Original Article

Apoptotic and mitotic indices in oral epithelial dysplasia and squamous cell carcinoma: A comparative study

Suhasini PD, Shaila Mulki, Supriya H

Department of Oral Pathology and Microbiology, KVG Dental College and Hospital, Sullia, Karnataka, India

Abstract

Background: Assessment of apoptotic cells and mitotic figures using light microscopy is an easy and viable alternative to assess tumour behaviour.

Aims: To evaluate apoptotic index (AI), mitotic index (MI) and apoptotic to mitotic index ratio (AI: MI) in different grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) in haematoxylin and eosin-stained (H&E) sections.

Settings and Design: The study included 45 cases each of OED and OSCC cases which were further subgrouped into groups of 15 each based on their grades. AI, MI and AI/MI were assessed and compared with 15 cases of normal mucosa.

Methods and Material: Apoptotic cells and mitotic figures were counted using a binocular light microscope equipped with an oculometer grid (20 × 20 squares) on the eyepiece. Cells were counted in 15 grid fields under oil immersion lenses (×100) in a stepladder fashion. AI/MI ratio was calculated.

Statistical Analysis: The results obtained were statistically analysed using Analysis of variance and Tukey Honestly Significant Difference tests with SPSS 20 software at a 0.05 significance level.

Results: AI increased with increasing grades of dysplasia and decreased with increasing grades of OSCC. MI increased with increasing grades of OED and OSCC. AI/MI increased with increasing grades of OED but decreased with increasing grades of OSCC.

Conclusion: In the light of the current observations, AI, MI and AI: MI can be considered as valuable parameters to assess the biological behaviour of OED and OSCC.

Keywords: Apoptosis, mitosis, oral epithelial dysplasia, oral squamous cell carcinoma

Address for correspondence: Dr. Supriya H, Department of Oral Pathology and Microbiology, KVG Dental College and Hospital, Sullia, Karnataka - 574 327, India.
E-mail: supriyakarunakar@gmail.com

Submitted: 18-Dec-2021, **Revised:** 11-Feb-2022, **Accepted:** 28-Feb-2022, **Published:** 22-Dec-2022

INTRODUCTION

Squamous cell carcinoma accounts for 90% of all oral cancers. It usually arises from a pre-existing potentially malignant lesion, and infrequently de novo, but in either case from within a field of precancerized epithelium.[1]

Mitosis and apoptosis are two processes that oppose each other but are essential for the survival of an organism. The cell count is tightly regulated not simply by controlling the rate of cell division but also cell death. Oral carcinogenesis is correlated with a progressive accumulation of genetic alterations in molecules that play crucial roles during

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_442_21

How to cite this article: Suhasini PD, Mulki S, Supriya H. Apoptotic and mitotic indices in oral epithelial dysplasia and squamous cell carcinoma: A comparative study. J Oral Maxillofac Pathol 2022;26:598-9.

apoptosis.[2] The relationship between cell growth in the form of mitosis and cell death in the form of apoptosis in cancer will predict the growth rate of the tumour.[3]

As counting of apoptotic bodies using light microscopy is possible, the technique has been used and described by several authors, thus making it a putative prognostic marker.[4]

Mitosis is the fundamental biological process because of its role in the growth and maintenance of tissue homeostasis, and its phases can be observed in tissue sections.[5,6]

Apoptotic index (AI) to mitotic index (MI) ratio (AI: MI) provides a conceptual framework to link cancer genetics, tumour initiation progression or metastasis with cancer therapy and prognosis.[7] Therefore, research continues to focus on the cell cycle machinery and signalling pathways that control cell cycle arrest and apoptosis. The present study was undertaken to evaluate AI, MI and AI: MI in different grades of OED and OSCC.

AIM OF THE STUDY

To evaluate AI, MI and AI: MI in different grades of OED and OSCC on H&E stained sections.

Objectives of the study

1. To assess and compare the AI in normal oral mucosa, different grades of OED and OSCC.
2. To assess and compare the MI in normal oral mucosa, different grades of OED and OSCC.
3. To calculate and compare the AI: MI in normal oral mucosa, different grades of OED and OSCC.

Source of the data

The study design was approved by the Institutional Research Ethics committee. Study sample included 105 formalin-fixed paraffin-embedded tissue sections of the normal oral mucosa (15), histopathologically diagnosed cases OED (45) and OSCC (45) from the archives of Department of Oral Pathology and Microbiology, KVG Dental College and Hospital, Sullia. Specimen of normal oral mucosa was obtained during surgical removal of impacted teeth.

Method of collection of data:

They study sample were grouped and subgrouped as following:

- Group 1: Fifteen normal mucosa
- Group 2: Forty-five cases of OED with 15 cases each of Mild, Moderate and Severe dysplasia.
- Group 3: Forty-five cases of OSCC with 15 cases each of Well, Moderate and Poor

METHODOLOGY

Preparation of the slides and staining

A uniform section of 3–4 μm thickness was cut from the selected blocks and routinely stained with H&E stain.

Quantification of Apoptotic cells and Mitotic figures

Apoptotic cells and Mitotic figures were counted using a binocular light microscope equipped with an oculometer grid (20 × 20 squares) on the eyepiece. A minimum of 1000 cells were counted in 15 grid fields under oil immersion lenses (×100) in a stepladder fashion. The area selected for counting apoptotic cells and mitotic figures included the most invasive and the most cellular part of the epithelial tissue. Apoptotic cells and mitotic figures were counted according to the morphological criteria proposed by Kerr *et al*.[8] and Van Diest *et al*.[5], Liu et al.[9] respectively. respectively.

Calculation

Finally, AI, MI and AI to MI ratio were calculated as follows,

AI = Number of apoptotic cells X 100/Total number of cells counted (1000)

MI = Number of mitotic figures X 100/Total number of cells counted (1000)

AI:MI = AI/MI

Statistical analysis

The results obtained were statistically analysed using ANOVA and Tukey HSD tests with SPSS 20 software at $P < 0.05$.

RESULTS

The study group of 105 cases comprised 68 male patients and 37 female patients with ages ranging from 30 to 80 years. The majority of the cases were seen in the buccal mucosa. Descriptive analysis of the parameters is tabulated in Tables 1, 4 and 7. The differences between and within the group are tabulated in Table 2 and intragroup comparisons are noted in Table 3. The differences between and within each grade of OED and OSCC were analysed using ANOVA. [Table 5] The mean AI, MI and AI: MI between the grades of OED and OSCC were compared by using the Tukey HSD test [Table 6]. AI within OED groups was found to be significant whereas MI was significant only between mild and severe dysplasia and AI: MI within the OED groups was not significant [Table 6]. Photomicrograph of mitotic

Table 1: Descriptive analysis of AI, MI and AI: MI in normal mucosa, OED and OSCC

Parameter	Groups	*n*	Mean	Std. deviation
AI	Normal	15	0.3067	0.07988
	OED	45	0.8444	0.29738
	OSCC	45	1.0156	0.39425
	TOTAL	105	0.8410	0.39800
MI	Normal	15	0.0000	0.00000
	OED	45	0.4400	0.25531
	OSCC	45	1.3378	0.37190
	TOTAL	105	0.7619	0.59846
AI: MI	Normal	15	0.0000	0.00000
	OED	45	2.1738	0.76739
	OSCC	45	0.8536	0.45130
	TOTAL	105	1.2974	0.99783

Table 2: Analysis of variance between the normal, OED and OSCC group

Parameter	Groups	*df*	Mean square	*F*	Significance
AI	Between Groups	2	2.827	26.653	0.000
	Within Groups	102	0.106		
	Total	104			
MI	Between Groups	2	14.147	161.159	0.000
	Within Groups	102	0.088		
	Total	104			
AI: MI	Between Groups	2	34.338	100.434	0.000
	Within Groups	102	0.342		
	Total	104			

Table 3: Intergroup comparison of AI, MI and AI: MI between Normal mucosa, OED and OSCC groups using Tukey HSD

Parameters	Groups	Groups	Significance
AI	Normal	OED	0.000
		OSCC	
	OED	Normal	0.000
		OSCC	0.038
	OSCC	Normal	0.000
		OED	0.038
MI	Normal	OED	0.000
		OSCC	
	OED	Normal	0.000
		OSCC	
	OSCC	Normal	0.000
		OED	
AI: MI	Normal	OED	0.000
		OSCC	
	OED	Normal	0.000
		OSCC	
	OSCC	Normal	0.000
		OED	

figures and apoptotic cell are featured in Figure 1 and Figure 2 respectively.

DISCUSSION

Oral lesions with epithelial dysplasia may be a morphological phenotype of the various steps within the progression from normal to malignant tissue.[10] Apoptosis is linked to the elimination of potentially malignant cells, hyperplasia and tumour progression.[3] Apoptosis prevents the development of aneuploidy and other genetic aberrations

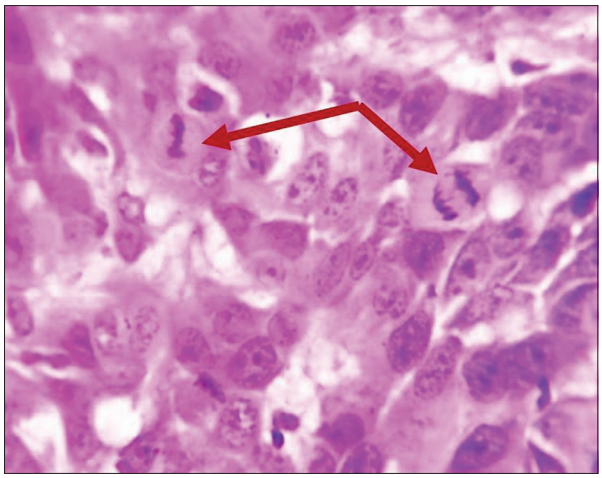


Figure 1: WDSCC showing mitotic figures (H&E stain: x100)

that are associated with the development and progression of OPMD.[11] A malignant cell can acquire a reduction in apoptosis or apoptosis resistance.[3]

Apoptosis plays a great role in cancer dynamics, hence, induction of apoptosis as a treatment mode in cancer is initiated.

Assessment of the number of apoptotic cells and mitotic figures and formulation of apoptotic to mitosis ratio provides a figure which reflects tumour dynamics at the light microscopic level and may provide a numerical index of biological behaviour.[12]

A statistically significant increase in AI from normal mucosa to OED to OSCC was observed in the present study [Table 6], which is in accordance but a higher mean AI than those reported by Jain *et al*.[4] Yet another study showed a progressive increase in AI from normal to carcinoma *in situ* but decreased in OSCC.[13] However the study did not analyse AI in different grades of OSCC. Our study evidenced a gradual increase in AI from group 2 to group 3, showing a gradual decrease in advancing grades of OSCC.

Also, the mean AI showed an ascending increase from mild to moderate and to severe dysplasia. The increase in mean AI with the increasing grades of dysplasia was found to be statistically significant [Table 6]. This was as per another study that acknowledged an increased number of apoptotic bodies from mild-to-severe dysplasia suggesting a mechanism whereby apoptosis aids in eliminating those cells which have proliferated due to increased or abnormal mitosis.[4] Thus, it may be assumed that epithelium is trying to maintain balance by removing cells with damaged genomes that are induced

Table 4: Descriptive analysis of AI, MI and AI: MI within the different grades of OED

Parameter	Groups	N	Mean	Std. Deviation
AI	Mild	15	0.6067	0.14376
	Moderate	15	0.8533	0.32704
	Severe	15	1.0733	0.18310
	Total	45	0.8444	0.29738
MI	Mild	15	0.3200	0.12071
	Moderate	15	0.4400	0.27723
	Severe	15	0.5600	0.28735
	Total	45	0.4400	0.25531
AI: MI	Mild	15	2.0907	0.74293
	Moderate	15	2.1640	0.63222
	Severe	15	2.2667	0.93937
	Total	45	2.1738	0.76739

Table 5: Analysis of variance between subgroups of OED and OSCC

Groups	Parameters	*df*	*F*	Significance
OED	AI			
	Between Groups	2	15.220	0.000
	Within Groups	42		
	Total	44		
	MI			
	Between Groups	2	3.724	0.032
	Within Groups	42		
	Total	44		
	AI: MI			
	Between Groups	2	0.192	0.826
	Within Groups	42		
	Total	44		
OSCC	AI			
	Between Groups	2	46.960	0.000
	Within Groups	42		
	Total	44		
	MI			
	Between Groups	2	30.148	0.000
	Within Groups	42		
	Total	44		
	AI: MI			
	Between Groups	2	102.741	0.000
	Within Groups	42		
	Total	44		

by adjacent normal or infiltrating inflammatory cells. It has been suggested that factors responsible for dysplastic change may themselves be converting increased situations of apoptosis.[11]

The mean AI showed an inverse relation with the histological grades of OSCC. A significant difference was found between well, moderate and poorly differentiated carcinoma [Table 7], which is harmonious with the findings of another study.[14] An additional study also showed a drop in mean AI with progression towards advanced grades, still, their study showed a significant difference only between WDSCC and PDSCC.[4]

Yet another study that assessed the impact of the AI, MI and turnover index in OED and OSCC showed no significant difference among the grades of oral dysplasia,

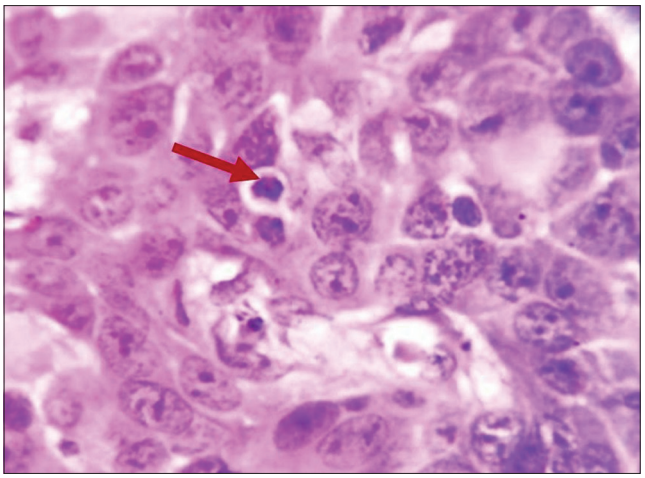


Figure 2: MDSCC showing apoptotic cell (H&E stain: x100)

whereas premalignant and malignant cases showed a significant difference.[15]

An immunohistochemical study conducted in OSCC showed that the ratio of bcl-2/bax mRNA was advanced in carcinomas than in the adjacent normal oral epithelium, and higher ratios were seen in most of PDSCCs. In both normal and tumour tissues, the distribution of bax are inversely related to that of bcl-2 and bax plays a role as a dominant inhibitor of bcl-2. When bcl-2 is present in redundant, cells are defended, and when bax is in redundant, cells are susceptible to apoptosis.[16] The results showed that inhibition of apoptosis had an upper hand in carcinoma and PDSCC in particular. The present study shows reduced apoptosis with increasing grades of OSCC and the inference of the above study justifies the same using IHC, where a decrease in bax/bcl2 (proapoptotic/antiapoptotic) suggests higher grade of OSCC.

Mitotic figures were readily identifiable in prophase, metaphase, anaphase and telophase among OED and OSCC cases. Mitotic numbers increased incrementally from OED to OSCC and a statistically significant difference was observed between groups [Table 3]. This is consistent with other studies which showed a significant increase in MI from normal through OED to OSCC.[6,17,18] Statistical significance noted in the present study might be due to a considerable increase in the sample size when compared to other studies.

The mean MI increased with advancing grades of dysplasia but statistically, a significant difference was not found between mild to moderate dysplasia and moderate to severe dysplasia. But there was a significant difference between mild and severe dysplasia [Table 6]. This may

be due to inter-observer variability in grading dysplasia of epithelial lesions. Analogous results were observed in several studies.[10,18] The mean MI increased with the histological grades of OSCC. The difference between WDSCC and PDSCC, MDSCC and PDSCC were found to be significant [Table 6].

Table 6: Intergroup comparison of AI, MI and AI: MI in subgroups of OED and OSCC using Tukey HSD

Parameters	Groups	Groups	Significance
OED			
AI	Mild	Moderate	0.015
		Severe	0.000
	Moderate	Mild	0.015
		Severe	0.034
	Severe	Mild	0.000
		Moderate	0.034
MI	Mild	Moderate	0.368
		Severe	0.025
	Moderate	Mild	0.368
		Severe	0.368
	Severe	Mild	0.025
		Moderate	0.368
AI: MI	Mild	Moderate	0.964
		Severe	0.812
	Moderate	Mild	0.964
		Severe	0.931
	Severe	Mild	0.812
		Moderate	0.931
OSCC			
AI	WDSCC	MDSCC	0.000
		PDSCC	
	MDSCC	WDSCC	0.000
		PDSCC	
	PDSCC	WDSCC	0.000
		MDSCC	
MI	WDSCC	MDSCC	0.075
		PDSCC	0.000
	MDSCC	WDSCC	0.075
		PDSCC	0.000
	PDSCC	WDSCC	0.000
		MDSCC	
AI: MI	WDSCC	MDSCC	0.000
		PDSCC	
	MDSCC	WDSCC	0.000
		PDSCC	
	PDSCC	WDSCC	0.000
		MDSCC	

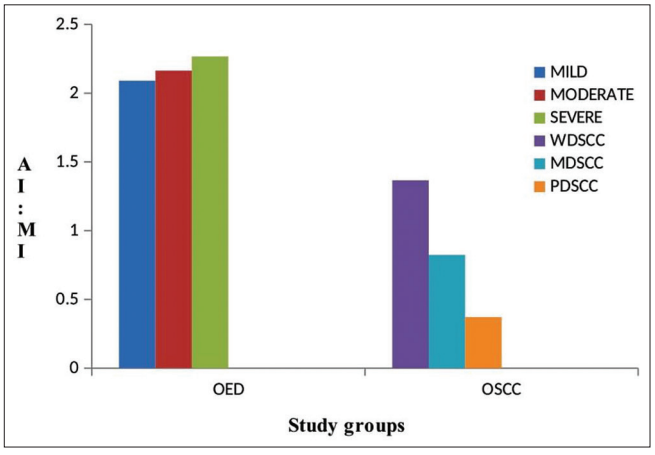
Table 7: Descriptive analysis of AI, MI and AI: MI within different grades of OSCC

Parameters	Groups	*n*	Mean	Std. deviation
AI	WDSCC	15	1.4200	0.30519
	MDSCC	15	1.0000	0.20702
	PDSCC	15	0.6267	0.12228
	TOTAL	45	1.0156	0.39425
MI	WDSCC	15	1.0467	0.20307
	MDSCC	15	1.2467	0.25317
	PDSCC	15	1.7200	0.27045
	TOTAL	45	1.3378	0.37190
AI: MI	WDSCC	15	1.3660	0.22658
	MDSCC	15	0.8233	0.21480
	PDSCC	15	0.3713	0.10562
	TOTAL	45	0.8536	0.45130

A statistically significant difference in AI to MI ratio was found between OED and OSCC. A1: MI dropped significantly from OED to OSCC [Table 3]. Similar results were observed in a former study.[17] The high AI: MI attained in OED indicates the increased rate of apoptosis in OED when compared to mitosis. This may be related to the vulnerable immune system of the body to counteract the tumour progression. The process involved in the robotic regression of tumours is substantially related to the process of apoptosis and the activity of the immune system, as well as to conditions in the tumour microenvironment.[19] Kurita *et al*.[20] reported a case of squamous cell carcinoma undergoing spontaneous regression in which enhanced apoptosis was demonstrated quantitatively.

The mean AI: MI was found to be increased with advancing grades of dysplasia but a statistically significant difference was not found [Table 6, Graph 1]. The results of the present study showed a drop in the mean AI: MI with increasing grades of OSCC [Table 6, Graph 1]. There was a decrease in apoptosis and an increase in mitosis as the tumour progressed to higher grades. This suggests that evasion of apoptosis and increase in mitosis play an important role in tumour progression and hence the ratio can be used to assess tumour progression.

Tumour growth is a summation of cell production and cell death. Although, it is not possible to determine which particular dysplastic lesion will progress to carcinoma, the AI: MI may provide a good model of tumour progression. Therefore, a high AI to MI ratio obtained in OED compared to OSCC in the present study conceivably suggests lesions exhibiting an increase in apoptosis may be slower growing and thus be biologically less aggressive.



Graph 1: AI: MI in subgroups of OED and OSCC

CONCLUSION

In the light of the current observations, AI, MI and AI: MI can be considered as valuable parameters to assess the biological behaviour of OED and OSCC. In future, histopathological reports can include AI/MI as an insight into tumour behavior, which will invariably aid in treatment planning.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Feller L, Lemmer J. Oral squamous cell carcinoma: Epidemiology, clinical presentation and treatment. J Cancer Ther 2012;3:263-8.
2. Ghatage DD, Gosavi SR, Ganvir SM, Hazarey VK. Apoptosis: Molecular mechanism. J Orofac Sci 2012;4:103-7.
3. Wong RS. Apoptosis in cancer: From pathogenesis to treatment. J Exp Clin Cancer Res 2011;30:87.
4. Jain A, Maheshwari V, Alam K, Mehdi G, Sharma SC. Apoptosis in premalignant and malignant squamous cell lesions of the oral cavity: A light microscopic study. Indian J Pathol Microbiol 2009;52:164-6.
5. Van Diest PJ, Brugal G, Baak JPA. Proliferation markers in tumours: Interpretation and clinical value. Clin Pathol 1998;51:716-24.
6. Ankle MR, Kale AD, Charantimath S. Comparison of staining of mitotic figures by haematoxylin and eosin-and crystal violet stains, in oral epithelial dysplasia and squamous cell carcinoma. Indian J Dent Res 2007;18;101-5.
7. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2007;35:495-516.
8. Kerr JFR, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239-57.
9. Liu S, Edgerton SM, Moore II DH, Thor AD. Measures of cell turnover (proliferation and apoptosis) and their association with survival in breast cancer. Clin Cancer Res 2001;7:1716-23.
10. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol 2006;42:987-93.
11. Pawar RB, Palaskar SJ, Kalavankar SS. Assessment of apoptotic index in various grades of oral epithelial dysplasia: A cross-sectional study. Indian J Pathol Microbiol 2020;63:534-7.
12. Mooney EE, Peris JMR, O'Neill A, Sweeney EC. Apoptotic and mitotic indices in malignant melanoma and basal cell carcinoma. J Clin Pathol 1995;48:242-4.
13. Birchall MA, Winterford CM, Allan DJ, Harmon BV. Apoptosis in normal epithelium, premalignant and malignant lesions of the oropharynx and oral cavity: A preliminary study. Eur J Cancer B Oral Oncol 1995;31:380-3.
14. Mandal AK, Verma D, Mohanta PK, Saha R, Maeda T, Sugino T, *et al*. Prognostic significance of apoptosis in squamous cell carcinoma of oral cavity with special reference to TNM stage, histological grade and survival. Indian J Pathol Microbiol 2001;44:257-9.
15. Singh P, Kumar V, Singh G, Singh A. Significance of the apoptotic index, mitotic index, and turnover index in premalignant and malignant squamous cell lesion of the oral cavity. How much is their diagnostic and prognostic role? J Sci Soc 2019;46:49-52.
16. Chen Y, Kayano T, Takagi M. Dysregulated expression of bcl-2 and bax in oral carcinomas: Evidence of post-transcriptional control. J Oral Pathol Med 2000;29:63-9.
17. Birchall MA, Winterford CM, Tripconi L, Gob G, Harmon BV. Apoptosis and mitosis in oral and oropharyngeal epithelia: Evidence for a topographical switch in premalignant lesions. Cell Prolif 1996;29:441-56.
18. Jadhav KB, Mujib ABR, Gupta N. Crystal violet stain as a selective stain for the assessment of mitotic figures in oral epithelial dsplasia and oral squamous cell carcinoma. Indian J Pathol Microbiol 2012;55:283-7.
19. Ricci SB, Cerchiari U. Spontaneous regression of malignant tumors: Importance of the immune system and other factors. Oncol Lett 2010;1:941-5.
20. Kurita M, Hirano K, Ebihara S, Takushima A, Harii K, Fujino T, *et al*. Spontaneous regression of cervical lymph node metastasis in a patient with nasopharyngeal squamous cell carcinoma of the tongue: Possible association between apoptosis and tumor regression. Int J Clin Oncol 2007;12:448-54.