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Original Research Article

Expression of EGFR and HER2 in Oral Dysplastic Epithelium and Squamous Cell Carcinoma

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Abstract

Introduction: A better understanding of molecular mechanisms of oral squamous cell carcinoma (OSCC) and identification of potential oncogenes may provide new therapeutic decisions such as targeted therapy. Most of the studies suggest that OSCCs expressing Epidermal growth factor receptor (EGFR) display pathological features of more aggressive tumors and its overexpression is associated with poor prognosis.[1] Studies have also shown that the overexpression of HER2/neu increased metastatic potential and that cell proliferation, as measured by the Ki67 labelling index at the invasive tumor front is directly related to the histological grade in OSCC.[2,3] *Objectives:* To study the expression of EGFR and HER2/neu in oral mucosal dysplasia and SCC, to evaluate their correlation, to compare their expression in different histological grades of the tumor, to evaluate the relationship between their expression and tumor proliferation index demonstrated by Ki67 labelling with an aim of emphasizing the role of these biomarkers in oral carcinogenesis as well as their role as potential therapeutic target. *Materials and methods:* This study was conducted on 68 patients having oral dysplasia or SCC. Immunohistochemistry was done for EGFR, HER2/neu and Ki67 and their expression was evaluated under the microscope. *Results:* EGFR overexpression is associated with higher grades and poorly differentiated tumors. Expression of EGFR is also more in superficial layer in higher grades of dysplasia. There is statistically significant difference in EGFR expression between cases of dysplasia and OSCC. However no such correlation was found with respect to HER2 expression. *Conclusions:* We conclude that EGFR overexpression is associated with higher grades and higher intrinsic proliferative activity. Hence EGFR can serve as a good prognostic indicator and a target for anti-cancer therapy. However no similar role of HER2 could be proved.

Keywords: EGFR; HER2/neu; Ki67; Oral dysplastic epithelium; Oral squamous cell carcinoma.

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Introduction

Oral cancer ranks eighth in the cancer incidence worldwide. In India, it ranks number one in terms of incidence among men and third among women.[4] More than 90% of all oral neoplasms are oral squamous cell carcinoma (OSCC)[1,5,6] accounting for 2–4% of all cancer cases worldwide. Morbidity and mortality in males are 6.6/100,000 and 3.1/100,000 respectively, while in females these are 2.9/100,000 and 1.4/100,000.[3] Leukoplakia and erythroplakia are the most commonly diagnosed oral premalignant lesions (OPL), with a 17% to 24% rate of malignant transformation over a period of up to 30 years.[7,8]

The greatest risk of oral cancer is the use of tobacco and alcohol. Other factors include an impaired ability to repair DNA damaged by mutagens and to metabolize carcinogens, deficiencies of vitamins A, E or C or trace elements and immune defects such as in HIV infection, organ transplantations and immunosuppressive therapy.[9]

Unless oral mucosal cells are exposed to chemical carcinogens, only HPV infection cannot turn them malignant.[10–14]

Genetic predisposition combined with exposure to environmental carcinogens, such as, tobacco, alcohol, chemical carcinogens, ultraviolet or ionizing radiation and microorganisms lead to multiple molecular events that ultimately gives rise to OSCC.[15–18] Chronic exposure to carcinogens may cause genetic damage which may activate mutations or amplification of oncogenes.

The ErbB family of receptors consists of a subfamily of four closely related receptor tyrosine kinases: Epidermal growth factor receptor (EGFR) (ErbB-1), human epidermal growth factor receptor 2(HER2/neu) (ErbB2), HER3 (ErbB-3) and HER4 (ErbB-4). Activated EGFR recruits a number of downstream signaling molecules, leading to the activation of several major pathways crucial for tumor growth, progression and survival. Overexpression of signaling of the receptor tyrosine kinases including EGFR is associated with the development of various kinds of tumors in humans.

HER2/neu, is a protein encoded by the ERBB2 gene, with tyrosine kinase activity. In several malignancies, HER2/neu oncogene amplification or overexpression of its protein has been proved.

High Ki67 index has been correlated with high degree of malignancy and histological grading in the invasive front and poor survival in OSCC.[19,20]

Despite advances in therapeutic modalities, the mortality rate has remained largely unchanged for decades, with a 5-year survival rate of around 50%.[21]

Currently, targeted molecular therapy has been applied to oral cancer patients which has limited or nonexistent side effects on normal cells and can also act as a complement to other existing cancer therapies and has been mainly focused on four molecules associated with the proliferation and the differentiation of OSCC.[22] After the identification of EGFR as an oncogene, anticancer therapeutics directed against EGFR ("EGFR inhibitors") have been developed.

As for chemotherapy, cisplatin-based chemoradiation remains the standard for loco regionally advanced head and neck SCC. However, its effectiveness in the treatment of recurrent/ metastatic tumors is limited because of acquired or intrinsic resistance. Several studies have suggested that enhanced expression of EGFR may be associated with cisplatin resistance in a variety of solid tumors including oral cancers.[23,24] Increased availability of EGFR inhibitors in cisplatin resistant cells has also been reported.[24] EGFR inhibitors have shown significant activity in cases failing cisplatin-based therapy.[25,26] Therefore, EGFR blockade may be a useful therapeutic tool in the treatment of patients with acquired cisplatin resistance.

Materials and Methods

This hospital based retrospective and prospective study was done on 68 patients who have undergone any surgery of oral cavity for the suspicion of malignancy and whose histopathological specimens have been received in the department of Pathology, ESI-PGIMSR, Manicktala, Kolkata showing dysplasia or SCC on routine histopathological examination done by H&E staining after obtaining ethical clearance from the institutional ethical committee. It took approximately one and a half years (February 2016 to October 2017) to complete.

Sections with fixation artefacts were excluded from the study. For EGFR, positive control consisted of paraffin embedded sections of human placental tissue with known antigenic reactivity to EGFR; known HER2 positive sections validated by FISH served as positive control for HER2 and sections from tonsil served as positive control for Ki67. Grading and staging of carcinoma were done according to WHO criteria.

For retrospective study, slides were reviewed from archival material, cases with OSCC were identified and the corresponding blocks were retrieved from archives for IHC (EGFR, HER2, and Ki67).

For prospective study, tissue specimens obtained from patients undergoing maxillofacio-mandible surgery were fixed in 10% neutral buffered formalin for 6–48 hours. After grossing and processing, paraffin blocks were prepared and 3–4 micron thick sections were taken and stained with Harris hematoxylin and eosin. Slides were screened for carcinomatous changes. Sections of 3–5 μm thickness of paraffin embedded tissue were taken for IHC. A known positive and negative control section is included in each run to ensure proper staining. The immunostain clones used for EGFR, HER2 and Ki67 were rabbit monoclonal antibody SP1, SP3 and SP6 respectively.

Reporting results of EGFR testing by immunohistochemistry:

Result	Criteria
Negative (Score 0)	No labeling OR Labelling in < 10% of Invasive Tumor Cells
Negative (Score 1)	Weak labeling, homogeneous or patchy in >10% of Invasive tumor Cells
Positive (Score 2)	Moderate labeling, homogeneous or patchy in >10% of the invasive tumor cells.
Positive (Score 3)	Intense labeling, homogeneous or patchy in >10% of the invasive tumor cells.

Reporting results of HER2 testing by immunohistochemistry:

Result	Criteria
Negative (Score 0)	No Staining Observed OR Incomplete faint / barely perceptible membrane staining ≤10% of Invasive Tumor Cells
Negative (Score 1+)	Incomplete, faint / barely perceptible membrane staining > 10% of Invasive Tumor Cells.
Equivocal (Score 2+)	Incomplete and / or weak to moderate circumferential membrane staining in > 10% of Invasive Tumor Cells. OR Complete, intense circumferential membrane staining in ≤10% of Invasive Tumor Cells.
Positive (Score 3+)	Complete, intense circumferential membrane staining in > 10% of Invasive Tumor Cells.

The statistical analysis was done by using SPSS 17 software.

Results

Among 68 cases studied, there were 18 dysplastic lesions (26.47%) and 50 SCCs (73.53%). Males were predominant accounting for 55 (80.9%) patients and 13 (19.11%) were females.

The mean age is 53.86 with median age of 55 years ranging from 31 to 77 years. Majority of the patients 33.82% (23) were in the age group of 51–60 age group.

The commonest site was found to be buccal mucosa [29.41% (20)]. The commonest site in males was buccal mucosa whereas in females it was tongue. Commonest site for dysplastic lesion in both was tongue (33.33%) but for OSCC was buccal mucosa (32%).

Well-differentiated SCC (WDSCC) cases were 34 in number (50%), whereas 18 (26.47%) were dysplastic lesions, amongst which majority were found to have moderate dysplasia (55.55%).

EGFR was expressed up to superficial layer in 44% cases (8 out of 18), up to upper spinous layer in 33.33% cases and up to deep spinous layer in 16.66% cases. In both moderate and severe dysplasia EGFR expression was more up to superficial layer being 60% and 50% respectively whereas in mild dysplastic epithelium 50% of cases show expression up to upper spinous, 50% up to deep spinous layers and no expression was observed in superficial layer. (Table 1) (Fig. 1).

EGFR score was found to be positively expressed (Score 2 & 3) in 76.46% cases whereas it was negative (score 0 & 1) in 23.52% cases (Fig. 2). EGFR positivity was found to be 25% in mild dysplasia but 75% in severe dysplasia and in 76.4% of cases of WDSCC. When EGFR expression of different grades of oral lesions (including all dysplastic and SCC) were analyzed, it was found to be statistically significant (p value = 0.000127). HER2 expression is negative in majority of the dysplastic lesions (Table 2). Both mild and severe dysplastic lesion show 75% of HER2 negativity and in moderate dysplasia showed negativity in 80% cases.

Majority of cases 72% (36) were found to be HER2 negative and 28% (14) were HER2 positive. Among the 34 cases of WDSCC, 24 (70.58%) were found to be HER2 negative and 10(29.41%) were found to be HER2 positive, whereas all cases (100%) of PDSCC showed HER2 negativity. (Table 2) (Fig. 3)

On comparison of HER2 expression with EGFR expression, 26.15% cases (including preneoplastic lesions) showed both EGFR and HER2 over

expression, the coexpression being 16.6% and 28% in dysplasia and OSCC respectively. Majority of cases; i.e. 10 out of 17 cases (58.8%) were WDSCC. Amongst the different grades 29.4% of WDSCC, 33.33% of MDSCC, and none of PDSCC showed coexpression of EGFR and HER2 (Table 3).

HER2 expression for both dysplastic epithelium and OSCC was not statistically significant ($p = 0.633755$).

Study showed high proliferation (Ki67> 20%) in all cases of severe dysplastic lesion whereas 50% of cases showed high proliferation index in mild dysplastic lesion (Table 4). Ki67 labelling index is found to be higher in OSCC as compared to that of dysplastic lesion. All the cases of PDSCC (4) and MDSCC (12) showed high Ki67 index (i.e, > 20%) whereas 76.47% cases of WDSCC expressed high Ki67 proliferation (Fig. 4). It was found to be statistically significant (p value < 0.05).

Both Ki67 index and EGFR positivity increase with increased histological grade (Table 2).

Table 4 depicts overall expression of biomarkers (EGFR, HER2 and Ki67) in the spectrum of oral squamous lesion which showed EGFR positivity in 77.94%, HER2 positivity of 26.47% and high proliferation in 83.82% of cases respectively.

Different stages of OSCC showed different expressions of EGFR, HER2 and Ki67 proliferation. Stage T1 showed overexpression of EGFR in 81.8%, HER2 in 27.3% and high Ki67 in 91% whereas stage T4 showed EGFR in 66.7%, HER2 in 22.3% and high Ki67 in 100% (Table 5).

Out of 50 cases of OSCC, 3 cases showed nodal metastasis, one of them coexpress EGFR and HER2, another showed EGFR positivity but HER2 negativity and both of them showed high Ki67 proliferation. The 3rd one was negative for HER2 and EGFR with low Ki67 proliferation.

Table 1: Extent of EGFR expression of mucosal epithelial cell layers in cases of dysplasia

Type of dysplasia	EGFR score (0)	EGFR score (1/2/3)		
		Up to deep spinous layer	Up to upper spinous layer	Up to superficial layer
Mild dysplasia (4)		2 (50%)	2 (50%)	-
Moderate dysplasia (10)		1 (10%)	3 (30%)	6 (60%)
Severe dysplasia (4)	1 (25%)	-	1 (25%)	2 (50%)
Total (18)	1 (5.55%)	3 (16.66%)	6 (33.33%)	8 (44.44%)

Table 2: Biomarkers (EGFR, HER2, Ki67) positivity

Histological grade	EGFR +ve	Percentage	HER2 +ve	Percentage	High Ki67	Percentage
Dysplasia (18)	12	66.66%	04	22.22%	15	83.33%
WDSCC (34)	26	76.47%	10	29.41%	26	76.47%
MDSCC (12)	11	91.66%	04	33.33%	12	100%
PDSCC (04)	04	100%	00	00	04	100%
Total (68)	53	77.94%	18	26.47%	57	83.82%

Table 3: Number of cases showing both high EGFR and HER2 overexpression

Histological type	No. of cases	Both EGFR & HER2+ve	% of positivity
Dysplasia	18	03	16.66%
WDSCC	34	10	29.4%
MDSCC	12	04	33.33%
PDSCC	04	00	00
Total	68	17	25%

Table 4: Ki67 expression in oral dysplastic epithelium

Type of dysplasia	Low Ki67 (≤20%)	Percentage	High Ki67 (>20%)	Percentage
Mild dysplasia (4)	2	50%	2	50%
Moderate dysplasia (10)	1	10%	9	90%
Severe dysplasia (4)	0	00	4	100%
Total (18)	3	16.66%	15	83.33%

Table 5: Biomarkers in different stages of tumor

Stage	No. of cases	EGFR score		HER2 score		Ki67 proliferation	
		(0 & 1)	(2 & 3)	(0 & 1)	(2 & 3)	Low Ki67	High Ki67
T1	11	18.2%(2)	81.8%(9)	72.7%(8)	27.3%(3)	9%(1)	91%(10)
T2	6	0	100%(6)	50%(3)	50%(3)	16.7%(1)	83.3%(5)
T3	3	33.3%(1)	66.7%(2)	33.3%(1)	66.7%(2)	0	100%(3)
T4	9	33.3%(3)	66.7%(6)	77.7%(7)	22.3%(2)	0	100%(9)
Total	29	24.1%(7)	75.8%(22)	65.5%(19)	34.5%(10)	6.9%(2)	93.1%(27)

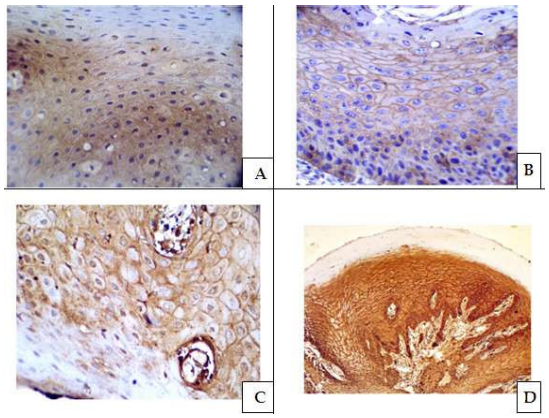

Fig 1: (A) EGFR expression Score 1 in moderate dysplastic epithelium extend up to deep spinous layer, (B) EGFR expression Score 2 in moderate dysplastic epithelium extend up to upper spinous layer, (C) EGFR expression (Score 2) in moderate dysplastic epithelium extend up to superficial layer, (D) EGFR expression (Score 2) in moderate dysplastic epithelium extend up to superficial layer (40X).

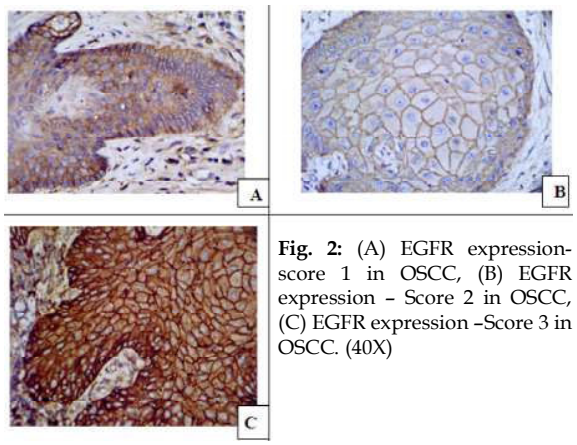

Fig. 2: (A) EGFR expression-score 1 in OSCC, (B) EGFR expression – Score 2 in OSCC, (C) EGFR expression –Score 3 in OSCC. (40X)

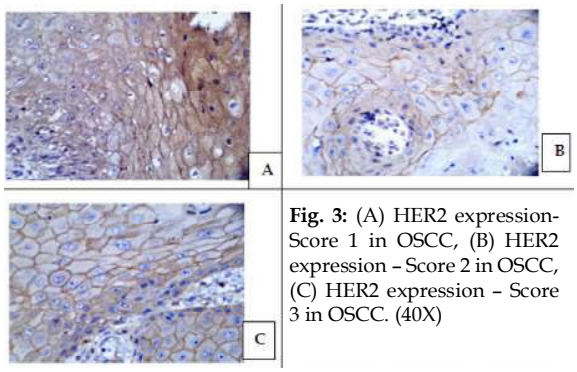

Fig. 3: (A) HER2 expression-Score 1 in OSCC, (B) HER2 expression – Score 2 in OSCC, (C) HER2 expression – Score 3 in OSCC. (40X)

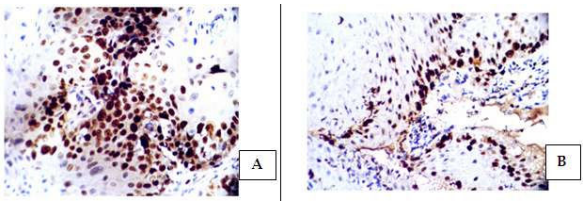

Fig. 4: (A) High Ki67 proliferation index, (B) Low Ki67 proliferation index (40X).

Discussion

Present study evaluates EGFR expression in different layers of dysplastic epithelium. It was observed that in both moderate and severe dysplasia EGFR overexpression are more in superficial layer (60% & 50% respectively) whereas in mild dysplastic lesion 50% of cases show expression in upper spinous and 50% in deep spinous layers, no expression is observed in superficial layer. Similarly Rajeswari M et al.[27] showed that in mild dysplasia, 80% cases showed moderate staining reactions in the basal layer and 15% of cases showed intense staining reactions in the deep spinous layers. Severely dysplastic lesions showed an intense staining reaction not only in the basal, deep spinous layer but also in 50% of cases of superficial spinous layer. These observations show that expression of EGFR is more in superficial layer in higher grades of dysplasia. It implies increased proliferative activity in the abnormal location, i.e. the nonproliferative compartment also which can be explained by altered regulation of cell growth and formation of abnormal receptors, which may be due to gene mutation or gene rearrangements.

In one case of severe dysplasia EGFR expression was completely lacking. The lesion was located in the retromolar region involving tonsil which is likely to be HPV driven.

It has been found that most OSCC are histologically diagnosed as MD or WD tumors[28–31] as was also shown by the present results. In contrast, Effiom et al.[32] have shown that 47.6% of their cases were histologically classified as PD tumors, while WD tumors represented 32.6% of their sample.

In present study varying degrees of expression

of EGFR was observed in oral dysplasia (66.67%) and SCC (82%). Dalal et al.[33] showed similar results i.e., 79.16% (19/24) in dysplasia and 84.4% (38/45) in OSCC.

Our study has shown that EGFR overexpression is associated with higher grades and lesser differentiated tumors (76.47% in WDSCC, 91.66% in MDSCC and 100% in PDSCC). This observation is also reflected in dysplastic lesions where EGFR positivity is observed in 25% of mild dysplastic lesion in contrast to 75% in severe dysplasia. Present study also established statistically significant difference in EGFR expression between cases of dysplasia and OSCC. In the study by Dalal et al.,[33] out of 22 cases of well-differentiated carcinoma, 6 (27.3%) showed high expression while 16 cases (72.7%) showed low expression of EGFR. On the other hand, 15 of 23 cases (65.2%) of the lesser-differentiated forms showed high expression while 8 cases (34.8%) showed low expression. On the contrary, Bernardes et al.[34] and Yamada et al.[35] found the EGFR expressing carcinomas to be well differentiated in most of the cases. These studies documented those EGFR-positive lesions presented as low-grade tumors, revealing no association with patient outcome.

In the present study in 29 cases of OSCC staging could be performed. Majority belonged to stage T1 (37.9%) followed by Stage T4 (31%). In contrast, Gervásio et al.[36], reviewing 740 OSCC patients, revealed that almost 50% of their patients with OSCC were diagnosed as T4 tumors. It seems that tumors are being diagnosed in earlier stages in recent years.

Dalal et al.[33] showed statistically significant correlation between EGFR expression, tumor size, node status and stage of tumor. However no specific correlation between EGFR overexpression and tumor stage could be established in the present study.

In contrast to other studies, Kimura et al.[37] in their study showed inverse correlation between EGFR expression and invasiveness of the tumor.

Kim et al.[20] showed that the higher immunoexpression of Ki67 was associated with worse survival rate suggesting that may be useful in predicting the prognosis. Myoung et al.[38] demonstrated that immunostaining of Ki67 increased in tumors with cervical lymph node metastasis. They have shown that Ki67 and clinical stage are independent prognostic factors in evaluating survival in patients with OSCC. In accordance with these studies our study showed higher Ki67 labeling index with higher grades of tumor.

Most of the cases of dysplasia as well as OSCC were negative for HER2 expression. Positivity was noted in 22.22% of dysplasia and 28% of OSCC with no statistical significance. This is in contrast with Dalal et al.[33] who identified a relatively high positivity of 66.7% (16/24) in cases of dysplasia and 62.2% (28/45) in cases of OSCCs. High expression was found in 18% (2/11) cases of mild, 50% (4/8) of moderate and 60% (3/5) of severe dysplasia while 82% (9/11), 50% (4/8) and 40% (2/5) cases respectively showed low expression. However no significant correlation was seen between the severity of dysplasia and HER2 neu and EGFR expression.

There was no correlation between percentage of HER2/neu staining and tumor differentiation.

Xia et al.[39] studied a series of 111 patients with SCC and found that the expression of all four EGFR members was significantly associated with shortened patient survival, and the association was strongest for HER2/neu. They reported HER2/neu to be the most significant single factor in predicting disease outcome. Conflicting results in different studies might be due to using different immunohistochemical methods or type of antibody used.

Hence further study with larger number of samples of both dysplasia and OSCC along with long-term follow up is required to establish the correlation of EGFR and HER2 with grade, stage, overall survival, disease free survival, rate of recurrence with introduction of targeted therapy.

Moreover molecular sub classification of OSCC based on EGFR status may serve as important information for appropriate therapeutic strategies.

Conclusion

OSCC is a growing cancer burden worldwide. The situation is even more threatening in our country. Studies have found that morbidity and mortality have not decreased substantially in spite of improved surgical treatment. This mandates the need for better understanding of molecular mechanism and therapeutic targets as an adjunct to the surgical modalities.

We have evaluated expression of biomarkers (EGFR, HER2, Ki67) in dysplasia and OSCC and observed that EGFR expression increases with higher grade and lesser-differentiated lesions expression of which also corroborated with the proliferation activity of the tumor.

Present study also evaluated EGFR expression in different layers of dysplastic epithelium, which shows that expression of EGFR is more in superficial layer along with expression in lower layers in higher grades.

However no such correlation was found with respect to HER2 expression, hence its role could not be established as a prognostic indicator.

Therefore we conclude that EGFR overexpression is associated with higher grades and higher intrinsic proliferative activity. Hence EGFR can serve as a good prognostic indicator and a target for anti-cancer therapy.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

1. Choi S, Myers J. Molecular Pathogenesis of Oral Squamous Cell Carcinoma: Implications for Therapy. Journal of Dental Research 2008;87(1):14–32.
2. Williams H. Molecular pathogenesis of oral squamous carcinoma. Molecular Pathology 2000;53(4):165–72.
3. Scully C, Felix D. Oral Medicine — Update for the dental practitioner Oral Cancer. British Dental Journal 2006;200(1):13–17.
4. Byakodi R, Byakodi S, Hiremath S, Byakodi J, Adaki S, Marathe K et al. Oral Cancer in India: An Epidemiologic and Clinical Review. Journal of Community Health 2011;37(2):316–19.
5. Way, L. Current surgical diagnosis and treatment. 11th ed. New York; London: Lange Medical Books/McGraw Hill, c2003.pp.282–97.
6. Schwartz S, Shires G. Principles of surgery. 7th ed. New York: McGraw-Hill, Health Professions Division 1999.
7. Warnakulasuriya S, Johnson N, Van Der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. Journal of Oral Pathology and Medicine 2007;36(10):575–80.
8. Jr S, Gorsky M, Ms F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer 1984;53(3):563–68.
9. Kulkarni D, Wadia P, Pradhan T, et al. Mechanisms involved in the down-regulation of TCR ζ chain in tumor versus peripheral blood of oral cancer patients. International Journal of Cancer 2009;124(7):1605–13.
10. Angiero F, Gatta L, Seramondi R, et al. Frequency and role of HPV in the progression of epithelial dysplasia to oral cancer. Anticancer Res 2010; 30(9):3435–40.
11. Nuovo G. In situ detection of human papillomavirus DNA after PCR amplification. Methods Mol Biol 2011;688:35–46.
12. Scully C, Felix D. Oral Medicine — Update for the dental practitioner Oral Cancer. British Dental Journal 2006;200(1):13–17.
13. Syrjanen S. The role of human papillomavirus infection in head and neck cancers. Annals of Oncology 2010;21(Supplement 7):243–245.
14. Jalouli J, Ibrahim S, Mehrotra R, et al. Prevalence of viral (HPV, EBV, HSV) infections in oral submucous fibrosis and oral cancer from India. Acta Oto-laryngologica. 2010;130(11):1306–11.
15. Califano J, Van der Riet P, Westra W, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996;56(11):2488-92.
16. Markopoulos A, Albanidou-Farmaki E, Kayavis I. Actinic cheilitis: clinical and pathologic characteristics in 65 cases. Oral Diseases. 2004;10(4):212–16.
17. Preston D, Ron E, Tokuoka S, et al. Solid cancer incidence in atomic bomb survivors: 1958-1998. Radiat Res 2007;168(1):1–64.
18. O'Grady J, Reade P. Candida albicans as a promoter of oral mucosal neoplasia. Carcinogenesis 1992;13(5):783–86.
19. Bettendorf O, Herrmann G. Prognostic Relevance of Ki-67 Antigen Expression in 329 Cases of Oral Squamous Cell Carcinoma. ORL. 2002;64(3):200–205.
20. Kim S, Shin H, Jung K, et al. Prognostic Value of Carbonic Anhydrase IX and Ki-67 Expression in Squamous Cell Carcinoma of the Tongue. Japanese Journal of Clinical Oncology 2007;37(11):812–19.
21. Marsh D, Suchak K, Moutasim K, et al. Stromal features are predictive of disease mortality in oral cancer patients. The Journal of Pathology 2011;223(4):470–81.
22. Hamakawa H, Nakashiro K, Sumida T, et al. Basic evidence of molecular targeted therapy for oral cancer and salivary gland cancer. Head and Neck 2008;30(6):800–09.
23. Aboud-Pirak E, Hurwitz E, Pirak M, et al. Efficacy of Antibodies to Epidermal Growth Factor Receptor Against KB Carcinoma In Vitro and in Nude Mice. JNCI Journal of the National Cancer Institute 1988;80(20):1605–11.
24. Dai Q. Enhanced Sensitivity to the HER1/ Epidermal Growth Factor Receptor Tyrosine

Kinase Inhibitor Erlotinib Hydrochloride in Chemotherapy-Resistant Tumor Cell Lines. Clinical Cancer Research 2005;11(4):1572–78.

25. Bourhis J. New approaches to enhance chemotherapy in SCCHN. Annals of Oncology 2005;16(suppl_6):vi20–vi24.
26. Chan J, Pham H, You X, et al. Suppression of Ovarian Cancer Cell Tumorigenicity and Evasion of Cisplatin Resistance Using a Truncated Epidermal Growth Factor Receptor in a Rat Model. Cancer Research 2005;65(8):3243–48.
27. Rajeswari M, Saraswathi T. Expression of epithelial growth factor receptor in oral epithelial dysplastic lesions. Journal of Oral and Maxillofacial Pathology 2012;16(2):183.
28. Al-Rawi N, Talabani N. Squamous cell carcinoma of the oral cavity: a case series analysis of clinical presentation and histological grading of 1,425 cases from Iraq. Clinical Oral Investigations 2007;12(1):15–18.
29. Andisheh-Tadbir A, Mehrabani D, Heydari S. Epidemiology of Squamous Cell Carcinoma of the Oral Cavity in Iran. Journal of Craniofacial Surgery 2008;19(6):1699–1702.
30. Langlais R, Jainkittivong A, Swasdison S, et al. Oral Squamous Cell Carcinoma: A Clinicopathological Study of 342 Thai Cases. The Journal of Contemporary Dental Practice 2009;10(5):33–41.
31. Larsen S, Johansen J, Sørensen J, et al. The prognostic significance of histological features in oral squamous cell carcinoma. Journal of Oral Pathology and Medicine 2009;38(8):657–62.
32. Effiom O, Adeyemo W, Omitola O, et al. Oral Squamous Cell Carcinoma: A Clinicopathologic Review of 233 Cases in Lagos, Nigeria. Journal of Oral and Maxillofacial Surgery 2008;66(8):1595–99.
33. Dalal V, Alam K, Maheshwari V, et al. Correlation of clinicopathological parameters with EGFR and HER2 status in oral cancer patients in a tertiary care center. International Journal of Biomedical Research 2015;6(11):897–902.
34. Bernardes V, Gleber-Netto F, Sousa S, et al. EGFR status in oral squamous cell carcinoma: comparing immunohistochemistry, FISH and CISH detection in a case series study. BMJ Open 2013;3(1):e002077.
35. Yamada T, Takagi M, Shioda S. Evaluation of epidermal growth factor receptor in squamous cell carcinoma of the oral cavity. Oral Surgery, Oral Medicine, Oral Pathology 1992;73(1):67–70.
36. Gervásio OL, Dutra R, Tartaglia S, et al. Oral squamous cell carcinoma: a retrospective study of 740 cases in a Brazilian population. Braz Dent J. 2001;12(1):57–61.
37. Kimura I, Kitahara H, Ooi K, et al. Loss of epidermal growth factor receptor expression in oral squamous cell carcinoma is associated with invasiveness and epithelial-mesenchymal transition. Oncology Letters 2015;11(1):201–07.
38. Myoung H, Kim M, Lee J, et al. Correlation of proliferative markers (Ki-67 and PCNA) with survival and lymph node metastasis in oral squamous cell carcinoma: a clinical and histopathological analysis of 113 patients. International Journal of Oral and Maxillofacial Surgery 2006;35(11):1005–10.
39. Xia W, Lau Y, Zhang H, et al. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. Clin Cancer Res 1999;5(12):4164–74.