Original Article

IHC expression of AKT in different grades of epithelial dysplasia: An *in vitro* study

A. Sandhya Rani[1], Manay Srinivas Munisekhar[2], S. Shylaja[3], A. Krishna[4], Bhavani N. Sangala[5], Sharath Kumar Reddy E[3]

[1]Department of Oral and Maxillofacial Pathology, Care Dental College, Guntur, Andhra Pradesh, India, [2]Departments of Oral Pathology and [4]Preventive Dentistry, College of Dentistry, Aljouf University, Sakaka, Kingdom of Saudi Arabia, [3]Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahbubnagar, Telangana, [5]Department of Oral Pathology and Microbiology, Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai, Maharashtra, India

Abstract

Introduction: Akt, also known as protein kinase B, is a serine/threonine-specific protein-kinase which plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, transcription and cell migration. The activation of Akt is one of the most frequent alterations observed in human cancer and tumour cells. Akt regulates cellular survival and metabolism by binding and regulating many downstream effectors, e.g., Nuclear Factor-kB, murine double minute 2(MDM2).

Aims: To evaluate and compare immunohistochemical expression of Akt in normal epithelium and different histological grades of epithelial dysplasias.

Materials and Methodology: Forty paraffin-embedded tissue sections were used for the immunohistochemical study of which 10 cases of normal epithelium, 10 cases of each mild, moderate and severe epithelial dysplasia which were diagnosed by haematoxylin and eosin procedures. The tissue sections were immunohistochemically analysed for expression of Akt in different grades of epithelial dysplasia by using anti-Akt-1 monoclonal antibody. Statistical analysis was carried out using statistical package for social science (SPSS, V 10.5). The data were analysed using Chi-square test and $P < 0.05$ was considered statistically significant.

Results and Conclusion: An overall significant difference was observed when normal tissues were compared with epithelial dysplasia with a Chi-square value of 21.04, but there was no statistical significance between the three grades of epithelial dysplasias. In conclusion, this study suggests that Akt-1 overexpression can be one of the useful diagnostic markers for predicting the potential behaviour of oral dysplasias transforming into oral squamous cell carcinoma (OSCC).

Keywords: Akt, epithelial dysplasia, immunohistochemistry, OSCC

Address for correspondence: Dr. A. Sandhya Rani, Department of Oral and Maxillofacial Pathology, Care Dental College, Potturu, NH-5, Guntur -522 005, Andhra Pradesh, India.
E-mail: dr.sandhyarani11@gmail.com

Submitted: 17-Feb-2022, **Revised:** 25-Mar-2022, **Accepted:** 28-Mar-2022, **Published:** 17-Oct-2022

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_88_22

How to cite this article: Rani AS, Munisekhar MS, Shylaja S, Krishna A, Sangala BN, Reddy ES. IHC expression of AKT in different grades of epithelial dysplasia: An *in vitro* study. J Oral Maxillofac Pathol 2022;26:311-5.

INTRODUCTION

Epithelial dysplasia is defined as 'a precancerous lesion of stratified squamous epithelium characterized by cellular atypia and loss of normal maturation and stratification short of carcinoma in situ'**.** The term 'Dysplasia' was introduced by the Reagonin 1958 in relation to the cells exfoliated from the lesions of the uterine cervix.[1] Oral epithelial dysplasia (OED) is considered to be the first change noticed in the development of carcinoma. It is considered as a gold standard for the assessment of oral potentially malignant lesions in haematoxylin and eosin stained tissue sections.[2] Malignant transformation of oral epithelial dysplasia into squamous cell carcinoma ranges from 6 to 36%.[3] Oral mucosa undergoes transformation into OSCC, due to chronic exposure to carcinogens in the form of tobacco, betel nut, with alcohol as a co-carcinogen. There are a wide variety of molecular alterations that have been associated with carcinogenesis.[4] Transition from normal epithelium to premalignancy to oral carcinoma is a result of surface alterations, accumulation of genetic and epigenetic alterations.[5]

Several markers have been implicated in transition from normal to precancer to cancer such as tumour suppressor genes and oncogenes.[6] Many potential markers have been used to diagnose the epithelial dysplasia such as p53 targets and family members: p21, p27, MDM2, p14, p16, p63, p73.[7] Akt is also one of these potential markers used to diagnose epithelial dysplasia. The serine/threonine protein kinase Akt, also known as PKB, is a downstream target of phosphatidylinositol 3 kinase (PI3K) and has been shown as a key regulator of various cellular processes including normal and aberrant cell growth and cell fate decisions such as differentiation and cell survival or death by apoptosis. Activated Akt has been shown to be a frequent event in several cancer types such as breast, ovarian cancer, prostate cancer and squamous cell carcinomas of oral cavity.[8] High phosphorylated-Akt activity in oral epithelia leads to the formation of dysplastic lesions that eventually proceed to oral SCC.[9] Therefore, understanding Akt and its pathways is important for the creation of better therapies to treat cancer and tumour cells.

This study was undertaken to determine the role of Akt in varying histopathological grades of oral epithelial dysplasia as most cases of oral squamous cell carcinomas are preceded by visible changes of oral mucosa. Therefore, it would be important to identify those changes that represent an early stage of the process of malignant transformation. The aim of this study was to evaluate and compare immunohistochemical expression of Akt in normal epithelium and different histological grades of epithelial dysplasia.

MATERIALS AND METHODS

Histopathologically diagnosed cases of epithelial dysplasias were retrieved from the Department of Oral Pathology and Microbiology, SVS Institute of Dental Sciences, Mahabubnagar. A total of 40 tissues were assessed immunohistochemically for Akt-1 expression. These included 10 normal tissues, 10 cases of mild, 10 cases of moderate and 10 cases of severe dysplasia. Epithelial dysplasia was graded according to the WHO (2005) criteria into mild, moderate and severe dysplasia.

Immunohistochemistry staining

Immunohistochemistry (IHC) staining was performed using the Immunocruz ABC universal staining kit (Santa Cruz Biotechnology Inc., California) according to the manufacturer's instructions. Three to 4 μ thick sections were taken onto poly-L-lysine adhesive-coated slide and incubated for 3 hours at 50-60 degrees centigrade in a slide warmer for proper adhesion of the section to the slide. The sections were deparaffinized by heating on the slide warmer at 60°C for 15–20 minutes. The sections were rehydrated by taking them through 2 changes of absolute alcohol and 95% alcohol for 3 minutes each. Then the slides were kept immersed in distilled water for 30 seconds. For antigen retrieval, microsections were immersed in citrate buffer and boiled twice for 5 minutes each in a microwave oven. This increases the immunoreactivity of antigens in formalin-fixed paraffin–embedded tissues. Slides were then allowed to cool down in citrate buffer and wash in distilled water for 5 minutes followed by wash in 3 changes of PBS wash buffer for 5 minutes each. Microsections were then dipped in freshly prepared 3% H_2O_2 for 10 min, to block endogenous peroxidase activity. The sections were then washed in PBS wash buffer for 2 changes of 5 minutes each. Sections were then treated with protein block for 20 min, to block non-specific antigen binding sites. Sections were then incubated with primary antibody (anti-Akt-1) for 30 minutes at room temperature in a humid chamber. Then sections are washed with three changes of PBS for 5 minutes each. Excess buffer is tapped off and then the sections were incubated for 30 minutes with biotinylated secondary antibody. Then the sections were washed with three changes of PBS for 5 minutes each. Sections were then incubated for 30 minutes with AB enzyme reagent (avidin and biotinylated (HRP). Then the sections were washed with three changes of PBS for 5 minutes each. Sections were then incubated in 1–3 drops peroxidase substrate (substrate buffer, DAB chromogen and peroxidise

substrate) for 30 seconds–10 minutes or until desired stain intensity develops. The sections were then counterstained by using Harri's haematoxylin for 10 seconds, after which sections were gently washed in running tap water for 60 seconds. Mounting was done in DPX mounting media.

Evaluation of the staining for Akt-1

Presence of brown-coloured end product at the site of target antigen was indicative of positive immunoreactivity. Tissue sections of breast carcinoma were taken as positive control. The study cases were evaluated in a similar way by observing the immunoreactivity or positively stained cells in the epithelium and were graded as follows: grade 0 (weak expression) if less than 20% of cells were stained, grade 2 (moderate expression) if staining is between 20 and 50% and grade 3 (strong expression) if staining of cells is more than 50%. All these observations were carried out in randomly selected five fields by three observers to eliminate interobserver bias.

Statistical analysis

Data analysis was carried out using statistical package for social science (SPSS, V 10.5). The data were analysed using Chi-square test and $P < 0.05$ was considered statistically significant.

RESULTS

Immunohistochemical expression of Akt-1 varied in proportion of stained cells and the distribution of positive cells was different in normal epithelium [Figure 1] and three grades of epithelial dysplasia, mild [Figure 2], moderate [Figure 3], severe [Figure 4]. When the sections of normal mucosa and epithelial dysplasia were analysed, it was noted that epithelial cells from all normal tissues and different grades of epithelial dysplasia stained positively. However, the intensity of staining varied from normal to different grades of epithelial dysplasia and that there was an increase in the mean percentage of positive cells that expressed Akt-1 in epithelial dysplasia in general as compared to normal mucosa which was statistically significant with a Chi-square value of 21.04 and *P* value of 0.002 [Table 1]. There was no statistically significant difference in expression of Akt-1 between normal epithelium and mild dysplasia. The expression of Akt-1 in normal tissues when compared with moderate dysplasia and severe dysplasia, 90% of normal tissues showed weak expression and 10% showed moderate expression, whereas in moderate dysplasia, 20% showed weak expression, 50% showed moderate expression and 30% showed strong expression indicating that there is an increased expression of Akt-1 from normal to moderate dysplasia which was statistically significant with *P* value of 0.006 [Table 2]. In severe dysplasia, 30% showed weak expression, 30% showed moderate expression and 60%

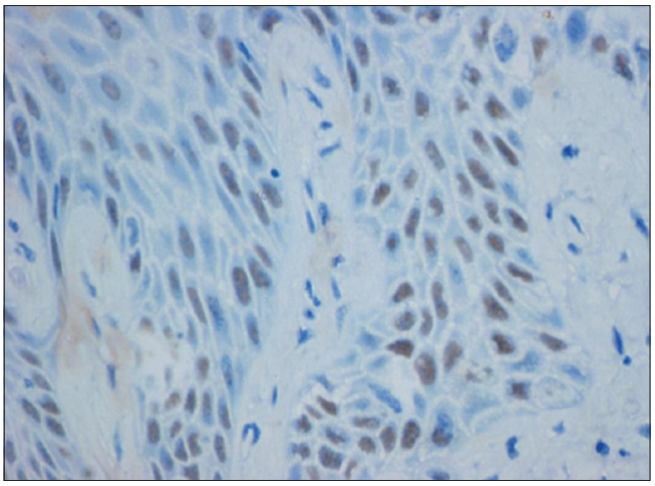

Figure 1: Normal epithelium showing expression of Akt-1 positive nuclear staining in the epithelium under 40X magnification

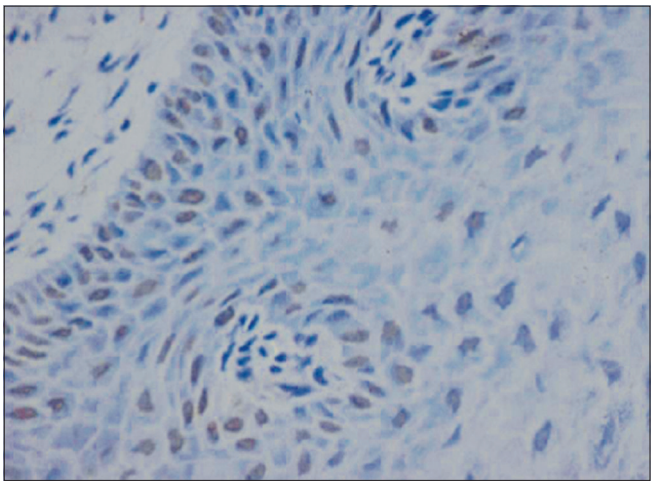

Figure 2: Mild dysplasia showing expression of Akt-1 positive nuclear staining in the epithelium under 40X magnification

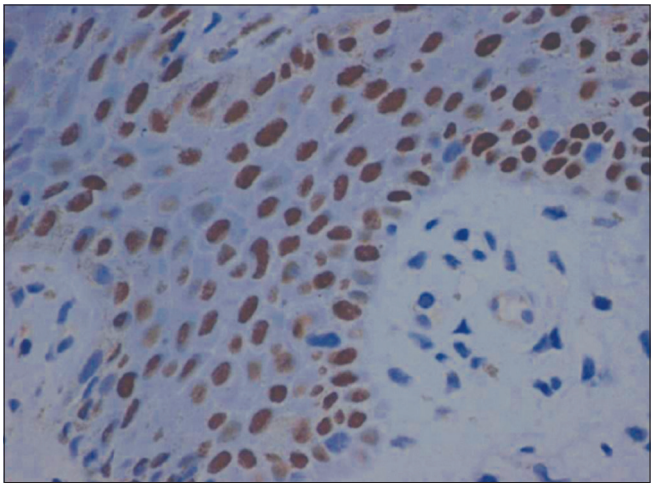

Figure 3: Moderate dysplasia showing expression of Akt-1 positive nuclear staining in the epithelium under 40X magnification

showed strong expression indicating that more percentage of cells showed increased expression of Akt as normal cells progressed to severe dysplasia and statistical significance was observed with *P* value of 0.001 [Table 3]. The results showed a gradual increase in the expression of Akt-1 by the cells from normal mucosa through the different grades of epithelial dysplasia, but there was no statistical significance between the three grades of epithelial dysplasias.

DISCUSSION

A significant proportion of squamous cell carcinoma of the oral mucosa arises from the precancerous lesions. Identification of the histologic degree of epithelial dysplasia is considered the most important way of predicting the risk of malignant transformation. However, the actual mechanism by which dysplasia progresses into cancer is poorly understood and it is not inevitable that a dysplastic lesion will progress into cancer. Moreover, there are no molecular markers which enable us to distinguish lesions that may progress from those that will not. At present, the degree of dysplasia is therefore the best guide to progression of oral lesions.[10,11] An overall malignant transformation of severe epithelial dysplasias is about 16% but studies have shown a wide range of 7–50%. The malignant transformation of moderate dysplasias ranges from 3 to 15%, whereas mild epithelial dysplasia shows a very low risk (<5%). However, it is always assumed that there is a temporal progression of disease, analogous to multistage carcinogenesis and that mild dysplasia will progress to severe dysplasia and then to carcinoma.[11,12] A major proportion of the research implicated in transition from normal to precancer to cancer is centred on oncogenes and tumour suppressor genes.[7]

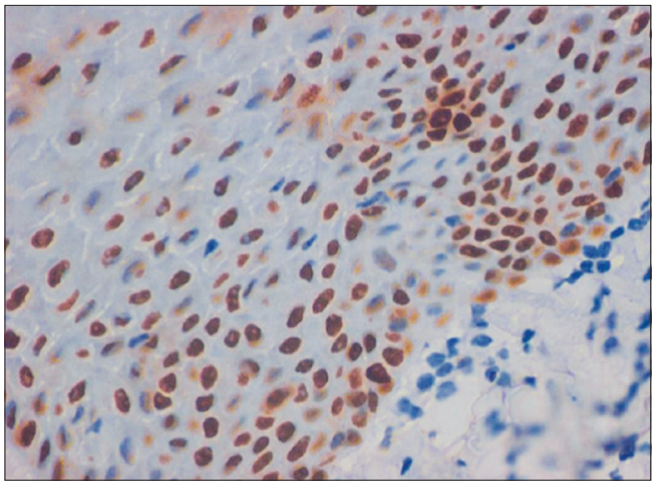

Figure 4: Severe dysplasia showing expression of Akt-1 positive nuclear staining in the epithelium under 40X magnification

In the present study, there was a statistically significant difference in expression of Akt-1 between normal epithelium and epithelial dysplasias. These results are in accordance with H.A.R. Pontes *et al.*[13] (2009) who conducted a study on OSCCs, normal and dysplastic cells to evaluate the immunoexpression of p-Akt and metallothionein proteins (MT) and observed that there was a significant increase in positive immunohistochemical staining for p-Akt and MT. Also no significant difference was observed among the three histological grades of oral dysplasia which is in accordance with the present study. These results were also in accordance with Brunno-Santos de Freitas Silva *et al.*[14] (2012) who evaluated the immunoexpression of TWIST and p-Akt proteins in oral leukoplakia (OL) and oral squamous cell carcinoma (OSCC) correlating their expressions with the histological features of the lesions. A significant difference in p-Aktimmuno-expression in normal oral mucosa when

Table 1: Mean percentage of positive cells in normal, different grades of epithelial dysplasia

	% Of Positively Stained Cells			Total	χ^2	*P*
	Weak (0-20%)	Moderate (20-50%)	Strong (>50%)			
Normal	9	1	0	10	21.04	0.002
	90%	10%	0%	100%		
Mild dysplasia	4	5	1	10		
	40%	50%	10%	100%		
Moderate	2	5	3	10		
dysplasia	20%	50%	30%	100%		
Severe dysplasia	1	3	6	10		
	10%	30%	60%	100%		
Total	16	14	10	40		
	40%	35%	25%	100%		

Table 2: Mean percentage of positive cells in normal and moderate dysplasia

	% Of Positively Stained Cells			Total	χ^2	*P*
	Weak (<20%0)	Moderate (20-50%)	Strong (>50%)			
Normal	9	1	0	10	10.12	0.006
	90%	10%	0%	100%		
Moderate	2	5	3	10		
dysplasia	20%	50%	30%	10%		
Total	11	6	3	20		
	55%	30%	15%	100%		

Table 3: Mean percentage of positive cells in normal and severe dysplasia

	% Of Positively Stained Cells			Total	χ^2	*P*
	Weak (0-20%)	Moderate (20-50%)	Strong (>50%)			
Normal	9	1	0	10	13.4	0.001
	90%	10%	0%	100%		
Severe	1	3	6	10		
dysplasia	10%	30%	60%	100%		
Total	10	4	6	20		
	50%	20%	30%	100%		

compared with three OL groups (mild, moderate and severe epithelial dysplasia) and OSCC was observed. However, there was no statistical difference among the three OL groups.

Shinya Watanabe (2009) performed histochemistry by using p-Akt antibody revealed no positive cells in normal epithelium which is in contrast to the present study as positive staining was observed in all the normal tissues. However, it was observed that there were positive cells present in epithelial dysplasia tissues and early cancer tissues in their study which is in accordance with the present study.[15] The present study showed a statistically significant difference in expression of Akt-1 when normal epithelium was compared to both moderate dysplasia and severe dysplasia. These results are in accordance with H.A.R. Pontes *et al*.[13] (2009) who observed that the expression of p-Akt was statistically significant from normal to severe epithelial dysplasia. Also similar to the study done by Brunno-Santos de Freitas Silva *et al*.[14] (2012) in which there was a statistical significance from normal to severe epithelial dysplasia.

In the present study, there was an overall significant difference when normal tissues were compared with epithelial dysplasia, but there was no statistical significance among the three grades of epithelial dysplasias. These results are in accordance with the observation done by H.A.R. Pontes *et al*. (2009).[13] Also it is correlating with the observations of Brunno-Santos de Freitas Silva *et al*.[14] (2012) in which there was no statistically significant difference between three grades of dysplasias.

The results of the present study showed that there is a gradual increase in the expression of Akt-1 by the cells from normal mucosa through the different grades of epithelial dysplasia. Studies in the past have also shown that there was an increase in the expression of Akt from normal mucosa to oral carcinoma like in dysplasia. Hence, it is possible that Akt may be involved in the multistep process of oral carcinogenesis. Akt activation may be considered as an early cellular response to nicotine and other tobacco-related exposure. However, the exact mechanism as to how such activation contributes to the tumorigenic potential of the lesions is still obscure. However, it has been suggested that Akt activation may induce epithelial mesenchymal transition. Moreover, since it is an established fact that Akt regulates cell growth, proliferation, differentiation and migration, the results of our study strongly suggest that Akt may be involved in progression from normal to dysplasia through any of mechanisms mentioned above.

CONCLUSION

The results of this study suggest that Akt-1 over-expression can be one of the useful diagnostic markers for predicting the potential behaviour of oral dysplasias transforming into OSCC. Its over-expression gives a clue regarding the initiation or promotion of carcinogenesis. However, a large sample is required to predict the expression of Akt-1 in different histological grades.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pinborg JJ, Riechart P, Smith CJ, Vander Waal I. World Health Organization: Histological Typing of Cancer and Precancer of the Oral Mucosa. Berlin: Springer-Verlag; 1997. p. 21-6.
2. Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC; 2017. p. 91-2.
3. Smith J, Rattay T, McConkey C, Helliwell T, Mehanna H. Biomarkers in dysplasia of the oral cavity: A systemic review. Oral Oncol 2009;45:647-93.
4. Mithani SK, Mydlarz WK, Grumbine FL, Smith IM. Molecular genetics of premalignant oral lesions. Oral Dis 2007;13:126-33.
5. Rastogi V, Puri N, Mishra S, Arora S, Kaur G, Yadav L. An insight to oral epithelial dysplasia. Int J Head Neck Surg 2013;4:74-82.
6. Williams HK. Molecular pathogenesis of oral squamous carcinoma. J ClinPathol MolPathol 2000;53:165-72.
7. Pitiyage G, Tilakaratne WM, Tavassoli M, Warnakulasuriya S. Molecular markers in oral epithelial dysplasia: Review. J Oral Pathol Med 2009;38:737-52.
8. Testa JR, Tsichlis PN. Akt signaling in normal and malignant cells. Oncogene 2005;24:7391-3.
9. Massarelli E, Liu DD, Lee JJ, El-Naggar AK, Lo Muzio L, Staibano S, *et al*.Massarelli E, Liu DD. Akt activation correlates with adverse outcome in tongue cancer. Cancer 2005;104:2430-6.
10. Pindborg JJ. Reibel J, Holmstrup P. Subjectivity in evaluating oral epithelil dysplasia, carcinoma *in situ* and initial carcinoma. J Oral Pathol 1985;14:689-708.
11. Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head Neck Pathol 2007;1:61-7.
12. Bouquot J, Speight PM, Farthing PM. Epithelial dysplasia of the oral mucosa— diagnostic problems and prognostic features. Curr Diagn Pathol 2006;12:11-22.
13. Pontes HA, de Aquino Xavier FC, da Silva TS, Fonseca FP, Paiva HB, Pontes FS, *et al*. Metallothionein and p-Akt proteins in oral dysplasia and in oral squamous cell carcinoma: An immunohistochemical study. J Oral Pathol Med 2009;38:644-50.
14. Silva BS, Yamamoto FP, Pontes FS, Cury SE, Fonseca FP, Pontes HA, *et al*. TWIST and p-Akt immunoexpression in normal oral epithelium, oral dysplasia and in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2012;17:29-34.
15. Watanabe S, Sato K, Okazaki Y, Tonogi M, Tanaka Y, Yamane GY. Activation of P13K-AKT pathway in oral epithelial dysplasia and early cancer of tongue. Bull Tokyo Dent Coll 2009;50:125-33.