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EGFR Gene Mutations in Tongue Squamous Cell Carcinoma: Frequency and Association with Clinicopathological Features

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ABSTRACT

Background & Objective: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity. Epidermal growth factor receptor (EGFR) mutations are well recognized in lung cancer and several other epithelial tumors, but their role in tongue squamous cell carcinoma (TSCC) remains unclear. Understanding their frequency and clinical relevance may provide insight into their potential therapeutic significance.

Methods: In this study, 30 paraffin-embedded tissue samples from patients with TSCC were examined. Demographic and clinicopathological data, including age, sex, tumor stage, histological grade, and recurrence, were collected. DNA was extracted, and mutations in exons 18 to 21 of the EGFR gene were analyzed using real-time polymerase chain reaction. Statistical analyses were performed to assess possible associations between EGFR mutation status and clinical or pathological features, as well as patient survival.

Results: The mean age of the patients was 58.77 years, with an equal male-to-female distribution. EGFR mutation was identified in only one patient (3.3%), consisting of a deletion in exon 19. No significant associations were observed between EGFR mutation status and age, sex, tumor stage, histological grade, or recurrence. Survival analysis also showed no prognostic impact of EGFR mutation in this cohort.

Conclusion: EGFR mutation appears to be rare in TSCC and was not significantly associated with clinicopathological features or patient survival in this study. Larger studies are needed to confirm these findings and to clarify any potential therapeutic implications.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, with the tongue being one of the most frequently affected subsites (1, 2). Tongue squamous cell carcinoma (TSCC) is characterized by aggressive behavior due to the rich lymphatic network of the tongue, which facilitates regional metastasis and recurrence (3). This malignancy also disrupts essential functions such as speech and swallowing, leading to a significant decline in patients' quality of life (4). Despite advances in therapeutic strategies, the five-year survival rate for TSCC remains low, with most cases diagnosed at advanced stages (5).

The epidermal growth factor receptor (EGFR) gene is a key molecular driver in the pathogenesis of various carcinomas (5). EGFR encodes a transmembrane receptor tyrosine kinase that plays a central role in regulating cell proliferation, survival, angiogenesis, and motility. Mutations in EGFR—particularly within exons 18 to 21 of the tyrosine kinase domain—can result in constitutive activation of downstream signaling pathways, including RAS/RAF/MEK and PI3K/AKT pathways, thereby promoting tumor growth and resistance to treatment (6).

Although EGFR mutations have been extensively studied in non-small cell lung carcinoma (NSCLC), their prevalence and clinical significance in TSCC remain unclear. Some studies report low mutation rates in OSCC, while others suggest potential prognostic or therapeutic value for EGFR-targeted therapies (7–9). Such variability may stem from differences in tumor location, population characteristics, sample size, and detection methods.

Given the limited and conflicting evidence, the present study aimed to determine the prevalence and

specific types of EGFR mutations in TSCC and to evaluate their correlation with clinicopathological parameters, including age, sex, tumor stage, histological grade, and recurrence.

Materials and Methods

This retrospective cross-sectional study included 30 formalin-fixed, paraffin-embedded (FFPE) tissue samples from patients with pathologically confirmed TSCC. Samples were retrieved from the pathology archives of Omid and Ghaem hospitals following ethical approval. All cases were confirmed by two independent pathologists. Clinical data (age, sex, tumor stage, histological grade), along with recurrence and survival information, were obtained from medical records.

DNA was extracted from FFPE tissues, and its quality was assessed using NanoDrop spectrophotometry. Samples with adequate DNA yield and quality (A260/280 ratio >1.8) were selected for downstream analysis.

EGFR mutations in exons 18 to 21 were analyzed using the Easy® EGFR Mutation Detection Kit, which utilizes real-time PCR based on allele-specific amplification. The reactions were performed using a Rotor-Gene Q real-time PCR system (Qiagen, Germany). Each reaction mixture included specific primers and probes targeting the most common EGFR mutations: exon 19 deletions, L858R (exon 21), T790M (exon 20), G719X (exon 18), L861Q (exon 21), S768I (exon 20), and exon 20 insertions such as c.2319_2320insCAC, c.2310_2311insGGT, and c.2307_2308insGCCAGCGTG. PCR cycling conditions were applied according to the kit protocol, and results were interpreted using the system's analysis software.

Statistical analysis included the Chi-square test to assess associations between variables. Kaplan–Meier analysis was used to evaluate overall survival (OS) and disease-free survival (DFS). A P-value <0.05 was considered statistically significant.

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Results

A total of 30 patients with TSCC were included in this study. The mean age of the patients was 58.77 ± 14.7 years, ranging from 31 to 80 years. There was an equal distribution between males and females (50% each).

The most common tumor stage was Stage II (33.3%), followed by Stage I (26.7%), Stage III (20%), and Stage IV (20%). The majority of tumors were moderately differentiated (Grade II, 56.7%), while 43.3% were well differentiated (Grade I).

Half of the patients (50%) experienced recurrence, with local recurrence accounting for 66.7% of those cases.

EGFR mutation was detected in 1 out of 30 cases (3.3%), identified as a deletion in exon 19. The remaining 29 patients (96.7%) showed no mutation. The patient with the EGFR mutation was a female over 60 years old with an advanced-stage, Grade II tumor and local recurrence (Table 1).

Statistical analysis showed no significant association between EGFR mutation status and sex ($p = 0.309$), age group ($p = 0.309$), tumor stage ($p = 0.213$), grade ($p = 0.374$), or recurrence status ($p = 0.471$) (Table 2).

The median overall survival (OS) was 27 months. The 1-year, 3-year, and 5-year OS rates were 83.3% ± 6.8%, 46.2% ± 9.2%, and 41.1% ± 9.5%, respectively. The median disease-free survival (DFS) was 18 months, with 1-year, 3-year, and 5-year DFS rates of 63.3% ± 8.8%, 35.6% ± 9%, and 35.6% ± 9%, respectively. In the EGFR-mutant case, OS was 16 months and DFS was 3 months (Table 3, Fig. 1).

Table 1. Clinicopathological characteristics of the TSCC patients.

Characteristic		number	percentage
gender	male	15	50
	female	15	50
Age group	≤ 60 years	15	50
	> 60 years	15	50
Stage	I	8	26.7
	II	10	33.3
	III	6	20
	IV	6	20
Grade	I	13	43.3
	II	17	56.7
Recurrence	yes	15	50
	no	15	50
Recurrence Type	Local	10	66.7
	Metastatic	5	33.3
EGFR mutation	positive	1	3.3
	Negative	29	96.7

Table 2. Association between EGFR mutation and clinicopathological features of the TSCCs.

Variable	Category	EGFR Neg	EGFR Pos	Chi-square	P-value
Gender	Female	14(93.3%)	1(6.7%)	1.034	0.309
	male	15(100%)	0(0%)		
Age	≤ 60 years	15(100%)	0(0%)	1.034	0.309
	> 60 years	14(93.3%)	1(6.7%)		
Tumor stage	Stage I,II (Early stage)	18(100%)	0(0%)	1.552	0.213
	Stage III,IV (advanced stage)	11(91.7%)	1(8.3%)		
Tumor grade	Grade I	13(100%)	0(0%)	0.791	0.374
	Grade II	16(94.1%)	1(5.9%)		
Recurrence/metastasis	No recurrence	10(100%)	0(0%)	0.517	0.471
	With recurrence or metastasis	19(95%)	1(5%)		

Table 3. DFS and OS in the studied cases.

	DFS				OS			
	Median	Std. Error	Chi-Square	P-value	Median	Std. Error	Chi-Square	P-value
neg	35.000	12.665	14.000	0.000	27.000	16.983	1.325	0.250
pos	3.000	-			16.000	-		

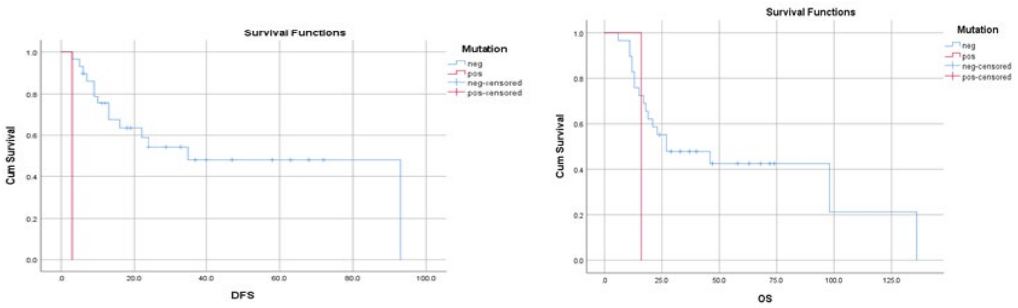

Fig. 1. OS and DFS in the studied patients

Discussion

The low frequency of EGFR mutations observed in the present study (3.3%) is consistent with much of the current literature, which generally indicates that EGFR gene alterations are uncommon in OSCC and TSCC populations. However, the biological basis for this low prevalence remains insufficiently understood and warrants further investigation. Several biological mechanisms, including tumor microenvironmental differences, cellular differentiation patterns, and variability in downstream signaling pathway activation, may influence the likelihood of EGFR pathway alterations. Moreover, environmental exposures such as tobacco use, alcohol consumption, and betel nut chewing show substantial geographic variability and have been suggested as contributing factors to the heterogeneous mutation patterns reported across studies. Geographic and ethnic diversity must also be considered; studies from South and East Asian countries, where betel nut chewing and certain lifestyle risk factors are more prevalent, have reported higher EGFR mutation rates in oral cavity cancers compared with Western cohorts (7, 8, 18).

The identification of a single exon 19 deletion in this study is consistent with previous analyses demonstrating that mutations within exons 18–21 are relatively rare in head and neck squamous cell carcinoma (HNSCC) (16). While Kaur et al. reported a slightly higher mutation rate of 6.7% in exon 20 (10), other studies, such as Mehta et al., found no EGFR mutations in OSCC samples (17). In contrast, markedly higher frequencies—up to 81%—have been described by Nagalakshmi et al. in HNSCC cohorts (18). These discrepancies likely reflect differences in sample size, anatomical site specificity (TSCC versus heterogeneous HNSCC cohorts), population characteristics, and methodological variations across studies. The present investigation focused exclusively on TSCC, which may partially explain the lower mutation rate compared with mixed-site studies. Although Vatte et al. reported significant associations between EGFR mutations and tumor grade and stage (8), no such correlations were observed in the current cohort, likely due to the very small number of mutation-positive cases.

The prognostic significance of EGFR mutations in TSCC remains unclear. Only a limited number of studies have evaluated survival outcomes in relation to EGFR mutational status in TSCC or OSCC. In the present study, no meaningful survival analysis could be performed because of the extremely low mutation frequency. Nevertheless, given the established therapeutic relevance of EGFR tyrosine kinase inhibitors in other malignancies, such as lung carcinoma (6), even rare actionable mutations may have clinical importance. Larger, well-powered studies are required to determine whether EGFR mutation status influences treatment response or long-term outcomes in TSCC.

An important limitation of this study is the small sample size (n = 30), which substantially limits statistical power and the ability to detect meaningful associations between EGFR mutations and clinicopathological variables. Furthermore, no formal power analysis was performed to estimate the required sample size, further restricting the interpretability of nonsignificant findings. These limitations should be considered when generalizing the results. Future investigations should include larger, multi-center cohorts with adequately powered study designs to validate these findings.

Overall, the present findings support the growing evidence that EGFR mutations play a limited role in TSCC pathogenesis, at least within this population. Nonetheless, molecular profiling remains clinically relevant, as the identification of even rare actionable mutations may offer therapeutic opportunities. Future studies should incorporate multi-institutional cohorts, larger sample sizes, advanced mutation detection techniques, and robust survival analyses to better clarify the biological and clinical significance of EGFR alterations in TSCC.

Conclusion

Based on the findings of this study, EGFR gene mutations appear to be rare in TSCC patients. Although their frequency is considerably lower than that reported in other malignancies, such as lung adenocarcinoma, these alterations may still contribute to tumorigenesis and disease progression. Given that the only detected mutation occurred in a patient with advanced-stage disease and poor survival, larger and adequately

powered studies are required to clarify whether EGFR mutation status has prognostic or therapeutic significance in TSCC.

Despite their low prevalence, the identification of EGFR mutations may help define a small subset of TSCC patients who could potentially benefit from targeted therapies, pending further validation in larger multicenter cohorts. The small sample size of the present study remains a major limitation and has been acknowledged.

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Authors' Contributors

None.

Data Availability

The data supporting the results of this study are available upon request from the corresponding author.

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Ethics Approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTR.REC.1403.082).

Consent to Participate:

Follow-up data, including recurrence status, were obtained through direct contact with patients or their companions. The study utilized an open-access article distribution under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Conflict of Interest

The authors declared no conflict of interest.

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