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Immunohistochemical expression of p53 and c-Myc at the invasive front of oral squamous cell carcinoma and its relation with clinicopathologic characteristics

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1. Introduction

The invasive tumor front (ITF) is the most progressed part of the tumor which consists of three to six tumor cell layers or detached tumor cell groups at the advancing edge of the oral squamous cell carcinoma (OSCC) [1]. It frequently shows a lower degree of differentiation and a higher grade of cellular dissociation in comparison with other parts of the tumor. It is believed that the integral prognostic information about the tumor's invasive and metastatic capacity can be deduced from ITF, where the deepest and presumably aggressive cells reside [2,3]. Although ITF has been exhaustively studied in OSCC, only a few studies have assessed the expression of cell cycle regulatory proteins in this area [4,5,6].

p53 is a multifunctional protein, which plays a role in modulating gene transcription, thereby policing cell cycle checkpoints, activating apoptosis, controlling DNA replication and repair, thus maintaining genomic stability in response to genetic insults [7]. Alteration in *p53* through loss of heterozygosity, point mutations, deletions, insertions or interactions with viral proteins are common and mark early events in head and neck carcinogenesis [8]. Mutations of the core domains (exons 5–9) of the *p53* gene, have been reported in about 60% OSCC and are strongly associated with immunohistochemically (IHC) detectable accumulation of the p53 protein product [4].

Among the oncogenes which are frequently involved in OSCC is *c-Myc*. Tumorigenicity of c-Myc protein is ascribed to the promotion of cell proliferation and inhibition of apoptosis. Amplification and overexpression of c-Myc has been observed in 10–40% of human OSCC [9]. *p53* and *c-Myc* have been shown to collaborate in the regulation of both cell proliferation and apoptosis, however, when altered by mutational events, they seem to be key regulatory elements of oncogenesis [10]. *p53* and *c-Myc* genes were frequently deregulated in head and neck squamous cell carcinoma (HNSCC) [11]. Currently, limited data exist on the association of p53 and c-Myc expression at the ITF of OSCC. Hence, the purpose of this study was to assess the expression of p53 and c-Myc in OSCC and to analyze and compare this expression in the Whole Tumor (WT) and ITF. Additional objectives were to evaluate the correlation between p53 and c-Myc expression in each region and also to study the association between p53 and c-Myc expression at the ITF with clinicopathologic features in OSCC patients.

2. Methodology

2.1. Patients' characteristics

The study protocol was approved by the institutional ethics committee SDMCDSH-IEC (IRB·No.2013/P/OP/15). Sixty patients (49 men, 11 women) with primary OSCC treated between 2009 and 2014 in the Cranio-facial unit (CFU) of our institution were studied. The mean age at presentation was 49 years (age range, 25–72 years) with 47% of them being < 45 years. Sixty two percent of the lesions involved the buccal mucosa and/ retro molar trigone and the rest were involving the tongue and gingivo buccal sulcus. Seventy three percent of patients had the lesion involving single sites whereas 27% had the lesion involving multiple sites by local extension. Nearly 85% of patients consumed tobacco and areca nut in various forms. Majority practiced the habit for at least 1–10 years duration. Eighty five percent of tumors were exophytic/ulcer proliferative type and remaining were endophytic/ulcerative. Sixty eight percent of tumors were < 4 cm in size. The clinical staging of patients was defined according to the criteria of the International Union against Cancer TNM classification. There were five stage I tumors, nineteen stage II tumors, twenty five stage III tumors and eleven stage IV tumors. About 40% of them were in early stage and 60% were in advanced. LNM was noted in 55% of patients. Tumors were scored according to Bryne's Invasive front grading (1992) – invasive front area, including connective tissue stroma. These included eight with grade I (well differentiated), seventeen with grade II (moderately differentiated) and thirty five with grade III (poorly differentiated).

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2.2. Tissue specimens

Sixty biopsy specimens of OSCC were obtained from patients who received no previous treatment and had undergone surgical resection in the CFU of institution. Formalin fixed, routinely processed tissue sections from the lesion proper which showed WT and ITF in Hematoxylin & Eosin (H & E) staining was used for the histopathological examination and for the study.

2.3. Immunohistochemical studies

Tissue sections were de-waxed with xylene, hydrated using graded alcohols and treated with Hydrogen peroxide (H_2O_2) in methanol for 10 min to eliminate endogenous peroxidase activity. The following monoclonal antibodies were used: Anti-p53 [Clone DO 7, IgG2b] and Anti-c-Myc [Clone 9E 10, IgG] were used after antigen retrieval. Super sensitive polymer - Horseradish Peroxidase (HRP) biotin-free detection system was used for application of the secondary antibody. The reactive products were visualized by immersing the sections in diaminobenzidine solution, containing H_2O_2. The sections were then counterstained with Mayer's hematoxylin. Sections of breast carcinoma with known p53 and sections of adenocarcinoma with known c-Myc were used as a positive control. Negative control was made by omission of each primary antibody. A brown precipitate seen in the nucleus confirmed the presence of p53, for c-Myc both nuclear and cytoplasmic immunostaining were considered positive. Tumor cell were independently counted in the WT and at ITF. A minimum of 500 cells were counted in each slide at the ITF and in the WT under 40 × magnification, using an eyepiece grid. Quantitative labelling index (LI) was assessed for each region. The p53 and c-Myc immunostaining at the ITF were classified as overexpressed when LI > 10% [4,6,12].

2.4. Statistical analysis

The data were analysed by means of SPSS-11 software (SPSS Inc., Chicago, IL, USA). Differences in p53 and c-Myc expression between the two regions were analysed using the Wilcoxon test. The correlation between p53 and c-Myc expression in each region was analysed using the Spearman rank correlation test. The association between expressions p53 and c-Myc was examined using Chi-square. The relation between the expression of p53 and c-Myc with clinicopathologic characteristics of OSCC patients was analysed using Mann-Whitney *U* test. $p < 0.05$ was considered significant.

3. Observations and results

A total of sixty cases of OSCC were evaluated for IHC expression of p53 and c-Myc. The percentage of expression was assessed in the WT and at the ITF for each case. The expression of p53 was observed in the nucleus while that of c-Myc was either in the nucleus and cytoplasm or only in the cytoplasm. Three observers evaluated each case and the average of the observations was considered.

Of the study samples only 63.3% (38/60) of them showed p53 expression and (54/60), 90% showed c-Myc expression both in the WT and at the ITF. The tumor was considered positive when at least 10% of tumor cells showed an appropriate staining pattern. The percentage of expression ranged from 0 to 59.4% for p53, 0–89.6% for c-Myc in the WT. The percentage of expression ranged from 0 to 78.6% for p53, 0–98.4% for c-Myc at the ITF (Table 1).

Overexpression of p53 was found in 38 of 60 specimens (63.3%). In 35 of the 38 specimens (92%), p53 LI was higher at ITF than in the WT. Statistically significant difference were found between p53 LI at ITF (Mean = 34.70 ± 31.47) and in the WT (Mean ± SD = 23.62 ± 22.97) ($p < 0.05$), Figs. 1–3).

Over expression of c-Myc was found in 54 of 60 specimens (90%). In 51 of 54 of the specimens (94.4%), c-Myc LI was higher at ITF than in the WT. Statistically significant difference were found between c-Myc LI at ITF (Mean ± SD = 69.16 ± 33.04) and in the WT (Mean ± SD = 57.59 ± 30.83) ($p < 0.05$), Figs. 4–8).

Statistically significant positive correlation was detected between WT and ITF with respect to p53 LI ($p < 0.05$) (Table 2). Statistically significant positive correlation was detected between WT and ITF with

Table 1
Comparison of mean, median value and range of p53 LI and c-Myc LI in the WT and at ITF of 60 OSCC samples.

IHC Marker		WT	ITF	*p*-Value	Significance
p53 LI	Mean ± SD	23.62 ± 22.97	34.70 ± 31.47	0.000	S
	Median	18.20	35.10		
	Range	0–59.40	0–78.6		
c-Myc LI	Mean ± SD	57.59 ± 30.83	69.16 ± 33.04	0.000	S
	Median	69.85	85.10		
	Range	0–89.60	0–98.4		

LI, labelling index; WT, whole tumor; ITF, invasive tumor front.
p-Values were obtained from the Wilcoxon signed rank test.

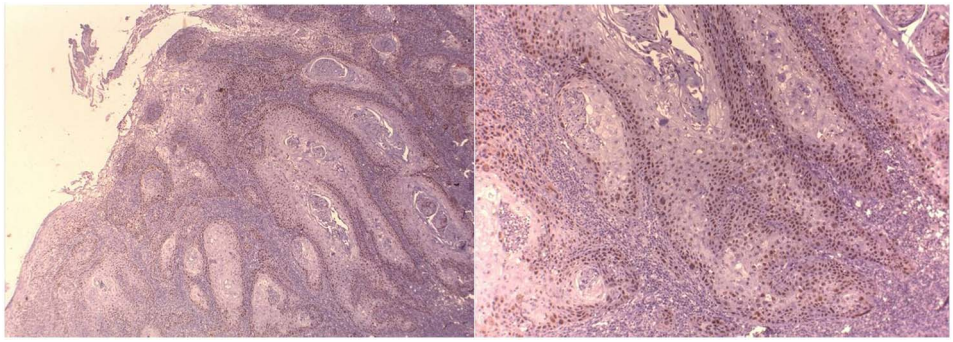

Fig. 1. OSCC depicting the whole tumor along with invasive front. Immunolocalization of p53 in the peripheral cells of long strands and islands of the tumor. (4 ×,10 × magnification, DAB chromogen, p53 monoclonal antibody).

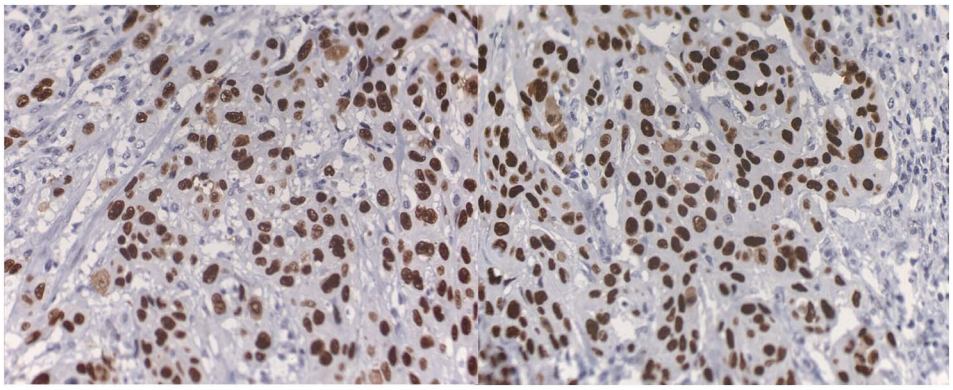

Fig. 2. Tumor cells at the invasive front of OSCC demonstrating strong nuclear staining of p53. (40 × magnification, DAB chromogen, p53 monoclonal antibody).

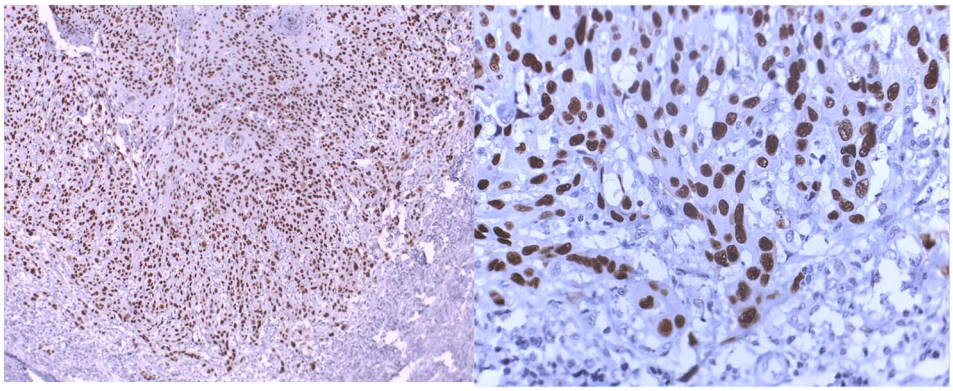

Fig. 3. Tumor cells at the invasive front of OSCC demonstrating strong nuclear staining of p53. (10 ×, 40 × magnification, DAB chromogen, p53 monoclonal antibody).

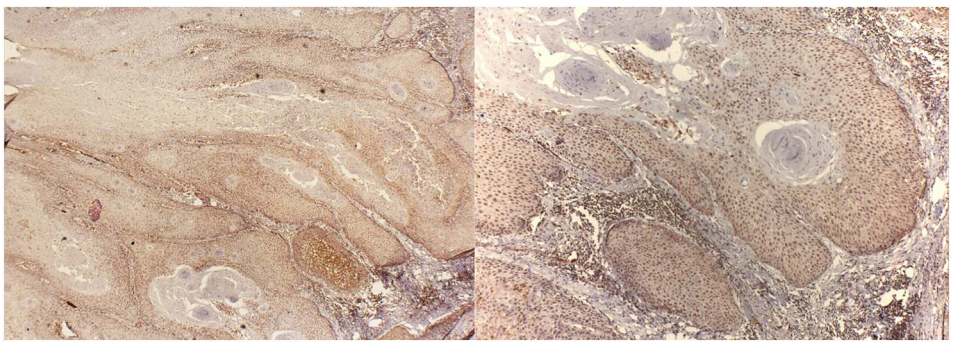

Fig. 4. OSCC representing whole tumor along with invasive front. Immunolocalization of c-Myc in the peripheral cells of long strands and islands of the tumor. (4 ×,10 × magnification, DAB chromogen, c-Myc monoclonal antibody).

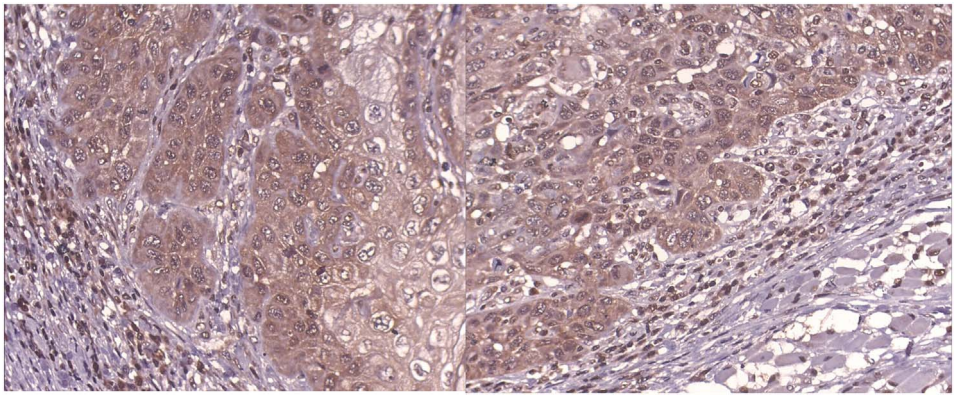

Fig. 5. Accumulation of c-Myc immunoreactive tumor cells at the invasive front of OSCC. (40 × magnification, DAB chromogen, c-Myc monoclonal antibody).

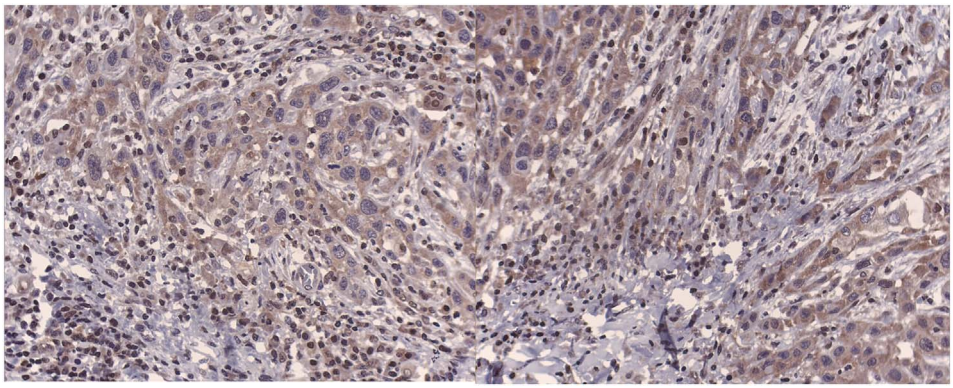

Fig. 6. Tumor cells in the form of cords, clusters and individual cells at the invasive front of OSCC showing cytoplasmic staining of c-Myc. (40 × magnification, DAB chromogen, c-Myc monoclonal antibody).

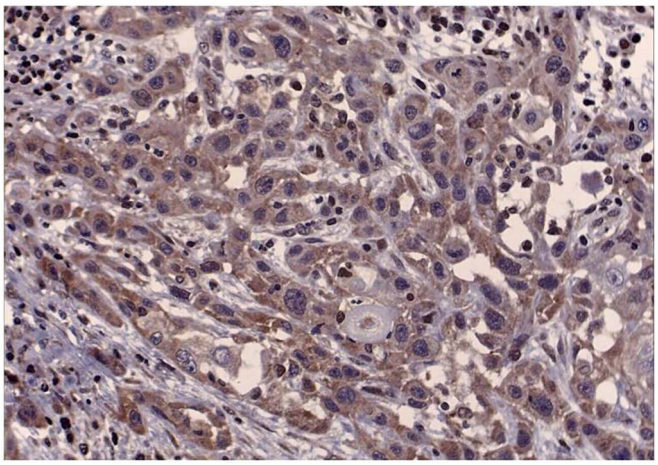

Fig. 7. Tumor cells at the invasive front of OSCC demonstrating cytoplasmic staining and nuclear staining of c-Myc. (40 × magnification, DAB chromogen, c-Myc monoclonal antibody).

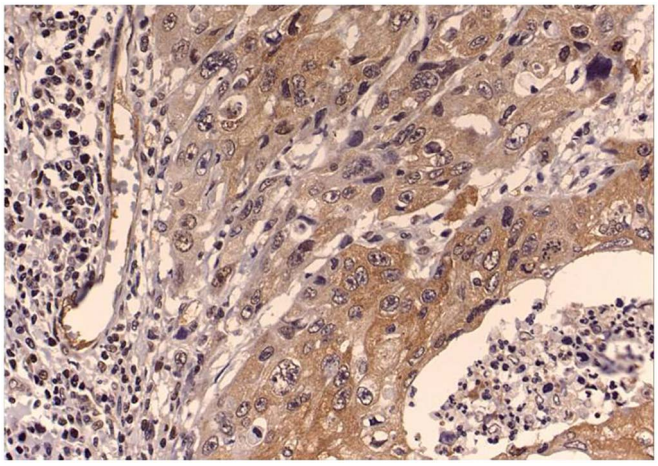

Fig. 8. Tumor cells at the invasive front of OSCC expressing cytoplasmic and nuclear staining of c-Myc. (40 × magnification, DAB chromogen, c-Myc monoclonal antibody).

Table 2
Correlation between p53 LI in the WT and at the ITF of OSCC samples.

			p53 WT	p53 ITF
Spearman's rho	p53 WT	Correlation coefficient	1.000	0.985[a]
		Sig. (2-tailed)	–	0.000
		N	60	60

[a] Correlation is significant at the 0.01 level (2-tailed).

Table 3
Correlation between c-Myc LI in the WT and at the ITF of OSCC samples.

			c-Myc WT	c-Myc ITF
Spearman's rho	c-Myc WT	Correlation coefficient	1.000	0.947[a]
		Sig. (2-tailed)	–	0.000
		N	60	60

[a] Correlation is significant at the 0.01 level (2-tailed).

respect to c-Myc LI ($p < 0.05$) (Table 3).

c-Myc LI was higher than p53 LI in the WT. Statistically significant differences were found between c-Myc (Mean ± SD = 57.59 ± 30.83) and p53 (Mean ± SD = 23.62 ± 22.97) in the WT ($p = 0.000$) (Table 1). c-Myc LI was higher than p53 LI at the ITF. Statistically significant differences were found between c-Myc (Mean ± SD = 69.16 ± 33.04) and p53 (Mean ± SD = 34.70 ± 31.47) at ITF ($p = 0.000$) (Table 1).

Statistically significant positive correlation was detected between p53 and c-Myc in the WT ($p = 0.007$) (Table 4) and at the ITF ($p = 0.019$) (Table 5). It was observed that p53 status significantly correlated with c-Myc IHC phenotype in the WT and at the ITF.

3.1. Relationship between p53 and c-Myc expression in OSCC

The association between expressions of p53 and c-Myc was examined using chi-square test. There was no statistically significant association between these markers ($p = 0.108$) (Table 6).

On the basis of p53 and c-Myc immunoreactions, OSCC cases were

Table 4
Correlation between p53 LI and c-Myc LI in the WT of OSCC samples.

			WT p53	WT c-Myc
Spearman's rho	WT p53	Correlation coefficient	1.000	0.345[a]
		Sig. (2-tailed)	–	0.007
		N	60	60

[a] Correlation is significant at the 0.01 level (2-tailed).

Table 5
Correlation between p53 LI and c-Myc LI at the ITF of OSCC samples.

			ITF p53	ITF c-Myc
Spearman's rho	ITF p53	Correlation coefficient	1.000	0.302[a]
		Sig. (2-tailed)	–	0.019
		N	60	60

[a] Correlation is significant at the 0.05 level (2-tailed).

Table 6
Relationship between p53 and c-Myc protein expression in OSCC.

Molecular marker	c-Myc +	c-Myc −	Total
p53 +	36 (60%)	2 (3%)	38 (63%)
p53 −	18 (30%)	4 (7%)	22 (37%)
Total	54 (90%)	6 (10%)	60 (100%)

Chi-square test $p = 0.108$.

divided into four groups: 1) Both p53 and c-Myc immunoreactive: Out of the 60 oral tumors, 36 (60%) were positive for both p53 and c-Myc. Of 36 tumors, 24 (66.7%) were in advanced stage and 22 (66.7%) had LNM. 2) & 3) Either p53 or c-Myc immunoreactive: A total of 20 (33%) cases fell in these two groups and they were distributed within different categories on the basis of extent of malignancy. 4) Neither p53 nor c-Myc immunoreactive: 4 (7%) were in this group.

3.2. Clinicopathological parameters in relation to p53-LI

Among the clinicopathologic parameters, p53 expression revealed statistically significant relation only with habits ($p < 0.05$). Median LI was higher in OSCC patients with areca nut and tobacco chewing than with other habits/without habits. c-Myc expression showed a statistically significant relation with location of lesion, Broder's grade and nuclear polymorphism ($p < 0.05$) (Table 7). Median LI was higher in OSCC patients with the lesion involving buccal mucosa and/ retromolar

Table 7
Relationship between p53 and c-Myc labelling index with clinicopathologic features in OSCC patients.

Parameters	Category	Total (N)%	Labelling Index p53 (median)	*p*-Value	Labelling index c-Myc (median)	*p*-Value
Age	< 45 years	28(46.7)	28.00	0.796	87.00	0.617
	≥ 45 years	32(53.3)	42.00		81.00	
Gender	Male	49(81.6)	47.00	0.505	85.00	0.903
	Female	11(18.3)	00.00		83.00	
Location	BM, RMT	37(61.6)	28.00	0.686	91.00	0.049
	Tongue	17(28.3)	28.00		77.00	
	GBS	6(10)	47.50		83.00	
Side	R	30(50)	47.50	0.071	84.00	1.000
	L	30(50)	6.00		87.50	
Site	S	44(73.3)	37.50	0.770	83.00	0.302
(extension)	M	16(26.6)	35.00		94.50	
Habits	No	9(15)	00.00	0.009	77.00	0.229
	TC	36(60)	48.50		90.00	
	TS	5(8.3)	00.00		48.00	
	C	10(16.6)	20.50		81.00	
Duration	0	9(15)	00.00	0.169	80.00	0.282
	1–10	25(41.6)	48.00		88.50	
	11–20	17(28.3)	47.00		90.00	
	21–30	7(11.6)	22.00		78.00	
	31–40	2(3.3)	62.00		94.50	
Tumor size	T1	12(20)	7.00	0.429	80.00	0.207
	T2	29(48.3)	28.00		79.00	
	T3	9(15)	49.00		96.00	
	T4	10(16.6)	53.50		93.00	
Clinical nodal status	N0	25(41.6)	47.00	0.601	85.00	0.621
	N1	29(48.3)	28.00		83.00	
	N2	6(10)	69.00		93.50	
Tumor stage	Early	24(40)	28.00	0.729	78.50	0.562
	Advanced	36(60)	35.00		88.00	
Type of growth	Endophytic	9(15)	48.00	1.000	91.00	0.120
	Exophytic	51(85)	28.00		83.00	
Lymph node metastasis	N0	27(45)	47.00	0.604	79.00	0.775
	N +	33(55)	28.00		86.00	
Broders grade	Well	38(63.3)	35.00	0.789	77.50	0.038
	Mod-poor	22(36.6)	35.00		91.50	
IFG	4–8	8(13.3)	28.00	0.957	76.00	0.204
(TMS)	9–12	17(28.3)	47.00		83.00	
	13–16	35(58.3)	28.00		90.00	
DK	1	4(6.66)	00.00	0.423	52.50	0.113
	2	12(20)	44.50		80.50	
	3	20(33.3)	57.00		81.00	
	4	24(40)	26.00		93.50	
NP	1	1(1.6)	14.00	0.160	83.00	0.038
	2	14(23.3)	21.00		71.00	
	3	17(28.3)	66.00		91.00	
	4	28(46.6)	26.00		87.50	
PI	1	4(6.6)	7.00	0.165	69.00	0.560
	2	3(5)	47.00		91.00	
	3	13(21.6)	56.00		83.00	
	4	40(66.6)	27.50		90.00	
LI	1	6(10)	35.00	0.397	85.50	0.430
	2	25(41.6)	48.00		83.00	
	3	24(40)	26.50		90.00	
	4	5(8.3)	00.00		85.00	

Mann-Whitney *U* test.
BM-Buccal Mucosa; RMT-Retromolar trigone; GBS-Gingivo buccal sulcus; TC-Tobacco and areca nut chewing, TS-Tobacco smoking; C-Combination of habits; IFG-Invasive front grading; TMS-Total malignancy score; DK-Degree of Keratinization; NP-Nuclear Polymorphism; LI-Lymphocytic infiltration.

area than with other sites. Median LI was higher in moderate-poorly differentiated tumors than well differentiated OSCCs.

4. Discussion

Abnormalities in cell-cycle-controlling genes are important in the malignant transformation and proliferation of tumors [13]. The inactivation of the *p53* tumor suppressor gene is the most common genetic alteration in all cancer types and is common in squamous cell carcinoma (SCC) with approximately 50% of the lesions expressing a mutant form of protein [14]. The positivity percentage for p53 in OSCC varies in different studies from 0 to 100% [15,16]. In this investigation, over expression of p53 was found in 63% of cases, a comparable range of p53 expression has been stated by various researchers [17-22]. Increased expression of p53 could be due to genetic inactivation of *p53* attributed to its conformational mutations and allelic deletion. Mutations of p53 result in a greatly extended protein half-life, thus permitting IHC detection [13]. Missense mutations can also be detected in IHC, due to prolonged half life time of p53 mutated protein leading to nuclear accumulation. The immunodetectable p53 expression can also result from a stabilized status of the protein due to its combination with viral or intracellular proteins [21]. Other potential mechanism for accumulations of p53 protein, by binding to cellular proteins such as 70 kD heat shock protein, Mdm2 or viral proteins i.e., Early gene (E6) of Human papilloma virus (HPV) may account for the higher prevalence of p53 over expression in malignant lesions [23].

Approximately 20% of oral cancers in patients that lack the classical risk factors of tobacco and alcohol abuse are also high risk HPV positive [24]. HPV is a risk ingredient for OSCC. The data about epidemiology of HPV positive in OSCC has a lot of variation [25]. The overall prevalence of HPV in OSCC in India has been reported as ranging from 20% to 50% [26]. HPV-16 is by far the most prevalent mucosal high-risk HPV type, followed by HPV-18 [24]. Among HNSCC with 90% of the HPV types identified as HPV16 [27]. In the disease with HPV infection, it is known that the viral genome integrates into the host genome. Many studies have shown that an integrated part of the genome corresponding to the Early gene (E6) and E7. E6 and E7 sequences are directly involved in the cellular cycle by inhibiting the normal function of p53 and pRb. In HPV-positive HNSCC, p53 interacts with the E6 protein, which leads to increased ubiquitin-dependent proteolysis of p53. Defective p53 could allow abnormal cells to proliferate, resulting in cancer [25].

In this study, 22 tumors were negative for p53. The reasons suggested for this negativity could be due to non-sense and frame-shift mutations of *p53* gene which may result in deletion or truncation of the protein, hence do not show detectable levels of p53 protein IHC. Another reason why loss of p53 activity may not be accompanied by accumulation of the p53 protein is that the underlying lesion may not be a point mutation but a gross deletion that abolishes all p53 protein production [23,28]. Also, tumors that follow different carcinogenic pathways in which the p53 abrogation does not play a role can be a reason for lacking p53 expression [29].

In 92% of tumors, p53 LI was higher at ITF than in the WT and with a significant positive correlation between WT and ITF with respect to p53 LI. These findings were in accordance with previous reports by several authors [5,6,13,15,20,30] who have observed increased p53 expression at the ITF when compared to remaining parts of the tumor. p53 LI was higher at ITF than in the WT which is indicative of noticeable accumulation of p53 positive cells at the IF of tumors. This analysis demonstrates a high incidence of expression of mutant p53 in OSCC at the ITF, underpinning genomic instability and increased cell proliferation due to loss of p53 function. Higher expression of p53 signifies actively proliferating malignant cells. Owing to the presence of amplified and uninhibited cell proliferation at ITF, tumor cells may accumulate essential genetic alterations for invasion and metastasis.

The incidence of proto-oncogene amplification in HNSCC patients is comparable to that reported for other solid tumors [31]. The expression of c-Myc is variable according to various studies [12]. Currently, only a limited number of studies have used an IHC technique to evaluate c-Myc protein expression [32]. Using IHC, over expression of Myc has been found to vary from 21% to 68% in HNSCC [33]. In the present investigation 90% of cases showed c-Myc expression. Results are in agreement with studies done by several researchers [34-37] who have observed similar range of c-Myc over expression in OSCC. Few studies found c-Myc positivity in all the cases of OSCC [38,39].

Over expression of c-Myc protein detected by IHC may reflect genetic alteration of the *c-Myc* gene such as gene amplification. Studies have shown that over expression of the c-Myc oncoprotein was significantly associated with amplification of the gene in breast cancer. Strong staining for c-Myc protein also could indicate accumulation of the protein because of stabilization or increased half-life of the c-Myc mRNA or the protein. Other mechanisms such as altered transcription rate or post-transcriptional modification may also be responsible for sustained c-Myc expression [32].

Several features of c-Myc and HPV-E7 suggest similarities in the means by which they regulate the cell cycle [40]. E7 protein interacts with pRb protein that is an important ingredient for the control of cellular cycle. This interaction causes the release of the E2F transcription factor that is now free to act and may stimulate cellular division via c-Myc protein. c-Myc codes for a protein that binds to the DNA of other genes and is therefore a transcription factor. Upon mitogen stimulation, Rb becomes phosphorylated and the Rb-E2F complex dissociates, freeing E2F to activate transcription of c-Myc and cell proliferation goes on [25]. The ability of c-*Myc* to promote cell proliferation suggests that its deregulation contributes to deregulated DNA synthesis and genomic instability [41] i.e., characterized by gene amplification, aneuploidy and polyploidy. Other studies suggest the c-Myc induces the production of ROS by mitochondria, leading to DNA damage and genomic instability [42].

In this study, only 6 cases were negative for c-Myc. This failure for samples to stain can probably be attributed to excessive heat during the processing of paraffin embedded tissues, particularly when paraffin or slides are subjected to heat in excess of 60 °C [37].

In 94% of cases, c-Myc LI was higher at ITF than in the WT with a significant positive correlation between WT and ITF with respect to c-Myc LI. Eversole et al. and Sakai et al. detected stronger staining at the ITF than remaining parts of the tumor [37,39]. Pietilainen et al. noted that expression of c-Myc protein in breast cancer was stronger at the invasive margin of the tumor, which indicates that c-Myc may facilitate invasive growth [43]. Studies have also shown that c-Myc is associated with loss of cell differentiation in OSCC [38]. It plays a key role in the switch from proliferation to differentiation. Decreased expression of c-*Myc* is evident following induction of differentiation in diverse cell types. But when there is loss of autoregulation of c-Myc because of mutations or genetic alteration, c-Myc is associated with loss of differentiation and uncontrolled proliferation leading to greater expression of c-Myc in actively proliferating cells [32]. A high level of c-Myc expression is known to accelerate the growth rate of the cells. ITF of OSCC is composed of tumor subpopulations with high proliferative activity and shows a lower degree of differentiation when compared to other parts of the tumor. This suggests the possibility of increased c-Myc expression at the ITF than in the WT in this investigation.

Principally the association of *c-Myc* with *p53* is less explored, though important [44]. In this evaluation 60% of tumors over expressed both p53 and c-Myc. Concordant p53 and c-Myc over expression may be indicative of aggressive tumors or anaplastic tumor zones which have collected too many alterations/mutations in genes which control the cell cycle. Over expression of c-Myc along with p53 may cause a rapid progression of the OSCC, possibly over expression of c-Myc actively participates in the p53 concert by accumulating different genetic lesions and thereby maintains the proliferative potential of cells.

Various hypotheses have been suggested by different researchers for the co-expression of c-Myc and p53 in oral oncogenesis which has been

mentioned in the literature [10,44,45]. Papakosta et al. have found expression of both p53 and c-Myc in the initial stages of oral oncogenesis, indicating a possible strong correlation between them, which can be further supported by the fact that human p53 promoter can be directly transactivated by c-Myc/Max heterodimers, or indirectly through the induction of *p19ARF* by c-Myc, which in turn inhibits the repressor of p53 Mdm2 [10]. The p53 promoter contains a conserved consensus recognition sequence for the basic-helix-loop-helix-containing proteins, identical to the specific binding site for c-Myc/Max heterodimers. In co-transfection assays, it has been observed that c-Myc transactivates the p53 promoter [45].

Tumors which over express c-Myc may, in some cases, over express mutant p53 or completely lack expression of the gene [45]. The above-mentioned data may be explained by the contribution made by p53 and c-Myc to the formation of OSCC. If a mutation in a single allele of *p53* that inactivated the tumor suppressor activity were the primary event, then a subsequent de-regulation or over-expression of c-Myc could result directly or indirectly in elevated transcription of the mutant gene. Since mutant p53 proteins generally are more stable than the wild proteins, the combined result would be an elevated level of mutant p53 protein in the cell. This would lead to an increased growth advantage for these cells, possibly by virtue of the ability of high concentrations of mutant p53 to complex with and inactivate the remaining wild p53 [10].

c-Myc overexpression and p53 loss share a common consequence, namely, the uncoupling of mitosis and subsequent S-phase initiation. Cells lacking p53 may show gene amplification as well as the polyploidy or aneuploidy typical of many tumors. In the absence of functional p53, cells arrested either in G2 or M by metabolic inhibitors undergo additional rounds of S-phase, without intervening mitoses, eventually resulting in the accumulation of cells with a polyploid DNA content. The combination of p53 inactivation and c-Myc overexpression in diploid cells markedly accelerates the spontaneous development of tetraploidy. Loss of p53 and overexpression of c-Myc permits the emergence and survival of cells with increasingly severe damage and the eventual of tetraploidy. Inherent genomic instability which ensues as a result of p53 inactivation is greatly accentuated by the co-expression of c-Myc [40].

An association between clinicopathologic parameters and p53 expression at the ITF was examined, and only habits showed significant association. Sixty percent (36/60) of OSCC patients in the present study were only chronic areca nut tobacco chewers. Among them over-expression of p53 was noted in 75% (27/36) of cases. A higher incidence of p53 protein overexpression was found in areca nut and tobacco chewers than with other habits suggesting that areca nut - tobacco chewing may play a part in p53 overexpression in OSCC. Indicating that carcinogens in smokeless tobacco use/areca-tobacco chewing could cause a mutation in the *p53* gene. This analysis also could not establish a significant association between age, sex, site, size, nodal status, stage, growth pattern, LNM, and grading with p53 expression. Similar findings were noted by several researchers [16,28,30].

Among the clinicopathologic parameters location, tumor grade and nuclear polymorphisms showed a significant association with the c-Myc expression at ITF. In this study, majority of the tumors exhibiting c-Myc positivity at the ITF about 58% were poorly differentiated (IFG). Incidence of aberrant expression of c-Myc protein increases from well differentiated to poorly differentiated tumors, suggests that c-*Myc* may be a contributing factor for loss of differentiation and increased aggressiveness [32]. Mishra et al. reported that c-Myc plays a role in higher stage of chewing tobacco mediated cancer development [46]. In this study, majority of OSCC patients who expressed c-Myc were areca nut-tobacco chewers 97% (35/36). In this analysis 87.5% (21/24) of early tumors and 92% (33/36) of advanced tumors expressed c-Myc. c-Myc expression was observed in tumors from all stages, suggesting possible involvement during the various stages of the neoplastic process. This could be due to the fact that c-Myc is a regulator of multiple cell signalling pathways [35]. Nguyen et al. noticed significant increase in c-Myc expression in advanced stage compared to early stage cancers and proposed that overexpression of cell cycle regulatory proteins correlates with advanced tumor stage in HNSCC [47]. The molecular pathways that modify the expression of c-Myc are complex and probably many which are still not described. Further studies are needed to analyze the relationship between the molecules that interact with c-Myc and clinicopathological parameters [12].

5. Conclusion

The present study, to the best of knowledge is first of its kind to assess the expression of p53 and c-Myc in OSCC and to analyze and compare this expression in the WT and at the ITF. The study confirms the over expression of p53 and c-Myc in OSCC thus emphasizing the fact that p53 and c-Myc genes are frequently deregulated in HNSCC. p53 LI was higher at ITF than in the WT which is indicative of noticeable accumulation of p53 positive cells at the IF of tumors. This study demonstrates a high incidence of expression of mutant p53 in OSCC at the ITF, underpinning genomic instability and an increased cell proliferation due to loss of p53 function. Higher expression of p53 signifies actively proliferating malignant cells. Owing to the presence of amplified and uninhibited cell proliferation at ITF, tumor cells may accumulate essential genetic alterations for invasion and metastasis.

c-Myc is associated with loss of differentiation and uncontrolled proliferation leading to greater expression of c-Myc in actively proliferating cells. A high level of c-Myc expression is known to accelerate the growth rate of the cells. ITF of OSCC is composed of tumor subpopulations with high proliferative activity and shows a lower degree of differentiation when compared to other parts of the tumor. This suggests the possibility of increased c-Myc expression at the ITF than in the WT in this investigation.

Over expression of c-Myc along with p53 may cause a rapid progression of the OSCC, possibly over expression of c-Myc actively participates in the p53 concert by accumulating different genetic lesions and thereby, maintains the proliferative potential of cells. Concordant p53 and c-Myc overexpression may be indicative of aggressive tumors or anaplastic tumor zones which have collected too many alterations/mutations in genes which control the cell cycle. Further investigations may provide more insights to better clarify this interesting field of study.

Conflict of interest

None.

Submission declaration

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