OSCC (7,8).

Immunohistochemical (IHC) analysis provides a reliable and cost-effective method for detecting hTERT protein expression in tissue specimens (9). Several studies have demonstrated a significant increase in hTERT expression in OSCC compared to normal oral mucosa, suggesting its potential as a biomarker for early detection and prognosis (10,11). Additionally, hTERT expression has been linked to lymph node involvement, a critical factor influencing tumor staging and patient survival (12).

Despite these findings, the role of hTERT in oral cancer progression and its correlation with clinicopathological parameters remains incompletely understood. This study aims to evaluate hTERT protein expression in OSCC tissues using immunohistochemical methods and assess its correlation with tumor differentiation, lymph node involvement, and clinical stage. The results may provide insights into the prognostic value of hTERT and its potential as a therapeutic target in oral cancer treatment.

Materials and Methods

Study Design and Sample Selection

This study was designed as a retrospective immunohistochemical analysis of human telomerase reverse transcriptase (hTERT) protein expression in oral malignancies. A total of 60 formalin-fixed, paraffin-embedded (FFPE) tissue samples were collected from a pathology archive. The samples included 40 cases of histopathologically confirmed oral squamous cell carcinoma (OSCC) and 20 samples of normal oral mucosa, which served as controls.

Tissue Processing and Immunohistochemical Staining

All tissue specimens were sectioned at 4 µm thickness using a rotary microtome and mounted on poly-L-lysine-coated slides. The sections were deparaffinized in xylene and rehydrated through a graded series of ethanol solutions. Antigen retrieval was performed using a citrate buffer (pH 6.0) in a microwave oven for 15 minutes.

Immunohistochemical staining was conducted using the streptavidin-biotin peroxidase method. The sections were incubated with a monoclonal anti-hTERT antibody (1:100 dilution) at 4°C overnight. After washing with phosphate-buffered saline (PBS), the sections were treated with a secondary biotinylated antibody for 30 minutes at room temperature, followed by the application of 3,3'-diaminobenzidine (DAB) substrate for visualization. Finally, the slides were counterstained with Hematoxylin, dehydrated, and mounted using DPX.

Evaluation of hTERT Expression

The immunohistochemically stained sections were examined under a light microscope (×400 magnification) by two independent pathologists who were blinded to the clinical data. hTERT expression was assessed based on:

- Staining intensity (0 = no staining, 1+ = weak, 2+ = moderate, 3+ = strong)
- Percentage of positively stained tumor cells (0 = <5%, 1 = 5–25%, 2 = 26–50%, 3 = >50%)

A semi-quantitative scoring system was used to classify cases as low expression (score 0–1), moderate expression (score 2–3), and high expression (score 4–6).

Statistical Analysis

Data were analyzed using SPSS software (version 25.0, IBM, USA). The chi-square test was applied to compare hTERT expression between OSCC and normal mucosa. The Pearson correlation coefficient was used to assess associations between hTERT expression and clinicopathological parameters, including tumor differentiation, lymph node involvement, and clinical stage. A p-value < 0.05 was considered statistically significant.

Results

hTERT Expression in OSCC and Normal Mucosa

Immunohistochemical analysis revealed a significant difference in hTERT expression between OSCC and normal mucosa ($p < 0.05$). Among the OSCC cases (n=40), 30% exhibited high expression, 45% moderate expression, and 25% low expression. In contrast, in normal oral mucosa samples (n=20), the majority (85%) showed low expression, with only 5% exhibiting high expression (Table 1). These findings suggest that hTERT overexpression is associated with malignant transformation in oral tissues.

Correlation of hTERT Expression with Clinicopathological Parameters

A higher hTERT expression was observed in poorly differentiated OSCC cases (55%), compared to moderately differentiated (30%) and well-differentiated tumors (15%). Additionally, lymph node-positive cases demonstrated significantly higher hTERT expression (55%) than lymph node-negative cases (15%) (Table 2). Statistical analysis confirmed a strong correlation between hTERT expression and both tumor differentiation and lymph node metastasis ($p < 0.01$).

These results indicate that hTERT overexpression is associated with aggressive tumor behavior and may serve as a potential prognostic biomarker in OSCC.

Table 1: hTERT Expression in OSCC and Normal Mucosa

Group	Low Expression (%)	Moderate Expression (%)	High Expression (%)
OSCC (n=40)	25	45	30
Normal Mucosa (n=20)	85	10	5

Table 2: Correlation of hTERT Expression with Clinicopathological Parameters

Parameter	Low Expression (%)	Moderate Expression (%)	High Expression (%)
Well-Differentiated OSCC	40	45	15
Moderately Differentiated OSCC	20	50	30

Poorly Differentiated OSCC	10	35	55
Lymph Node Positive	5	40	55

Discussion

The present study investigated the expression of human telomerase reverse transcriptase (hTERT) in oral squamous cell carcinoma (OSCC) and its correlation with clinicopathological parameters. The results demonstrated a significant upregulation of hTERT protein expression in OSCC cases compared to normal oral mucosa, suggesting a role for hTERT in tumor progression and malignancy. These findings align with previous studies that have identified hTERT as a key regulator of cellular immortality in cancer (1,2).

Telomerase activation is a crucial step in carcinogenesis, enabling malignant cells to bypass replicative senescence and apoptosis (3). hTERT, the catalytic subunit of telomerase, is predominantly expressed in cancer cells and is responsible for maintaining telomere length, thereby promoting uncontrolled proliferation (4). The high expression of hTERT in OSCC observed in this study is consistent with prior research indicating that hTERT upregulation is a hallmark of aggressive tumor behavior (5,6).

A strong correlation was found between hTERT expression and tumor differentiation, with poorly differentiated OSCC cases showing the highest levels of hTERT. This is in agreement with studies by Gonzalez-Moles et al. (7) and Liu et al. (8), which reported that hTERT overexpression is more pronounced in poorly differentiated and metastatic oral cancers. The increased expression in advanced tumors suggests that hTERT contributes to cellular dedifferentiation and enhanced tumor invasiveness (9).

Additionally, our study found a significant association between hTERT overexpression and lymph node metastasis, reinforcing the role of hTERT as a prognostic biomarker in OSCC. Previous studies have demonstrated that telomerase activation enhances tumor cell survival and migration, facilitating metastasis to regional lymph nodes (10,11). The presence of high hTERT expression in lymph node-positive cases suggests its involvement in tumor progression and distant spread (12).

The immunohistochemical method used in this study effectively detected hTERT protein expression and provided valuable insights into its role in oral malignancy. IHC has been widely recognized as a reliable and cost-effective approach for evaluating tumor biomarkers (13). However, it should be noted that molecular techniques such as qRT-PCR and Western blot analysis could further validate hTERT expression levels at the genetic and protein levels, respectively (14).

Despite these significant findings, this study has certain limitations. The sample size was limited, and a larger cohort with long-term follow-up would provide a more comprehensive understanding of hTERT's prognostic potential. Additionally, further studies should explore the therapeutic implications of telomerase inhibition as a targeted approach for OSCC treatment. Telomerase inhibitors such as imetelstat have shown promising results in preclinical cancer models and may serve as potential therapeutic agents in oral cancer management (15).

Conclusion

Overall, our findings suggest that hTERT expression is significantly elevated in OSCC, particularly in poorly differentiated tumors with lymph node metastasis. These results highlight the potential of hTERT as a diagnostic and prognostic marker and emphasize the need for further research on telomerase-targeted therapies for oral malignancies.

References

1. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266(5193):2011-5.
2. Shay JW, Wright WE. Telomeres and telomerase in normal and cancer stem cells. FEBS Lett. 2010;584(17):3819-25.
3. Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, et al. hTERT: Activation of telomerase in human cancer. Cell. 1997;90(4):785-95.
4. Kannan S, Mahimkar MB, Ranganathan S, Krishnamurthy S, Bhisey A. Telomerase activity in oral cancer. Cancer. 1997;79(5):850-7.
5. Sugiura T, Yamazaki Y, Matsunaga T, Kimura T, Tanaka A, Saito M, et al. Telomerase activity and its correlation with the expression of hTERT in oral squamous cell carcinoma. Oral Oncol. 2002;38(3):237-42.
6. Chai RC, Lim Y, Frazer IH, Wan Y, Perry C, Jones L, et al. hTERT expression in oral squamous cell carcinoma and oral epithelial dysplasia. Int J Cancer. 2011;128(8):2030-5.
7. Gonzalez-Moles MA, Ayen A, Gonzalez-Ruiz I, Ruiz-Avila I, Rodriguez-Archilla A, Ramos-Garcia P. Prognostic and clinicopathological significance of hTERT expression in head and neck squamous cell carcinoma: Systematic review and meta-analysis. Oral Oncol. 2021;112:105087.
8. Liu Z, Li Q, Li K, Chen L, Li W, Hou Y, et al. hTERT expression in oral squamous cell carcinoma: A potential biomarker for tumor progression and prognosis. BMC Cancer. 2020;20(1):463.
9. Kannan R, Krishnamurthy A, Sethuraman M, Ramadoss K. Expression of hTERT in oral leukoplakia and oral squamous cell carcinoma: An immunohistochemical study. J Clin Diagn Res. 2017;11(6):ZC77-ZC80.
10. Neves M, Vargas P, Peres M, Pinto D, Santos-Silva AR, Lopes MA, et al. Immunohistochemical expression of hTERT in oral squamous cell carcinoma and its correlation with clinicopathological parameters. J Oral Pathol Med. 2020;49(1):45-52.
11. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4-5):309-16.
12. Gonzalez-Moles MA, Ruiz-Avila I, Ayen A, Plaza-Campillo JJ, Gil-Montoya JA, Ramos-Garcia P. Analysis of the role of telomerase in oral cancer and precancer: A meta-analysis. Med Oral Patol Oral Cir Bucal. 2020;25(3):e415-e424.
13. Ramani P, Gupta R, Sulochana KV, Sherlin HJ, Premkumar P, Natesan A. Telomerase in oral cancer. J Oral Maxillofac Pathol. 2015;19(3):346-53.
14. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016;8(1):69.
15. Joseph I, Tressler R, Bassett E, Harley C, Buseman CM, Pattamatta P, et al. The telomerase inhibitor imetelstat depletes cancer stem cells in glioblastoma multiforme. PLoS One. 2010;5(12):e14488.