RESEARCH ARTICLE

Differential Expression of *c-fos* Proto-Oncogene in Normal Oral Mucosa versus Squamous Cell Carcinoma

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Abstract

Background: The c-Fos nuclear protein dimerizes with Jun family proteins to form the transcription factor AP-1 complex which participates in signal transduction and regulation of normal cellular processes. In tumorigenesis, c-Fos promotes invasive growth through down-regulation of tumor suppressor genes but its role in oral carcinogenesis is not clear. **Objectives:** This study concerned *c-fos* gene expression in normal and malignant tissues of the oral cavity, with attention to associations between expression status and clinico-pathological profiles of OSCC patients. **Method:** A total of 65 histopathologically confirmed OSCC tissue samples were included in case group along with an equal number of age and sex-matched normal tissue samples of oral cavity for the control group. c-Fos protein and m-RNA expressions were analyzed using immunohistochemistry and qRT-PCR, respectively. **Results:** A significant low expression of c-Fos protein was observed in OSCC cases than normal control subjects (p= <0.001). The mean percent positivity of c-Fos protein in cases vs. controls was 24.91± 2.7 vs. 49.68± 2.2 (p= <0.001). Most OSCC tissue samples showed weak or moderate c-Fos expression whereas 53.8% of normal tissue sections presented with strong immunostaining. Moreover, the relative m-RNA expression for the *c-fos* gene was significantly decreased in case group (0.93± 0.48) as compared to the control group (1.22± 0.87). Majority of c-Fos positive cases were diagnosed with well developed tumor. The mean percent positivity of c-Fos protein was significantly lower in higher grade tumor as compared with normal oral mucosa (p= < 0.001). **Conclusion:** The present study suggested that the *c-fos* gene is downregulated in oral carcinomas. The disparity of c-Fos protein levels in different pathological grades of tumor and normal oral tissue samples may indicate that loss of c-Fos expression is related with the progression of OSCC.

Keywords: OSCC- c-Fos- immunohistochemistry- gene expression

Introduction

OSCC (oral squamous cell carcinoma) is sixth most common cancer worldwide with an estimated 300,400 new cases and 145,400 deaths occur annually. However, in India, the incidence of oral cancer is recorded approx 83,000 new cases and over 46,000 deaths each year which is 3 to 7 times higher as compared to developed countries (Torre et al., 2015; Bray et al., 2013; ICMR-NCRP, 2016). The high incidence and poor survival of oral cancer patients are associated with popular oral habits of certain countries of Central and South East Asia such as Sri Lanka, India, Thailand, Indonesia, Pakistan and Bangladesh. Tobacco, which is widely used in India, is a significant cause of cancer of oral cavity (Goyal et al., 2014). Approx 91-95% of oral cancer cases are directly related to tobacco chewing and smoking habits in India (Krishna et al., 2015). Moreover, an increased involvement of Human Papilloma Virus in OSCC has also been reported in last decade (Chaturvedi et al., 2008; Singh V et al., 2015).

The pathogenesis and development of OSCC are closely related to the expression of some proto-oncogenes, in which fos multigene family (*c-fos*, *fos B*, *fra* 1 and 2) is well known. These fos proto-oncogenes encode nuclear proteins that dimerize with Jun family proteins (c-Jun, JunB, and JunD) to form the transcription factor complex i.e. Activating Protein 1 (AP-1). c-Fos and Jun dimmer activate the transcription by binding to core TGAC/GTC/AA sequences (AP-1 responsive elements or AP-1 sites), present in the promoter enhancer regions of other genes (Milde-Langosch , 2005; Angel and Karin 1991). Activation of AP-1 has been implicated in the control of a number of key cellular processes mainly cell differentiation, proliferation and also in signal transduction. The Activity of this complex is tightly regulated by the serine-threonine kinases of mitogen-activated protein kinase (MAPK) family (Whitmarsh and Davis, 1996; Shaulian and Karin, 2001). A dual mechanism of

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control by MAPKs operates over c-Fos, first activation of extracellular signal regulated kinases (ERKs) which leads to coordinated stimulation of *c-fos* gene expression by acting on transcription factors bounded at promoter region and second is the post translational modification through the direct phosphorylation within its NH_2-terminal transactivation domain (TAD), thereby enhancing the transcriptional activity of c-Fos protein (Treisman, 1994; Murphy et al., 2002; Monje et al., 2003). So, c-Fos protein is important for the activation of downstream genes regulated by AP-1.

Many studies focused on its oncogenic functions and found that c-Fos deregulates several target genes like cyclin D1, p16, Bcl-2, Bcl-xL, VEGF, EGFR, MMPs, osteopontin, and CD44 etc., promotes the invasive tumor growth (Bakin and Curran, 1999; Bancroft et al., 2002; Mishra et al., 2010). However, some recent studies have been identified the tumor suppressor activity of c-Fos and indicate its involvement in apoptosis (Teng, 2000; Mahner et al., 2008).

Furthermore, there is inconsistency in findings of *c-fos* gene expression in several human cancers. According to some in vivo investigations in oral cancer, c-Fos protein was overexpressed in dysplasia and well developed tumor (Ohyama et al., 2004; Sachdev et al., 2008). In contrast, Turatti et al., (2005) found the higher level of this protein in premalignant lesions and adjacent normal tissue against the cancerous tissue of oral cavity. Therefore, in this context of controversial findings of c-Fos protein expression in oral pre-cancer and cancer lesions were actuated us to access the *c-fos* gene expression in the normal and malignant tissue of oral cavity, and also explore its association with different clinico-pathological parameters of OSCC patients.

Materials and Methods

Sample collection and diagnosis

Clinically suspected patients with oral malignant and non-malignant lesions who presented for the pathological diagnosis or primary surgery at Departments of Oral and Maxillofacial Surgery, and Surgical Oncology, King George's Medical University (K.G.M.U), Lucknow, U.P., India between January 2013 to June 2016 were included in this study. The clinical and histopathological diagnosis were carried out according to AJCC cancer staging manual and WHO guidelines for the histological grading of the tumor (Barnes et al., 2005; Edge et al., 2010).

The tissue samples were collected after obtaining the informed consent either from patients or their next kin. All biopsies and surgically removed tissues were stored in 4% formaldehyde and RNA later solutions (Invitrogen, USA) for histopathological diagnosis and estimation of c- Fos protein and messenger-RNA (m-RNA). This study design was approved by the Institutional Ethics Committee, K.G.M.U, Lucknow, U.P., India. Patients who had undergone/completed therapy, diagnosed with secondary or any other malignancy were excluded from the present study.

A total of 150 patients were clinico-pathologically analyzed. In the Hematoxylin and Eosin (HE) staining,

out of total tissue samples 65 samples were confirmed for OSCC and included in the case group. Moreover, equal number of age and sex-matched tissue samples from non-malignant lesions of the oral cavity were checked and verified for normal epithelial cell morphology (without any sign of dysplastic conditions) considered for control group.

Immunohistochemistry (IHC)

The c-Fos protein expression was performed on 4μm paraffin-embedded tissue sections by using c-Fos rabbit polyclonal antibody (sc-52, Santa Cruz, USA, 1:200 dilution) and anti-rabbit horseradish peroxidase (HRP) labeled-secondary antibody detection kit (TL-015-QHD, Thermo Fisher Scientific, USA) as per the manufacturer's protocol. Three percent hydrogen peroxide solution and protein block reagent were used to stop the enzymatic activity and non-specific binding of the protein. Antigen retrieval of tissue sections was carried out in Tris EDTA buffer (pH 9.0). Positive staining was detected with diaminobenzidine (DAB) substrate and Mayer's hematoxylin counterstain. Normal adult skin tissue was used as positive control. The interpretation of c-Fos nucleoplasmic expression was carried out in the tumor hotspot and observing five hundred cells in high magnification power (40X). The stained tissue sections were scored by using the semi-quantitative method in at least five fields. The staining criteria was applied as 0 for <5% positive stained cells (negative expression), +1 for 5-20% positive stained cells (weak expression), +2 for 21-50% positive stained cells (moderate expression) and +3 for > 50% positive stained cells (strong expression). Scores of 2 and 3 were considered as c-Fos positive expression (Vairaktaris et al., 2008).

Quantitative Real Time PCR (qRT-PCR)

qRT-PCR was used to analyze relative m-RNA expression of the *c-fos* gene. Total RNA was isolated from all tissue samples by using RNeasy Mini Kit (Qiagen, Germany). Complimentary DNA (cDNA) was synthesized by reverse transcription with PCR amplification using Reverse Transcription kit (Applied Biosystem, USA). qRT-PCR was performed on Lightcycler 96 system (Roche, USA) using Power SYBR Green PCR Master Mix (Applied Biosystem, USA) and specific primer for *c-fos* (NM005252.3): forward 5'-AGAATCCGAAGGGAAAGGAA-3' reverse 5'-CTTCTCCTTCAGCAGGTTGG-3' (product size 150 bp) (Isabella et al., 2011). β-actin (XM006715764.1) was used as internal control and primer sequences used were: forward 5'-ATCGTGCGTGACATTAAGGAGAAG-3' reverse 5'-AGGAAGGAAGGCTGGAAGAGTG-3' (product size 179 bp). qRT-PCR reactions for each sample were performed in triplicates. The qRT-PCR thermal profile for *c-fos* and β-actin genes was 94°C:10min [94°C: 30sec, 58°C: 35sec, 72°C: 1min] x 45 cycles and 72°C for 5 min. m-RNA Fold change was calculated by relative gene quantification method ($2^{-\Delta\Delta CT}$ method) (Livak et al., 2001).

Table 1. c-Fos Protein Expression in Case and Control Groups

c-Fos Protein	Case n=65 (%)	Control n=65 (%)	p value
Positivity			
Positive	32 (49.2)	52 (80)	<0.001*
Negative	33 (50.8)	13 (20)	
Expression			
Negative	12 (18.5)	4 (6.2)	<0.001*
Weak	21 (32.3)	9 (13.8)	
Moderate	25 (38.5)	17 (26.2)	
Strong	7 (10.7)	35 (53.8)	

*Significant; p value obtained by chi-square test (χ^2)

Statistical analysis

The Chi - square (χ^2) test and student t-test (two-tailed) were used to examine the association of c-Fos protein and m-RNA expressions with study groups. Binary Logistic Regression method was carried out to observe the relation between c-Fos protein status and demographical characteristics, risk habits and clinico-pathological variables of patients. Odds Ratio (OR) with 95% confidence interval (CI) has determined the risk value of c-Fos positivity. Pearson's correlation coefficient test was used for observation of correlation between protein and m-RNA level of c-Fos. The Probability value (p) ≤ 0.05 was regarded as statistically significant. All statistical analyses were carried out by using SPSS software Version 16 (SPSS Inc., Chicago, IL, USA).

Results

In the present study, the prevalence of OSCC found higher in males with age group of 41-60 years. Majority of patients were attributed to tobacco-related risk habits. The almost similar frequency of age groups, gender, and risk habits was also observed in control group.

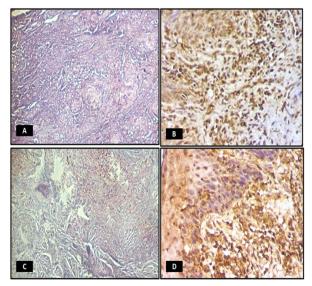In cases, buucal Mucosa (BM; 41.5%) was the most commonly affected oral cavity site followed by lower

Figure 2. Microphotograph Showing the HE and Immunohistochemistry Images for Diagnosis and c-Fos Protein Expression; A) HE stained tissue section of oral carcinoma, B) IHC image showing moderate nucleoplasmic immunoexpression in OSCC, C) HE stained section of normal tissue of oral cavity, D) IHC image with strong expression in normal tissue section. [HE images: digital magnification, x100, and IHC images: DAB, digital magnification, x100]

alveolus (LA; 21.6%), upper alveolus (UA; 13.9%), tongue (7.7%), palate (4.6%), retromolar trigone (RMT; 4.6%), lips (4.6%) and floor of mouth (FM; 1.5%). The non-malignant lesions with normal histopathology were mostly involved with BM (35.4%) site of oral mucosa, however LA, UA, RMT, Palate and lips regions were also diagnosed (Figure 1).

In the histopathological diagnosis, tissue sections showed the proliferated squamous epithelial cell with the high nucleus-cytoplasm ratio, inconsistent amount of cytoplasm presenting low-grade dysplasia arranged in tubercular and cluster patterns. The epithelial keratin pearl formation was also seen. Weak and moderate immunoreactivity of the c-Fos protein was quantified in the majority of cases. However, HE section of oral
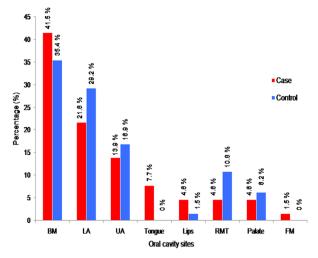

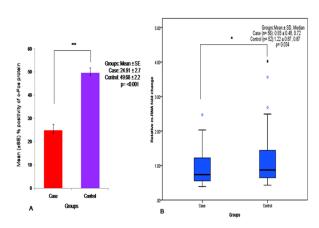Figure 1. Frequency of Oral Malignant and Non-Malignant Lesions Sites in Case and Control Groups

Figure 3. c-Fos Expression in Case and Control Groups; A) Mean (±SE) percent positivity of c-Fos protein, B) Mean (±SD) of relative m-RNA fold change of *c-fos* gene

Table 2. Association c-Fos Protein Expression with Demographic Characteristics and Risk Habits in Cases and Controls

Characteristics	c-Fos expression in cases (n= 65)		OR (95% CI)	p value	c-Fos expression in controls (n= 65)		OR (95% CI)	p value
	Positive n=32 (%)	Negative n=33 (%)			Positive n=52 (%)	Negative n=13 (%)		
Age (years)								
≤40	7 (21.9)	9 (27.3)	Ref		22 (42.3)	8 (61.5)	Ref	
41-60	20 (62.5)	21 (63.6)	1.22 (0.38-3.91)	0.73	24 (46.2)	4 (30.8)	2.18 (0.57-8.26)	0.25
>60	5 (15.6)	3 (9.1)	2.14 (0.37-12.19)	0.39	6 (11.5)	1 (7.7)	2.18 (0.22-21.04)	0.5
Gender								
Male	28 (87.5)	21 (63.6)	Ref		33 (63.5)	9 (69.2)	Ref	
Female	4 (12.5)	12 (36.4)	4.00 (1.13-14.2)	0.03*	19 (36.5)	4 (30.8)	0.77 (0.20-2.80)	0.69
Marital status								
Unmarried	2 (6.2)	4 (12.1)	Ref		11 (21.2)	2 (15.4)	Ref	
Married	30 (93.8)	29 (87.9)	2.07 (0.35-12.2)	0.42	41 (78.8)	11 (84.6)	0.68 (0.13-3.51)	0.64
Residence area								
Urban	10 (31.2)	14 (42.4)	Ref		27 (51.9)	7 (53.8)	Ref	
Rural	22 (68.8)	19 (57.6)	1.62 (0.58-4.48)	0.35	25 (48.1)	6 (46.2)	1.08 (0.32-3.65)	0.9
Risk Habits Tobacco chewing								
No	3 (9.4)	6 (18.2)	Ref		20 (38.5)	4 (30.8)	Ref	
Yes	29 (90.6)	27 (81.8)	2.15 (0.48-9.45)	0.31	32 (61.5)	9 (69.2)	0.71 (0.19-2.61)	0.6
Tobacco smoking								
No	14 (43.8)	12 (36.4)	Ref		24 (46.2)	5 (38.5)	Ref	
Yes	18 (56.2)	21 (63.6)	0.73 (0.27-1.98)	0.54	28 (53.8)	8 (61.5)	0.72 (0.21-2.52)	0.61
Alcohol consumption								
No	22 (68.8)	19 (57.6)	Ref		39 (75)	7 (53.8)	Ref	
Yes	10 (31.2)	14 (42.4)	0.62 (0.22-1.70)	0.35	13 (25)	6 (46.2)	0.38 (0.11-1.36)	0.14
Types of Risk Habit								
No habit	2 (6.3)	2 (6.1)	Ref		12 (23.1)	2 (15.4)	Ref	
Single habit	11 (34.4)	10 (30.3)	1.10 (0.14-8.63)	0.93	16 (30.8)	5 (38.5)	1.50 (0.26-8.57)	0.64
Multiple habits	19 (59.3)	21 (63.6)	1.21 (0.42-3.5)	0.92	24 (46.2)	6 (46.2)	0.80 (0.20-3.07)	0.74

*Significant, p value obtained by Binary Logistic Regression method; Ref, Reference; OR, Odds ratio
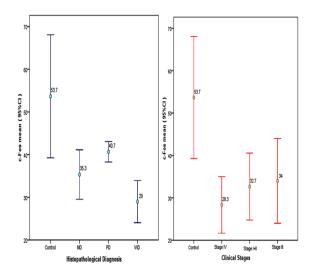

Figure 4. Mean Number of Percent Positivity of c-Fos Protein with 95% CI in Normal Controls and c-Fos Positive OSCC Cases, According to Histopathological Diagnosis and Clinical Stages

non-malignant lesions presented normal squamous epithelial lining based on an intact basement membrane. Most of the normal tissue sections showed strong c-Fos expression (Figure 2).

The immunohistochemical and m-RNA analysis of *c-fos* is shown in Table 1 and Figure 3. Out of 65 OSCC cases, 32 (49.2 %) cases were analyzed for positive immunostaining of c-Fos protein whereas 33 cases (50.8 %) observed for negative staining. However, in control group, c-Fos immunopositivity was found in 80% (52/65) normal oral tissues. This difference was statistically significant (p= <0.001). The mean (±SE) percent positivity of c-Fos protein in cases vs. controls was 24.91± 2.7 vs. 49.68± 2.2 (p= <0.001). Moreover, the c-Fos level according to subgroups of percent positivity denoted as negative (n=12), weak (n=21), moderate (n=25) and strong (n=7) expression in cases. Nevertheless, the majority (n=35) of normal tissue sections of control group were estimated to strong immunopositivity. This expression difference of both the groups was significantly associated (p= <0.001). The relative m-RNA fold change

Table 3. Association of c-Fos Expression with Clinico-Pathological Variables in OSCC Cases

Characteristics	c-Fos expression in cases (n=65)		OR (95% CI)	p value
	Positive	Negative		
	n=32 (%)	n=33 (%)		
Oral cavity sites				
BM	15 (46.9)	12 (36.4)		
LA	8 (25.0)	6 (18.2)	NA	0.41
UA	5 (15.6)	4 (12.1)		
Tongue	1 (3.1)	4 (12.1)		
Lips	2 (6.3)	1 (3.0)		
RMT	1 (3.1)	2 (6.1)		
Palate	0 (0.0)	3 (9.1)		
FM	0 (0.0)	1(3.0)		
Histological grade				
WD	18 (56.3)	27 (81.8)	Ref	
MD	11 (34.3)	6 (18.2)	2.75 (0.86-8.77)	0.08
PD	3 (9.4)	0 (0.0)	-	0.99
TNM stage				
I+II	7 (21.8)	5 (15.2)	Ref	
III	6 (18.8)	10 (30.3)	0.43 (0.09-1.98)	0.27
IV	19 (59.4)	18 (54.5)	0.75 (0.20-2.81)	0.67
Node				
Absent (N0)	12 (37.5)	7 (21.2)	Ref	
Present (N1-2)	20 (62.5)	26 (78.8)	2.23 (0.74-6.69)	0.15

p value obtained by Binary Logistic Regression method; Ref, Reference ; OR, odds ratio

of *c-fos* gene was also quantified in both OSCC case and control groups. Out of total tissue samples of both the groups, 9 samples were dropped out from cases (n=56) whereas 13 samples from controls (n=52) because of insufficient tissues or poor yield of isolated total RNA. The mean (±SD) fold change level of *c-fos* m-RNA was significantly lower in case group (0.93±0.48) in compared to control group (1.22±0.87). The Pearson's correlation analysis illustrated about poor correlation in between c-Fos protein and m-RNA level of OSCC cases (r=0.25, p=0.11) whereas a significant strong correlation observed in control subjects (r=0.61, p= <0.01).

The association of c-Fos protein expression with demographic characteristics and risk habits in both case and control groups was presented in Table 2. Among case group, 62.5% c-Fos positive carcinoma patients were belonging from the age group of 41-60 years. Similarly, the positivity was also higher in 41-60 years and ≤ 40 years age groups in controls. Overall the higher positivity of c-Fos protein was accounted mostly in males of both the groups. Furthermore, out of total c-Fos positive patients 87.5% males and 12.5% females were detected in this study. Despite that negative c-Fos expression has also been presented in 63.6 % male patients (OR= 4.00, p= 0.03).

More than 90 % c-Fos positive cases were attributed to tobacco chewing habit while 61.5% of c-Fos positive control subjects also had the similar habit. In addition, the risk of c-Fos positivity was 2.15 times higher in tobacco chewers as comparing with non-chewers of OSCC cases.

Furthermore, we categorized all cases and controls in subgroup of single, multiple (habituated with any two or more type of tobacco related and alcohol consumption habits) and no risk habit. Majority of c-Fos positive cases and controls were observed with multiple risk habits. The risk of c-Fos overexpression was 1.50 times higher in individuals with single risk habit as compared to no habit of control group.

The association of clinico-pathological profile of OSCC cases and c-Fos protein expression was exhibited that buccal mucosa was the most commonly affected site in both c-Fos positive and negative cases. The overexpression of c-Fos protein was analyzed in most of OSCC cases with stage IV (59.4%) and also in early stages (stage I+II; 21.8%). In histopathological investigations, positivity of c-Fos was enhanced in higher grade carcinoma with lymph node compromised patients. Interestingly, elevated level of c-Fos protein was analyzed in a large proportion of moderately differentiated carcinoma cases (11/17) and all cases of poorly differentiated carcinoma cases (3/3). Moreover, out of 12 OSCC cases with early stages (stage I+II) patients, 7 patients were presented the overexpression of c-Fos (Table 3).

Figure 4 presented the difference between mean number of percent positive cells for c-Fos protein (with 95% confidence interval) in normal tissue of oral cavity (mean 57.3%) and well differentiated (mean 29%, p= <0.001) and moderately differentiate (mean 35.3, p= <0.001) OSCC. A significant difference of c-Fos percent positivity was also analyzed in early stages of tumor and normal oral mucosa (p= 0.03). However, the c-Fos expression had distinctively reduced in stage III (p= <0.001) and stage IV of oral carcinoma (p= <0.001).

Discussion

Oral cancer is a significant health burden for the developing countries and accounted as third most common cancer in India (Bray et al., 2013). The basic characteristics of OSCC patients in this study have similar patterns as described in the previous reports, higher frequency in 41-60 years age group with male gender and mostly associated with the tobacco - related risk habits (Aruna et al., 2011; Kiran et al., 2012). The distribution of demographical variables and risk habits of controls were almost identical to cases. In our investigation, buccal mucosa was found the most common site of oral malignancy followed by lower alveolus, upper alveolus, tongue and other mucosal sites. These findings are in agreement with our previous report (Krishna et al., 2014) and some other studies as well (Su et al., 2007; Singhania et al., 2015).

According to the methodology of the previous literatures, we have also utilized the same selection criteria for control tissue samples of oral cavity (Partridge et al., 2000; Sachdev et al., 2008; Shirani et al., 2014). Only those oral biopsies which were clinico-pathologically diagnosed as non-malignant lesions and normal histology, considered as controls. In this study, buccal mucosa and both upper and lower alveolus were observed the most

frequent affected oral sites for non-malignant lesions. A literature reported that traumatic oral injury either by accidental biting (during mastication, talking, or sleeping) or thermal, electrical, the chemical may visualize like the patch which reflects the conditions like non-cancerous lesion (Mortazavi et al., 2016). According to Chen et al., (2010) traumatic lesions of the oral cavity were mostly seen in the buccal mucosa followed by tongue and lower lip. However, in contrast to our findings, a study reported that different types of non cancerous oral mucosal masses were most frequently affected the mobile tongue site followed by the labial mucosa, buccal mucosa, hard palate, gingival/ alveolar ridge and floor of mouth (Allon et al., 2014). In our control group, the majority of subjects with non-malignant lesions had tobacco chewing and smoking habits as like to a previous finding (Mirbod and Ahing, 2000). So, we hypothesized that different types of tobacco - related oral practices may cause irritation and ulceration in the oral sites and produces definite morphological changes in the oral mucosal cells which may leads to non-cancerous lesions.

The *c-fos* proto-oncogene has been reported as an immediate early gene, is significantly associated with cell proliferation and differentiation, induced by a number of mitogens (Nephew et al., 2000). In addition, c-Fos protein has been associated with apoptotic cell death induced by anti-proliferative conditions (Smeyne et al., 1993). The present work depicted that the expression level of *c-fos* mRNA and protein was significantly low in oral carcinoma compared with normal tissue of oral cavity. In the pattern of c-Fos immunostaining, most of the cancerous tissues expressed either weak or moderate protein levels, whereas eighty percent normal tissues showed the strong immunoexpression. The c-Fos mean percent positivity was significantly low in case group as compared with control groups. Our results are in concordance with De Sousa et al., (2002), who found higher expression in normal oral mucosa while decreased level in the least differentiated OSCC. Moreover, another investigation was reported the intense expression of the c-Fos protein in normal mucosa but diminished in mild dysplasia and high in moderate to severe dysplasia, and also in OSCC (Turatti et al., 2005). Contradictory, an in vivo study by Vairaktaris et al., (2008) analyzed the overexpression of c-Fos protein in pre-cancerous lesions compared to the normal oral mucosa, but gradually decreased in subsequent stages of OSCC. Similarly, Mangone et al., (2005) evaluated the c-Fos protein expression in assorted OSCC at various stages with adjacent normal mucosa and found no difference. However, these previous studies were not validated the c-Fos protein levels by either western blot or real time PCR techniques.

In our study, c-Fos protein expression in cases and controls were quantitatively checked and validated through its m-RNA level. The relative *c-fos* m-RNA fold change was also significantly reduced in OSCC group than control group. Thus, we firmly ratified our results about downregulation of *c-fos* gene in oral carcinoma. Furthermore, correlation analysis of *c-fos* m-RNA and protein levels in cases and controls was supported to conclude that c-Fos protein might be a factor that inhibits the proliferation of tumor cells.

Amirhossein et al., (2013) revealed that relative *c-fos* m-RNA was expressed at a significant higher level in adjacent tissue compared with tumor tissue of breast cancer and this is in agreement with our results. An analysis also demonstrated that c-Fos was highly expressed in normal epithelium of ovary and weakly expressed in ovarian carcinoma. Moreover, the *c-fos* gene expression was up-regulated in low grade carcinoma compared to intermediate and high grade carcinomas (Mahner et al., 2008). However, in contrast, Ming-Yao et al., (2002) detected low level of *c-fos* m-RNA and protein in simple hyperplasia and normal esophageal epithelia but its higher level in dysplasia and cancer tissues.

The present study showed that a higher proportion of low grade tumors with early stages were more prone to overexpression of c-Fos. Moreover, the mean level of c-Fos percent positivity was significantly lower in well differentiated and moderately differentiated carcinoma compared to the normal tissue of oral cavity. Further, the OSCC cases in advanced stages had presented weak expression of c-Fos in contrast of early stages of the tumor or normal oral epithelium and these findings are similar to previous reports (Ming-Yao et al., 2002; Vairaktaris et al., 2008). c-Fos overexpression was found in lymph node compromised patients than without lymph node involved patients. However, on contrary, no difference in c-Fos level was observed in with or without lymph node involved head and neck cancer patients (Mangone et al., 2005). The c-Fos positivity in our case and control groups was regardless of age, marital status and residential area of patients. In this investigation, the percentage of c-Fos positive expression was higher in patients who had tobacco related habits like chewing and smoking compared to patients with non tobacco-related habits. The previous reports have approved that nicotine and other tobacco derived carcinogens participate in oral carcinogenesis and induce *c-fos* proto-oncogene (Volm et al., 1992; Malpass et al., 2014).

Furthermore, some evidence about tumor suppression mechanism of *c-fos* proto-oncogene in contrast to cell proliferation seeks our attention more for its tumor suppressive nature. Some in vitro and in vivo studies suggested that c-Fos protein can act both as the transcriptional activator and transcriptional repressor. A report by Teng (2000) supported the c-Fos tumor suppressor activity and its role in apoptosis. Another observation in hepatocellular cancer indicated that c-Fos might induce apoptosis through the p38 MAPK pathway (Kalra and Kumar, 2004). Other investigations concluded that c-Fos functions as one of the targets of some tumor suppressor genes. The c-Fos was directly regulated the BRCA1 tumor suppressor gene and plays an antioncogenic role in breast and ovarian cancer (Graves et al., 2007; Li et al., 2013; Poi et al., 2013). So findings of these studies concluded that c-Fos might also have the function in tumor suppression in different types of cancer and associated with reduction of c-Fos protein expression. In this context, we can also conclude that c-Fos may also perform the tumor suppressor activity in oral carcinoma in this study population. The dissimilar findings in comparison with the

other studies for *c-fos* gene expression in oral carcinoma is ambiguous.

Therefore, some future studies should aim to elucidate the underlying molecular mechanisms of activation of *c-fos* gene expression with its exact mitogens and post translational modifications of c-Fos protein, which may contribute towards better understanding of the role of *c-fos* proto-oncogene in oral carcinogenesis.

In conclusion, this report documented that down-regulated *c-fos* gene expression is associated with oral carcinoma. The c-Fos positivity was higher in tobacco users as well as in multiple risk habited individuals. The highest score of mean percent positivity of c-Fos protein in poorly differentiated carcinoma and early stages of tumor may indicate that the loss of c-Fos protein is related with tumor progression in OSCC. We also suggest that additional studies are needed in the context of c-Fos protein expression with overall and progression free survival of oral cancer patients to attain better knowledge of its prognostic role.

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