# **Original Article**

# Elevated p63 Expression as an Indicator for Poorer Prognosis in Squamous Cell Carcinomas of the Oral Cavity: An Immunohistochemical Study

#### Abstract

Background: Oral cancer remains one of the most debilitating and disfiguring of all malignancies. The survival rates for oral cancer vary, depending on several factors. Although p63 is an accepted prognostic marker in various other carcinomas, no consensus has been obtained till date regarding the applicability of p63 as a prognostic marker in head and neck squamous cell carcinomas (SCC). Aim and Objectives: The present study was conducted to determine the applicability of p63 as a prognostic marker in oral squamous cell carcinomas (OSCC) using incisional biopsies. Materials and Methods: Incisional biopsies of 27 candidates who were histopathologically diagnosed with SCC (8070/3) of the oral cavity (C06.9) (OSCC) between January 2013 and June 2014 were included in the trial. Sections were subjected to immunohistochemistry with p63 as the primary antibody. The percentage p63 expression was calculated and compared based on their Broders' and Anneroth's multifactorial grading systems with the overall survival status of the patients. **Results and Observations:** A statistically significant increase (P = 0.0203) was found between p63 expression and the histological grading of the tumor (from Grade I OSCC to Grade III OSCC). Similarly, a statistically significant correlation (P = 0.013) was obtained between mean Anneroth score (MAS) and the Broders' grading. Log-rank (Mantel-Cox) test showed statistical significance for the survival curves when the candidates were classified based on % p63 expression (P = 0.0049) and MAS (P = 0.0003). Conclusion: We have shown expression of p63 to correlate with survival in OSCCs, where high expression was seen in tumors with poorer survival after treatment. Furthermore, the usage and importance of Anneroth's multifactorial grading system over Broders' grading system in routine histopathological reporting for incisional biopsies of OSCCs is stressed.

Arvind Venkatesh, Vijay Wadhwan, Pooja Aggarwal, Vandana Reddy, Preeti Sharma, Suhasini Palakshappa Gotur, Chitrapriya Saxena<sup>1</sup>

Department of Oral Pathology and Microbiology, Subharti Dental College and Hospital, 'Department of Dentistry, Lala Lajpat Rai Memorial Medical College, Meerut, Uttar Pradesh, India

Keywords: Carcinoma, human, immunohistochemistry, prognosis, squamous cell of the head and neck, TP63 protein

# Introduction

Oral cancer remains one of the most debilitating and disfiguring all of malignancies. Our knowledge on the prevention and treatment of cancer is increasing, yet the number of new cases grows every year.<sup>[1]</sup> The survival rates for oral cancer vary, depending on several factors: the stage of the lesion, the site of the primary tumor, the adequacy of initial treatment, and the histological differentiation of the malignancy.<sup>[2]</sup> Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by several genetic alterations.<sup>[3]</sup> In recent years, considerable progress has been made in understanding the genetic basis of the development of oral squamous cell carcinoma (OSCC). Alterations of

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

the p53 tumor suppressor gene are the most frequently documented genetic abnormalities in human cancer, especially OSCC.<sup>[4]</sup> p53 belongs to a family which includes p63 and p73, which are expected to play a role in cancer development due to their close homology to p53. A large data collected over the years have indicated that altered expression of p63 and p73 could be found in different neoplasia and play a role in its biology.<sup>[5]</sup>

Since p63, in tumorigenesis, is attributed to various roles such as, apoptosis,<sup>[6]</sup> cellular senescence,<sup>[7]</sup> tumor suppression,<sup>[6]</sup> interplay with NOTCH pathways,<sup>[8]</sup> cellular proliferation<sup>[7]</sup> and oncogenetic properties,<sup>[5,6]</sup> due to the diversity in the gene structure,<sup>[9]</sup> and availability of numerous isoforms,<sup>[6]</sup> studies conducted

How to cite this article: Venkatesh A, Wadhwan V, Aggarwal P, Reddy V, Sharma P, Gotur SP, *et al.* Elevated p63 expression as an indicator for poorer prognosis in squamous cell carcinomas of the oral cavity: An immunohistochemical study. Indian J Med Paediatr Oncol 2018;39:146-52. Address for correspondence: Dr. Arvind Venkatesh, Department of Oral Pathology and Microbiology, Subharti Dental College and Hospital, NH 58, Delhi Haridwar Bypass Road, Meerut - 250 005, Uttar Pradesh, India. E-mail: drarvindvenkatesh@ gmail.com



For reprints contact: reprints@medknow.com

on the applicability of p63 as a prognostic marker has delivered varied, contrasting results in different types of cancers.

Although p63 is an accepted prognostic marker in various other carcinomas, no consensus has been obtained till date regarding the applicability of p63 as a prognostic marker in head and neck squamous cell carcinomas (SCC).<sup>[5]</sup>

Hence, the present study was conducted to determine the applicability of p63 as a prognostic marker in OSCC using incisional biopsies and aid to mitigate the overall effect of various isoforms of p63 in the pathogenesis of OSCCs.

# **Materials and Methods**

The present study was duly cleared for implementation by the University Ethical Committee, and an informed consent was obtained from all the candidates included in the study.

Twenty-seven candidates who were histopathologically diagnosed with SCC (8070/3) of the oral cavity (C06.9) between January 2013 and June 2014 and decided to undergo treatment for the disease in our center were included in the trial. This sample excluded patients in whom mortality was encountered due to intra- and post-operative complications of surgery, history of other systemic/immunodeficiency disorders, and recurrent cases of OSCC.

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of the incisional biopsies of all the 27 included candidates were retrieved from the pathology archives, and fresh H and E-stained sections were interpreted by three qualified pathologists for confirmation of the histological grade of OSCC as per Broders' classification<sup>[10]</sup> and calculation of the mean Anneroth score (MAS) based on the morphological (degree of keratinization, nuclear pleomorphism, number of mitotic figures per high power field [HPF]), and histological (pattern of invasion, stage of invasion, and lymphoplasmacytic infiltrate) scoring parameters of Anneroth's multifactorial grading system.<sup>[11]</sup> The various clinicopathological variables of the included study candidates have been tabulated in Table 1.

FFPE tissue blocks of normal oral mucosa (n = 10) obtained during therapeutic or surgical extractions were included as a control group.

## Immunohistochemistry

From each FFPE tissue block selected, 3  $\mu$ m thick sections were made on poly-l-lysine (0.1% [w/v] in H<sub>2</sub>O) (Sigma-Aldrich, Missouri, USA) coated slides. Sections were deparaffinized and rehydrated with xylene and serial dilutions of ethanol to distilled water. Tissue sections were immersed in EDTA buffer at a pH of 9 (EZ 2, Biogenex, Fremont, USA, ready to use), and heat-induced epitome retrieval was done using autoclave method at 120°C, 12–15 psi for 15 min. For each sample, anti-p63 antibody (Clone: 4A4) (Biogenex, Fremont, USA, mouse IgG,

candidates						
Variables in study candidates	Number of cases (%)					
Sample size (OSCC)	27					
Age (years)						
10-19	1 (3.7)					
30-39	4 (14.81)					
40-49	5 (18.51)					
50-59	10 (37.03)					
60-69	6 (22.22)					
70-79	1 (3.7)					
Sex						
Male	21 (77.77)					
Female	6 (22.22)					
Site						
Oral cavity, NOS (C06.9)	27					
Cheek mucosa (C06.0)	19 (70.37)					
Gum (C03.9)	5 (18.51)					
Dorsal surface of tongue (C02.0)	3 (11)					
TNM staging						
Stage II (nT N M )	12 (44 44)					
Stage III ( $pT_2T_0^{(n)}$ )	6 (22 22)					
Stage III ( $\mathbf{p} \mathbf{T}_{3} \mathbf{V}_{0} \mathbf{W}_{0}$ )	4(14.81)					
Stage III ( $pT_2N_1N_0$ )	= (14.01)					
Stage III ( $p_{1_3}N_1M_0$ ) Treatment protocol	5 (18.51)					
	20(74.07)					
S alone	20(74.07)					
S T KI	7 (23.93)					
Grade L ( all differentiated OSCO)	10 (27.02)					
Grade I (well-differentiated USCC)	10 (37.03)					
Oscc)	10 (37.03)					
Grade III (poorly differentiated OSCC)	7 (25.93)					
Anneroth's multifactorial grading						
system						
Mean Anneroth score ( $\leq 2.5$ )	14 (51.85)					
Mean Anneroth score (2.6-4.0)	13 (48.15)					
Percentage p63 expression						
<50%	5 (18.52)					
50%-75%	9 (33.33)					
>75%	13 (48.15)					
Status at end date						
ADf	15 (55)					
AwD	Nil					
DoD	12 (45)					
DoC	Nil					
Follow-up period (days)						
Range	121-949					
Mean	479.15					

Table 1: Clinicopathological variables of included study

OSCC – Oral squamous cell carcinoma; NOS – Not otherwise specified; ADf – Alive, disease free; AwD – Alive, with disease; DoD – Dead of disease: DoC – Dead of any other cause; S + RT – Surgery + postoperative radiotherapy; TNM – Tumor, node, and metastasis

ready to use) was used as the primary antibody for 45 min incubation at room temperature in a humidity

chamber. The antigen–antibody binding was detected with labeled anti-mouse polymer-horseradish peroxidase detection system and 3, 3'-diaminobenzidine + chromogen (Biogenex, Fremont, USA). Tissue sections were briefly immersed in hematoxylin for counterstaining. In all cases, staining of dysplastic epithelial cells served as positive internal controls for anti-p63 antibody, and the antigenic potential of the tissue blocks was confirmed by applying pan-cytokeratin cocktail (Biogenex, Fremont, USA, mouse IgG, ready to use) as primary antibody on the subsequent sections. For negative control, the primary antibody was replaced by mouse-negative control (nonimmune serum in phosphate-buffered saline with 0.09% sodium azide).

#### Quantitative assessment of p63 expression

The p63-stained slides were initially analyzed at low magnification (original magnification  $\times$  100) to select cancer islands which were defined as cancer tissue without fibroblasts and vasculature. Five HPFs (original magnification  $\times$  400) were selected in tumor proper area for each case of experimental group and in the epithelium of the control group, and the percentage of immune-reactive dysplastic cells was calculated by counting the dysplastic epithelial cells using manual tag function in the selected HPFs using Image Pro Express ver. 6.0 (Media Cybernetics Inc. Rockville, Maryland, USA) analysis software. The percentage of immune-reactive dysplastic cells for each case was calculated using the following formula.

Percentage of p63 immune-reactive dysplastic cells = Total no. of p63-positive dysplastic epithelial cells/Total no. of dysplastic epithelial cells  $\times$  100%

The percentage p63 expression for each sample was calculated, and the results were tabulated against the corresponding data on the survival status of the respective study candidate [Table 2].

## Results

#### Statistical analysis

The data were statistically analyzed using GraphPad Prism 5.03 for Windows (GraphPad Software Inc., La Jolla, CA, USA). P < 0.05 was considered significant.

Table 2: Clinicopathological parameters of the study candidates along with their survival status									
Case number	Age/sex	Broders' classification	Mean Anneroth	TNM staging	Treatment protocol	Percentage p63 expression	Follow-up (days)	Survival status	
1	60/famala	Crada LSCC	score	Stage II (pT N M)	C	42 4512	040	ADf	
1	52/male	Grade I SCC	1.0007	Stage II ( $p_1 N_0 N_0$ )	5	42.4315	949	ADI	
2	52/male	Grade I SCC	2.3333	Stage III $(pI_3N_0M_0)$	S	46.03//	942	ADI	
3	65/male	Grade I SCC	2.6667	Stage III $(pI_3N_1M_0)$	S	87.9194	322	DoD	
4	39/male	Grade I SCC	1.8333	Stage II $(pT_2N_0M_0)$	S	58.3756	926	ADf	
5	50/male	Grade I SCC	2.6667	Stage III $(pT_3N_1M_0)$	S + RT	72.2599	128	DoD	
6	45/male	Grade I SCC	2	Stage II $(pT_2N_0M_0)$	S	37.4057	735	ADf	
7	32/male	Grade I SCC	2.1667	Stage II $(pT_2N_0M_0)$	S	52.9182	460	ADf	
8	58/female	Grade I SCC	2.3333	Stage II $(pT_2N_0M_0)$	S	79.1411	279	DoD	
9	40/male	Grade I SCC	1.8333	Stage III $(pT_3N_0M_0)$	S + RT	49.6977	856	ADf	
10	50/female	Grade I SCC	2.3333	Stage III $(pT_3N_1M_0)$	S	34.3103	772	ADf	
11	60/male	Grade II SCC	2.1667	Stage III $(pT_3N_0M_0)$	S	70.3557	874	ADf	
12	34/male	Grade II SCC	2.8333	Stage III (pT <sub>2</sub> N <sub>0</sub> M <sub>0</sub> )	S	83.5924	179	DoD	
13	30/male	Grade II SCC	1.6667	Stage II (pT <sub>2</sub> N <sub>0</sub> M <sub>0</sub> )	S	61.4661	543	ADf	
14	60/Male	Grade II SCC	2.6667	Stage III $(pT_1N_1M_0)$	S + RT	77.9951	168	DoD	
15	71/female	Grade II SCC	2.1667	Stage II $(pT_N M_0)$	S	81.7857	338	DoD	
16	41/male	Grade II SCC	3.6667	Stage II (pT <sub>2</sub> N <sub>0</sub> M <sub>0</sub> )	S	74.3065	298	DoD	
17	48/male	Grade II SCC	2.8333	Stage III $(pT_1N_1M_0)$	S + RT	72.1297	523	ADf	
18	61/male	Grade II SCC	2.5	Stage II $(pT_NM_0)$	S	78.8359	460	ADf	
19	56/male	Grade II SCC	2	Stage II (pT <sub>2</sub> N <sub>2</sub> M <sub>2</sub> )	S	67.1755	502	ADf	
20	19/male	Grade II SCC	2.6667	Stage III $(pT_1N_1M_0)$	S + RT	75.2562	462	ADf	
21	45/female	Grade III SCC	3.5	Stage III (pT <sub>2</sub> N <sub>2</sub> M <sub>2</sub> )	S	83.087	225	DoD	
22	55/male	Grade III SCC	3	Stage II (pT <sub>N</sub> M <sub>0</sub> )	S	86.0549	194	DoD	
23	50/male	Grade III SCC	2.6667	Stage III $(pT_N, M_0)$	S	91.7867	141	DoD	
24	55/male	Grade III SCC	3.1667	Stage III (pT <sub>2</sub> N <sub>1</sub> M <sub>0</sub> )	S + RT	87.7478	820	ADf	
25	60/male	Grade III SCC	2.8333	Stage III $(pT_2N_1M_0)$	S + RT	87.0114	121	DoD	
26	50/female	Grade III SCC	2.1667	Stage II $(pT_2N_0M_0)$	S	61.2959	510	ADf	
27	55/male	Grade III SCC	3	Stage III $(pT_3N_0M_0)$	S	83.2356	210	DoD	

ADf – Alive, disease free; DoD – Dead of disease; SCC – Squamous cell carcinoma; S + RT – Surgery + postoperative radiotherapy; TNM – Tumor, node, and metastasis

#### p63 expression in normal oral mucosa

Normal human oral mucous epithelium had a basal and parabasal pattern of p63 expression. The labeling was only nuclear, with nuclei showing an intense staining, stronger in the basal layer with respect to the parabasal layer (with nuclei of the parabasal layer showing only a faint staining). In general, keratinocytes of suprabasal layers were not immunolabeled by anti-p63 antibody although a slight expression of p63 was recorded in some areas [Figure 1d]. Thus, normal epithelium included a mean of 20.86% (range: 9.26%–36.59%) of stained cells.

# p63 expression in squamous cell carcinomas of the oral cavity

Various staining patterns were observed for p63 expression in OSCCs. It was observed that the pattern of staining differs between the grading of neoplasms. Grade I neoplasms [Figure 1a] showed a varied range of p63 expression (range: 34.31%–87.91%; mean = 56.05%). In Grade II neoplasms [Figure 1b], the mean % p63 expression was higher when compared to Grade I OSCCs (mean: 74.29%; range: 61.47–83.6%) and lesser when compared to poorly differentiated neoplasms (Group III) [Figure 1c] which showed the most intense and diffuse labeling (mean: 82.89%; range: 61.29%–91.79%) [Table 2]. Staining for p63 was



Figure 1: p63 expression in (a) Grade I oral squamous cell carcinoma, (b) Grade II oral squamous cell carcinoma, (c) Grade III oral squamous cell carcinoma, (d) Normal oral mucosa (immunoperoxidase, original magnification ×400)

not detected in the keratin pearl areas in both Grade I and Grade II neoplasms.

A statistically significant correlation (P = 0.0203) was found between p63 expression and the histological grading of the tumor; in fact, the percentage of cells expressing p63 was lower in well-differentiated tumors (Grade I) with respect to poorly differentiated tumors (Grade III) [Table 3].

Similarly, a statistically significant correlation (P = 0.013) was obtained between MAS and the Broders' histological grading of the tumor; the MAS was lower in well-differentiated tumors (Grade I) when compared to that of the poorly differentiated counterparts (Grade III) [Table 3].

To analyze the prognostic significance of p63, the study candidates were subclassified into three subgroups based on their percentage p63 expression (subgroup x [n = 5]: <50% p63 expression; subgroup y [n = 9]: 50%–75% p63 expression; subgroup z [n = 13]: >75% p63 expression).

In addition, the prognostic applicability of Broders' classification (Grade I SCC [n = 10]; Grade II SCC [n = 10]; and Grade III SCC [n = 07]) and Anneroth's multifactorial grading system (MAS:  $\leq 2.5 [n = 14]$ ; MAS: 2.6–4.0 [n = 13]).

The patients with increased p63 expression (subgroup z) had poorer survival rates than the patients with comparatively lesser p63 expression (subgroup x, subgroup y). Among participants of subgroup x (05/27), the survival proportion was 100.00 after 949 days whereas data of participants of subgroup y (9/27) showed a survival proportion of 77.78 after 926 days of follow-up. Whereas in participants with the highest p63 expression, subgroup z (13/27), the survival proportion after 820 days of follow-up was 23.07. The statistical comparison of the survival curves was done by log-rank (Mantel-Cox) test which showed statistical significance (P = 0.0049) between the survival curves of patients of subgroups x, y, and z, respectively [Figure 2].

Similarly, the patients with higher MAS (MAS = 2.6–4) had poorer survival rates when compared to the patients with lesser MAS (MAS  $\leq$ 2.5). Among patients with MAS  $\leq$ 2.5 (14/27), the survival proportion was 85.71 after 949 days of follow-up whereas data of patients with MAS = 2.6–4 showed a comparatively lower survival proportion of 23.07 after 820 days of maximum follow-up. The statistical comparison of the survival curves was done

Table 3: Percentage p63 expression and mean Anneroth scores of various grades of oral squamous cell carcinoma								
Factor analyzed	Grade I	Grade II	Grade III	Normal oral	One-way	Tukey's multiple comparison test		
	SCC ( <i>n</i> =10)	SCC ( <i>n</i> =10)	SCC ( <i>n</i> =07)	mucosa (n=10)	ANOVA(P)			
Mean Anneroth score (mean)	2.1833	2.5167	2.9047	Not applicable	0.013	<i>P</i> <0.05 between Grade I SCC and Grade III SCC		
Percentage p63 expression (mean)	56.0517	74.2899	82.8885	20.8655	0.0203	<i>P</i> <0.05 between Grade I SCC and Grade II SCC, Grade III SCC, normal mucosa; Grade II SCC, Grade III SCCs and normal mucosa		

SCC - Squamous cell carcinoma

by log-rank (Mantel-Cox) test which showed statistical significance (P = 0.0003) between the survival curves of patients with MAS  $\leq 2.5$  and MAS = 2.6-4 [Figure 2].

On the contrary, when the tumors were classified based on Broders' classification, although the survival proportion of poorly differentiated (Grade III) tumors (28.57 after 820 days) was comparatively lower than the moderately (Grade II) (60.00 after 874 days) and well-differentiated (Grade I) neoplasms (70.00 after 949 days), the statistical comparison of the survival curves (log-rank [Mantel-Cox] test) showed no statistical significance (P = 0.1016) [Figure 2].

Moreover, when the mean and standard error of mean (X  $\pm$  SEM) of the percentage p63 expression of the study participants classified based on their survival period following diagnosis, it was observed that there was a statistically significant increase (*P* = 0.0004) in the mean % p63 expression of patients with <479 days (mean no. of follow-up) of overall of Grade I SCC (79.77  $\pm$  4.532) and Grade II SCC (79.42  $\pm$  2.065) when compared to the participants with >479 days' survival (Grade I SCC = 45.89  $\pm$  3.228; Grade II SCC = 70.87  $\pm$  2.494). In Grade III SCC participants, although there was an increase in the mean % p63 expression in participants with <479 days of survival (86.24  $\pm$  1.587) when compared to those with >479 days of survival (74.52  $\pm$  13.23), no statistical significance was obtained [Table 4].

Similarly, when the  $X \pm$  SEM of MAS of study participants classified based on survival period following diagnosis, it was compared; although there was an increase in the MAS of patients with <479 days of survival when compared to those with >479 days of survival in all the three histological grades of the neoplasm (Grade I, II, III SCCs), statistical

significance was obtained only for well-differentiated neoplasms [Table 4].

# **Discussion**

The p63 proteins are important in the formation of the oral mucosa, and in normal oral mucosa, there is a balance between the six proteins belonging to the p63 family. In contrast, an imbalance in levels between them is seen in SCCs, in the same area.<sup>[12,13]</sup> Although numerous studies have preferably used semi-quantitative analysis and quick scoring methods for grading immunoperoxidase expression in immunohistochemistry,<sup>[12,14]</sup> our primary intent was to exactly quantify the p63 expression of every HPF assessed. Hence, quantitative assessment was done using manual tag function of Image Pro Express ver. 6.0 (Media Cybernetics Inc., Rockville, MD, USA) analysis software. Although, being a comparatively more time-consuming process than semi-quantitative analysis, the results could be stipulated to the exact percentage of immunoperoxidase expression in each HPF included, as compared to results expressed in "range" in semi-quantitative methods. This method can be preferred at centers with a limited access to automated quantification facilities.

Cancer arises in a multistep process resulting from the sequential accumulation of genetic and epigenetic defects and the clonal expansion of selected cell populations.<sup>[15]</sup> The p53 gene, first described in 1979, was the first tumor suppressor gene to be identified. It was originally believed to be an oncogene – a cell-cycle accelerator – but genetic and functional data obtained 10 years after its discovery showed it to be a tumor suppressor.<sup>[16]</sup> In 1997–1998, two additional members of the p53 family, namely, p63 and p73 which had a close structural homology with their predecessor were discovered.<sup>[17]</sup>



Figure 2: Kaplan-Meier survival curves based on various criteria of classification

Table 4: Study candidates tabulated based on mean survival (days)								
Broders'	Me	an Anneroth sc	ore (mean±SEM)	Percentage p63 expression (mean±SEM)				
grading	Survival		Inference (unpaired <i>t</i> -test)	Survival		Inference (unpaired <i>t</i> -test)		
	>479 days	<479 days		>479 days	<479 days			
Grade I SCC	2.024±0.0991	2.556±0.1111	Significant (P=0.0141)	45.89±3.228	79.77±4.532	Significant (P=0.0004)		
Grade II SCC	2.306±0.1796	2.833±0.3118	Nonsignificant (P=0.1521)	70.87±2.494	79.42±2.065	Significant (P=0.0412)		
Grade III SCC	2.667±0.5	3.000±0.1394	Nonsignificant (P=0.3881)	74.52±13.23	86.24±1.587	Nonsignificant (P=0.1784)		

SCC - Squamous cell carcinoma; SEM - Standard error of mean

The complexity of the study of p63 is due to the existence of multiple isoforms (six known isoforms, namely, TA-p63 $\alpha$ , TA-p63  $\beta$ , TA-p63  $\gamma$ ,  $\Delta$ N-p63 $\alpha$ ,  $\Delta$ N-p63  $\beta$ , and  $\Delta$ N-p63  $\gamma$ ) with opposing functions.<sup>[18]</sup> The multiple numbers of antibodies that are needed to be employed distinguish between these isoforms have made the ability to analyze the expression of p63 difficult in human tumors. Many studies have found that p63 is overexpressed in human tumors while other studies have shown a loss of expression of p63.<sup>[6]</sup>

The present study was intended to evaluate whether the amount of p63 expression (expressed as percentage expression) could be related to any of the histological grading which is generally used to define the aggressiveness of the tumor such as the Broders' classification and Anneroth's multifactorial grading system, which takes into consideration various morphological and histological parameters previously mentioned. Furthermore, the multifactorial grading system is considered to have greater significance in predicting the growth capacity and outcome of the tumor.<sup>[19]</sup> Although the multifactorial grading of invasive sites/front has shown highly significant prognostic value,<sup>[20,21]</sup> since the intent was to use incisional biopsy tissue, parameters of Anneroth's multifactorial grading system were preferred over Bryne's multifactorial grading system,<sup>[21]</sup> since the grading criteria of the latter were not applicable for most of the incisional biopsies included in the study since they contained only the tumor tissue.<sup>[20]</sup>

Interestingly, the survival curves showed a statistically significant correlation (P = 0.0003) between the study samples when categorized based on their MAS [Figure 2]. Hence, the Anneroth's multifactorial grading system can be preferred for initial assessment of prognosis and the aggressiveness of the tumor when incisional biopsy tissue alone is available for the pathologist. Moreover, we advocate the use of Anneroth's multifactorial grading system, since the survival curves showed no prognostic significance (P = 0.1016) [Figure 2]. This finding was in consensus with the results of various previously conducted studies.<sup>[19,20-23]</sup>

The survival curves showed a statistically significant correlation (P = 0.0049) when the study candidates were classified based on their percentage p63 expression [Figure 2]. Studies conducted by Lo Muzio *et al.* in 2005,<sup>[14]</sup> Gu *et al.* in 2008,<sup>[24]</sup> and Loljung *et al.* in 2014<sup>[12]</sup> found a significant correlation of p63 expression and patient survival in OSCCs, and Cho *et al.* in 2003<sup>[25]</sup> and Moergel *et al.* in 2010<sup>[26]</sup> associated increased p63 expression with radiation resistance whereas, on the other hand, the present study results are at odds with the findings of the studies conducted by Bortoluzzi *et al.* in 2004<sup>[27]</sup> and Monteiro *et al.* in 2016.<sup>[28]</sup>

Data from the p63 field currently demonstrate that p63 can act as a tumor suppressor or as an oncogene. The

data are most consistent with supporting the idea that the  $\Delta$ Np63 isoforms have oncogenic activities while the TAp63 isoforms have tumor suppressive activities.<sup>[5-7]</sup> In addition, discovery of the interactions of p63 in NOTCH pathway,<sup>[8]</sup> beta-catenin signaling pathway,<sup>[29]</sup> and control of growth signaling pathways involving cyclin kinase inhibitor, p21 and p57,<sup>[8]</sup> have validated the existence of a correlation between p63 expression and the invasive behavior of various tumors.

Flores, based on an extensive review of studies conducted on p63, put forth a hypothesis that the downregulation or loss of TAp63 and/or overexpression of  $\Delta$ Np63 may lead to inhibition of the functions of TAp63, p53, and TAp73 which, in turn, will result in the development of an invasive and metastatic tumor. Furthermore, it was evident that mutant p53 could bind to TAp63 and TAp73, which would inhibit their function leading to the development of an invasive and metastatic tumor.<sup>[6]</sup>

# **Summary and Conclusion**

In summary, we have shown expression of p63 to correlate with survival in OSCCs, where high expression was seen in tumors with poorer survival after treatment. Although the results of the present study have shown considerable promising evidence for the applicability of p63 as a prognostic marker for OSCCs, the completeness of the follow-up is crucial in any study of survival. Hence, it is intended to extend the follow-up for a longer period (5–10 years) and also accommodating additional cases, which we intend, will aid toward validating the applicability of p63 as a prognostic marker for OSCCs. Furthermore, the usage and importance of Anneroth's multifactorial grading system over Broders' grading system in routine histopathological reporting for incisional biopsies of OSCCs is stressed.

#### Acknowledgments

The authors would like to thank all the candidates and their family members who were a part of this trial.

#### Financial support and sponsorship

Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

#### References

- Speight PM, Palmer S, Moles DR, Downer MC, Smith DH, Henriksson M, *et al.* The cost-effectiveness of screening for oral cancer in primary care. Health Technol Assess 2006;10:1-144, iii-iv.
- Balasubramaniam AM, Sriraman R, Sindhuja P, Mohideen K, Parameswar RA, Muhamed Haris KT. Autofluorescence based diagnostic techniques for oral cancer. J Pharm Bioallied Sci 2015;7 Suppl 2:S374-7.
- 3. Tanaka T, Tanaka M, Tanaka T. Oral carcinogenesis and

oral cancer chemoprevention: A review. Patholog Res Int 2011;2011:431246.

- Fukuda M, Kusama K, Sakashita H. Molecular insights into the proliferation and progression mechanisms of the oral cancer: Strategies for the effective and personalized therapy. Jpn Dent Sci Rev 2012;48:23-41.
- 5. Graziano V, De Laurenzi V. Role of p63 in cancer development. Biochim Biophys Acta 2011;1816:57-66.
- 6. Flores ER. The roles of p63 in cancer. Cell Cycle 2007;6:300-4.
- 7. Guo X, Mills AA. p63, cellular senescence and tumor development. Cell Cycle 2007;6:305-11.
- Dotto GP. Crosstalk of Notch with p53 and p63 in cancer growth control. Nat Rev Cancer 2009;9:587-95.
- Müller M, Schleithoff ES, Stremmel W, Melino G, Krammer PH, Schilling T. One, two, three – p53, p63, p73 and chemosensitivity. Drug Resist Updat 2006;9:288-306.
- Broders AC. Carcinoma of the mouth: Types and degrees of malignancy. Am J Roentgenol Radium Ther Nucl Med 1927;17:90-3.
- 11. Anneroth G, Batsakis J, Luna M. Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. Scand J Dent Res 1987;95:229-49.
- Loljung L, Coates PJ, Nekulova M, Laurell G, Wahlgren M, Wilms T, *et al.* High expression of p63 is correlated to poor prognosis in squamous cell carcinoma of the tongue. J Oral Pathol Med 2014;43:14-9.
- Thurfjell N, Coates PJ, Uusitalo T, Mahani D, Dabelsteen E, Dahlqvist A, *et al.* Complex p63 mRNA isoform expression patterns in squamous cell carcinoma of the head and neck. Int J Oncol 2004;25:27-35.
- Lo Muzio L, Santarelli A, Caltabiano R, Rubini C, Pieramici T, Trevisiol L, *et al.* p63 overexpression associates with poor prognosis in head and neck squamous cell carcinoma. Hum Pathol 2005;36:187-94.
- Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, *et al.* Genetic progression model for head and neck cancer: Implications for field cancerization. Cancer Res 1996;56:2488-92.
- Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307-10.
- 17. Levine AJ, Oren M. The first 30 years of p53: Growing ever more complex. Nat Rev Cancer 2009;9:749-58.

- Yang A, McKeon F. p63 and p73: p53 mimics, menaces and more. Nat Rev Mol Cell Biol 2000;1:199-207.
- Anneroth G, Hansen LS. A methodologic study of histologic classification and grading of malignancy in oral squamous cell carcinoma. Scand J Dent Res 1984;92:448-68.
- Bryne M, Koppang HS, Lilleng R, Stene T, Bang G, Dabelsteen E. New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. J Oral Pathol Med 1989;18:432-7.
- Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol 1992;166:375-81.
- Jakobsson PA, Eneroth CM, Killander D, Moberger G, Mårtensson B. Histologic classification and grading of malignancy in carcinoma of the larynx. Acta Radiol Ther Phys Biol 1973;12:1-8.
- 23. Anneroth G, Hansen LS, Silverman S Jr. Malignancy grading in oral squamous cell carcinoma. I. Squamous cell carcinoma of the tongue and floor of mouth: Histologic grading in the clinical evaluation. J Oral Pathol 1986;15:162-8.
- Gu X, Coates PJ, Boldrup L, Nylander K. p63 contributes to cell invasion and migration in squamous cell carcinoma of the head and neck. Cancer Lett 2008;263:26-34.
- Cho NH, Kim YB, Park TK, Kim GE, Park K, Song KJ. P63 and EGFR as prognostic predictors in stage IIB radiation-treated cervical squamous cell carcinoma. Gynecol Oncol 2003;91:346-53.
- Moergel M, Abt E, Stockinger M, Kunkel M. Overexpression of p63 is associated with radiation resistance and prognosis in oral squamous cell carcinoma. Oral Oncol 2010;46:667-71.
- Bortoluzzi MC, Yurgel LS, Dekker NP, Jordan RC, Regezi JA. Assessment of p63 expression in oral squamous cell carcinomas and dysplasias. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:698-704.
- Monteiro LS, Delgado ML, Ricardo S, Amaral BD, Salazar F, Pacheco J, *et al.* Prognostic significance of CD44v6, p63, podoplanin and MMP-9 in oral squamous cell carcinomas. Oral Dis 2016;22:303-12.
- 29. Koga F, Kawakami S, Kumagai J, Takizawa T, Ando N, Arai G, et al. Impaired Delta Np63 expression associates with reduced beta-catenin and aggressive phenotypes of urothelial neoplasms. Br J Cancer 2003;88:740-7.