# ROLE OF ERK IN ORAL CANCER PROGRESSION IN PAKISTANI POPULATION

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## Abstract

***Objectives:*** *Oral cancer is highly prevalent in Pakistan and is becoming a global health priority. More than 90% of oral and oropharyngeal cancers are squamous cell carcinoma. The purpose of this study was to assess the role of the ERK pathway in oral cancer diagnosis and progression in the Pakistani population.*

***Materials & Methods:*** *A total of 53 samples comprising of 25 well-differentiated grade I OSCC and 25 samples of poorly differentiated grade III OSCC and 03 normal healthy samples were included in the study. All samples were processed for RNA extraction, and Real-Time PCR followed by RT PCR. The data so obtained are separately recorded for each gene, and the value was entered in the SPSS.*

***Results:*** *ERK 1/2 showed increased expression level with the proliferation of the tumour cells. Mean Ct values for Normal was 38.1+ 0.11 1/RFU, Grade I 20.43 + 1.08 1/RFU, & Grade III 9.11 + 1.25 1/RFU. Grade III has a significantly higher level of expression as compared to the healthy group (with $p<0.01$). No significant difference could be observed in the samples of healthy tissue as compared to grade I.*

***Conclusion:*** *This study provides valuable data regarding the expression of ERK among the Pakistani population. Genetic alterations have a significant role in the initiation and progression of OSCC. We can say our data demonstrate a basis for the molecular diagnosis of OSCC and this information can be beneficial in future.*

***Key Words:*** *Oral cancer, oral squamous cell carcinoma, MAPKinase, ERK1, ERK2*

## Introduction

Oral Squamous cell carcinoma is one of the most common malignant neoplasm representing about 90% of all oral malignancies[1,2]. The incidence of OSCC varies according to geographic region, being more common in developing countries[3]. According to the World Health Organization, it is the eighth most common cancer worldwide4 whereas in Southeast Asia OSCC is ranked as the second most frequent cancer[5]. It is a cause of great concern in Pakistan also, and the high incidence of oral cancer is seen in all areas of Pakistan[6].

Tobacco (betel quid, areca nut) and alcohol consumption are the main causes of developing OSCC[7]. Other predisposing factors include human papillomavirus (HPV) infection, dietary deficiencies, chronic trauma, exposure to radiations and genetic factors[8]. Recent advancements in research have enlightened the role of oncogenes and basic knowledge of the mechanisms controlling head and neck cancer development and progression[9]. Multiple signalling pathways, tumour suppressor genes and oncogenes

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are involved in the development of OSCC. Among these, the most common ones are p53, p16, Ras and Epidermal Growth Factor Receptor (EGFR)[10]. The overexpression of either EGFR or Ras can play a vital role in carcinogenesis by deregulating multiple intracellular pathways[10]. One of the major signalling routes downstream of EGFR is the mitogen-activated protein kinase (MAPK) pathway.

The MAPK signalling pathway is composed of four subfamilies, which includes the extracellular signal-regulated kinase (ERK)1/2, the c Jun N terminal kinase (JNK) and p38 MAPKs[11].

Up till now, among MAPK family, the ERK1 and ERK2 have received the most attention[12]. The ERK signalling is associated with the proliferation, differentiation, apoptosis, angiogenesis, invasion and metas¬tasis of tumour cells[13]. Usually, an extracellular stimulus such as binding of growth factor or hormone to cell surface receptors starts the ERK signalling pathway and causes activation of ERK which gets translocated in the nucleus, where ERK activates transcription factors that govern the cellular responses[14].

Development of oral squamous cell carcinoma (OSCC) is a multistep process which involves several environmental carcinogens causing genetic mutations and chromosomal abnormalities[15]. In spite of so many advances in the field of diagnosis and therapeutics in the past two to third decades still, the morbidity and mortality rate of OSCC has not improved significantly.

Better prognosis and survival rate is highly dependent on the stage at which a tumour is diagnosed. Early identification of lesion is necessary to reduce the morbidity and mortality associated with oral cancers[16]. Therefore, there is an urgent need to gain a better understanding of the molecular mechanisms underlying the development of OSCC for improving early diagnosis and predicting the progression of cancer. So that new therapeutic targets can be identified to inhibit unwanted cellular mechanisms. The present study was conducted with the aim of identifying molecular markers, which would be useful for diagnosing oral cancer at an early stage and predict the extent of malignant changes in OSCC in Pakistani population.

## Materials and Methods

The current study was conducted in Centre of Research of Molecular Medicine throughout three years. After taking ethical clearance, the collection of oral cancer samples were started. A total of fifty-three (53) samples were included in the study. Out of this fifty-three, twenty-five (25) were well-differentiated grade I OSCC, whereas another set of twenty-five (25) samples was of poorly differentiated grade III OSCC. Three samples of normal tissue were also included in the study.

Patients of all ages having oral cancer either Grade1 or Gerade III OSCC were included in the study, whereas patients are having a history of radiotherapy or chemotherapy were not included in the study. Grade 1 samples showed hyperchromatism, increased mitosis, altered nucleus cytoplasmic ratio and other features of dysplasia. Overall they had less than 25% of undifferentiated cells. Whereas Grade III samples show features of minimal keratinization, abundant nuclear polymorphism, increased mitosis. Further cells showed lack of orientation, loss of polarity, loss of cohesiveness and invasion. Overall OSCC Grade III tissue samples had 75% or more of undifferentiated cells.

All samples were processed for RNA extraction and Real-Time PCR (RT PCR). To run the samples of RNA in the Multiplex RT-PCR format, specific Evergreen primers were designed for ERK-1 and ERK-2. Extracted RNA was subjected to PCR for generating cDNA. This cDNA was used as a template for multiplex RT-PCR. Quantification was done with primers for 45cycles, and the mean Ct (Cycle Threshold) values of each group were used to draw the graph with standard error represented as vertical solid Bars. The computer data generated by Multiplex RT-PCR was represented by Relative Fluorescent Unit (RFU) as generated by CFX- Manager. The data so obtained are separately recorded for each gene, and the value was entered in the SPSS.

## Results

ERK 1/2 showed increased expression level with the proliferation of the tumour cells. Mean Ct values for normal samples were 38.1+ 0.11 1/RFU, whereas the mean values of Grade I OSCC samples were 20.43 + 1.08 1/RFU. Grade III OSCC samples had a significantly higher level of expression as

compared to a healthy group showing the mean value of & Grade III 9.11 + 1.25 1/RFU (with $p<0.01$).

The data of the genes showed progressive changes in two grades of oral carcinoma: well-differentiated Grade1 and Poorly differentiated Grade III OSCC tissue. It is seen that both Erk-1 and Erk- 2 expression raised to 1- fold from normal in well-differentiated tissues, and the rise continued to 4--folds in poorly differentiated tissues. This continues to rise between the two grades of oral cancer showed that ERK-1 and ERK- 2 plays an important role in promoting cell proliferation.

Fig1 shows expression of ERK-1 in Normal, Grade I and Grade III tissues samples of OSCC as histograms. Presence of * on a vertical bar is showing the significant/non-significant or highly significant value taken from the Oneway ANOVA after applying the tukeys. Results of our study clearly show that there are increased expression levels of ERK1 with high proliferation rate which is seen more in grade III as compared to grade I OSCC.

Figure2 In this figure the grades are shown in arbitrary units on the X-axis, and reciprocal of Ct values are shown on Y-axis. Curve estimation was plotted by taking mean Ct values of well-differentiated oral squamous cell carcinoma Grade I and poorly differentiated oral squamous cell carcinoma Grade III. Dotted lines represent the inverse of Ct values. A straight line is the trend line which shows the expression of genes in different grades of OSCC.

Figure: 3 Expression of ERK2 in Normal, Grade I and Grade III tissues samples of OSCC is shown as histograms in figure 4.16. Presence of * on a vertical bar is showing the significant/non-significant or highly significant value taken from the Oneway ANOVA after applying the Tukeys.

Figure: 4 In this figure the grades are shown in arbitrary units on the X-axis and reciprocal of Ct values are shown on Y-axis. Curve estimation was plotted by taking mean Ct values of well-differentiated oral squamous cell carcinoma Grade I and poorly differentiated oral squamous cell carcinoma Grade III. Dotted lines represent the inverse of Ct values. A straight line is the trend line which shows the expression of genes in different grades of OSCC.

## Discussion

Head and neck cancer is a global health concern with an approximate diagnosis of 630,000 new patients each year resulting in more than 350,000 deaths annually17. Almost 90% of head and neck cancers are squamous cell carcinomas arising either in the

**Fig: 1 Expression of ERK1 in well differentiated (Grade I) and poorly differentiated (Grade III) Oral Squamous Cell Carcinoma**

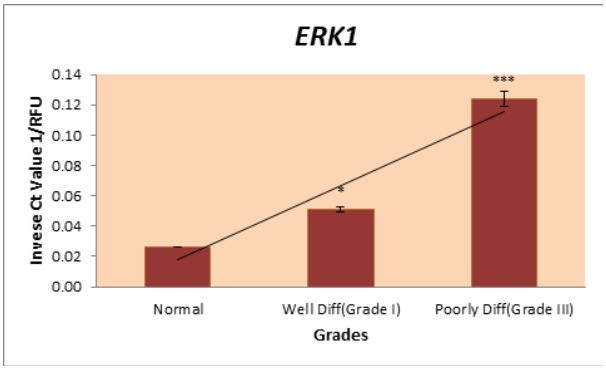



**Fig: 2 Curve Estimation: Expression of ERK1 in well-differentiated oral squamous cell carcinoma (Grade I) and poorly differentiated oral squamous cell carcinoma (Grade III)**

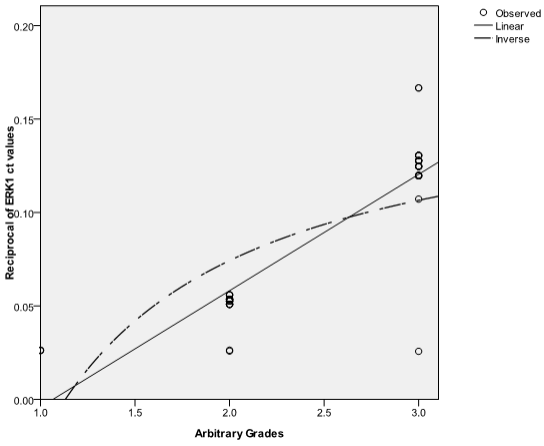



**Fig: 3 Expression of ERK2 in well differentiated (Grade I) and poorly differentiated (Grade III) Oral Squamous Cell Carcinoma**

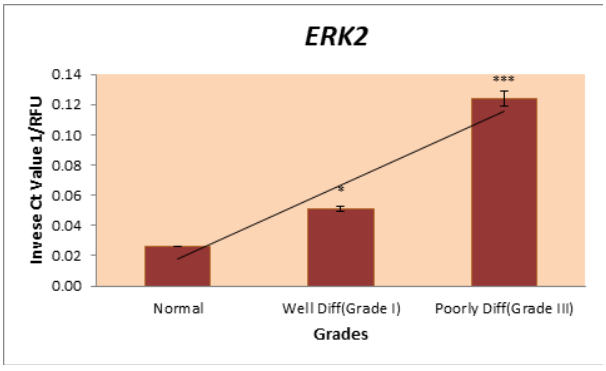
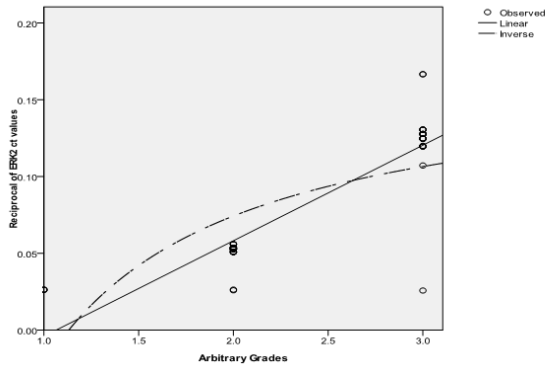


**Fig: 4 Curve Estimation: Expression of ERK2 in well-differentiated oral squamous cell carcinoma (Grade I) and poorly differentiated oral squamous cell carcinoma (Grade III)**




mucous membranes of the mouth or oropharynx 18. Sometimes oral cancer develops from precancerous lesions or potentially malignant disorders of the oral cavity and hence contributing towards the high burden of oral cancer19. Both the development of oral precancerous lesions or cancerous lesions depends on genetic and epigenetic factors.

Tobacco, alcohol, diet and nutrition, viruses, radiation, ethnicity, oral thrush, immunosuppression, use of mouthwash, syphilis, dental factors, occupational risks all come under epigenetic factors20. These risk factors may cause the initiation of genetic events through chromosomal alteration or mutations in genetic material leading towards progression and development of oral cancer through the propagation of events at both cellular and clinical levels21. Oral squamous cell carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumour suppressor genes.

EGFR is a cell surface tyrosine kinase transmembrane receptor. When cell surface receptor binds to its specific ligand, they initiate a cascade of intracellular biochemical steps causing downstream activation of complex interacting signalling pathways which include the Ras/Raf/MEK/ERK and the Ras/PI13K/PTEN/AKT/mTOR pathways22. Ras/Raf/MEK/ERK cascade reaction is an important signalling pathway in MAPKs23.

Mitogen-activated protein kinase (MAPK) is a serine-threonine kinase that is activated by various extracellular stimuli. An extracellular signal-regulated kinase (ERK1 and ERK2), a MAPK subfamily, are activated by many oncogenes24.

To understand the cross-talks between these pathways and the role of the Erk pathway in oral cancer progression in Pakistani population, we conducted this research project. Our study shows an almost 1fold increase of ERK1/2 in well-differentiated tissues which rise to 4-fold increase as the tissue progresses to poorly differentiated stage which is in line with the study of Wang. He also concluded that the expression of activated ERK1/2 and Ki-67 were significantly stronger in OSCC than in normal oral mucosa and these were more with a moderately or poorly differentiated grade of carcinoma25.

Results of our study are also following the study of Inoue Kazuya in 2003 as he observed that extracellular signal-regulated kinase (ERK), a MAPK subfamily, is activated by many oncogenes including Ras and Raf, which are closely associated with cell growth and differentiation. He concluded that ERK provides a good clue for assessing the progression or prognosis of OSCC26. The studies mentioned above are closely related to our findings as we also observed that ERK1/2 are expressed in both grades, but slight more expression has been noticed in poorly differentiated OSCC.

Genetic alterations play a vital role in the process of cancer development and progression. We can say that our data demonstrate a basis for the molecular diagnosis of OSCC and may be useful in innervating the pathways which are involved in the initiation and progression of tumours. This information combined with histopathological reports can further improve our understanding regarding the prognosis of oral squamous cell carcinoma.

The effects of mitogen-activated protein kinases (MAPKs) and particularly extracellular signal-regulated kinase 1/2 (ERK1/2) signalling in OSCC have not been thoroughly investigated. Further studies on larger sample size are needed to improve the understanding of genes and signalling pathways for better diagnosis and prognosis of oral squamous cell carcinoma.

## References

1. Attar E, Dey S, Hablas A, Seifeldin IA, Ramadan M, Rozek LS, Soliman AS. Head and Neck Cancer in a Developing Country: A Population-Based Perspective Across 8 Years. Oral Oncology. 2010; 46(8):591-596.

2. 2. Kapoor C, Vaidya S, Wadhwan V, Malik S. Lymph node metastasis: A bearing on prognosis in squamous cell carcinoma. Indian J Cancer. 2015;52:417-24.

3. Petersen PE, Oral cancer prevention and control – The approach of the World. Oral Oncol. 2008.05.023

4. Sharma P, Saxena S, Aggarwal P. Trends in the epidemiology of oral squamous cell carcinoma in western UP: An institutional study. Int J of Den Res. 2010; 21(3): 316-19.

5. Petti S, Masood M, Scully C. The magnitude of tobacco smoking-betel quid chewing-alcohol drinking interaction effect on oral cancer in South-East Asia. A meta-analysis of observational studies. PLoS One. 2013; 8 (11): e78999.

6. Akram S, Mirza T, Mirza A, Qureshi M. (2013). Emerging Patterns in Clinicopathological spectrum of Oral Cancers. Pakistan journal of medical sciences.2013; 29(3):783-7.

7. Dikshit R, Gupta PC, Rama SC, Gajalakshmi V, Aleksandrowicz L. Cancer mortality in India: A nationally representative survey. Lancet. 2012; 379:1807–16.

8. Singh MP, Kumar V, Agarwal A, Kumar R, Bhatt ML, Misra S. Clinical-epidemiological study of oral squamous cell carcinoma: A tertiary care centre study in North India. J Oral Biol Craniofac Res. 2016;6:31-4.

9. Wahid A, Ahmad S, Sajjad M. Pattern of carcinoma of oral cavity reporting at the dental department of Ayub Medical College. J Ayub Med Coll Abbottabad. 2005;17(1):65-6.

10. Aguzzi A, Maggioni D, Nicolini G, Tredici G, Gaini RM, Garavello W. MAP Kinase Modulation in Squamous Cell Carcinoma of the Oral Cavity. Anticancer Research.2009; 29: 303-308.

11. Gkouveris I, Nikitakis N, Karanikou M, Rassidakis G, Sklavounou A. Erk1/2 activation and modulation of STAT3 signalling in oral cancer. Oncol Rep. 2014;32(5):2175-82.

12. Crane EK and Wong KK: The therapeutic promise of anticancer drugs against the Ras/Raf/MEK/ERK pathway. Topics Anti Cancer Res 2: 63 94, 2013.

13. Arkun Y, Yasemi M. Dynamics and control of the ERK signalling pathway: Sensitivity, bistability, and oscillations PLoS One 13(4):e0195513

14. McCain J. The MAPK (ERK) Pathway: Investigational Combinations for the Treatment Of BRAF-Mutated Metastatic Melanoma. P & T: a peer-reviewed journal for formulary management. 2013;38(2): 96-108.

15. Jayasooriya PR, Pitakotuwage TN, Mendis BR, Lombardi T. Descriptive study of 896 oral squamous cell carcinomas from the only university-based Oral Pathology Diagnostic Service in Sri Lanka. BMC Oral Health. 2016;16:1

16. Garg P, Karjodkar F. Catch them before it Becomes Too Late -Oral Cancer Detection. Report of Two Cases and Review of Diagnostic AIDS in Cancer Detection. International journal of preventive medicine. 2012; 3(10): 737-41.

17. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral and maxillofacial surgery clinics of North America.2014; 26(2): 123-41.

18. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our Rural Health Care Centre in Loni, Maharashtra - a retrospective 10-year study. Contemporary Oncology. 2017;21(2):178-183.

19. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007; 36:575–580.

20. Malay K, Ronak N, Tapan M G, Chintan D. Oral cancer: Etiology and risk factors: A review. Journal of cancer research and therapeutics.2016:12(2):458-463.

21. Krishna A, Singh S, Kumar V, Pal US. The molecular concept of human oral cancer. National Journal of maxillofacial surgery.2015;6(1):9-15.

22. Vecchione L, Jacobs B, Normanno N, Ciardiello F, Tejpar S. EGFR-targeted therapy. Exp Cell Res. 2011; 317: 2765–71.

23. Chung E, Kondo M. Role of Ras/Raf/MEK/ERK signalling in physiological hematopoiesis and leukaemia development. Immunol Res. 2011; 49:248–268.

24. Li L, Zhao GD, Shi Z, Qi LL, Zhou LY, & Fu ZX. The Ras/Raf/MEK/ERK signalling pathway and its role in the occurrence and development of HCC. Oncology letters.2016;12(5): 3045-3050.

25. Wang L, Liu T, Nishioka M, Aquirre RL, Win SS, Okada N. Activation of ERK1/2 and cyclin D1 expression in oral tongue squamous cell carcinoma; Relationship between clinicopathological appearance and cell proliferation. Oral Oncol.2006; 42(6):625 -631.

26. Kazuya I, Yasunori E, Kunio I. Expression of phosphorylated ERK and MKP-1 in human oral squamous cell carcinoma .J Nara Med Assoc. 2003:54(1)33-42