ADVANCES IN BIORESEARCH
Adv. Biores., Vol 8 [3] May 2017: 91-95

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.3.9195

Advances in Bioresearch

ORIGINAL ARTICLE

Value of Ki67 in Differentiating between Keratoacantoma and squamous cell carcinoma

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ABSTRACT

Keratoakanthoma is a common skin neoplasm characterized by rapid growth and a similar histopathologic pattern to squamous cell carcinoma (SCC) which is the second common skin cancer. According to different prognoses and out comes diagnosis of both disease is essential; unfortunately there is no definite clinical or histopathological criteria for differentiating between these two diseases. Ki67 a high molecular weight non-histone protein is a reliable marker for cell proliferation. So, this study assessed value of Ki67 in differentiating between Keratoacanthoma and squamous cell carcinoma. This prospective study includes 15 cases of KA and 15 cases of SCC. Cases were randomly collected from archive of pathology. Ki67 immunohistochemical staining were performed in all biopsy specimens, positive staining and pattern of expression was analyzed. Slides were stained with H&E and reviewed and those had definite pattern of Keratoacanthoma or SCC were enrolled and after preparation were stained with Ki67 antibody. The results indicated there was significant relationship between the expression of Ki67 in these two types of lesion (P< 0.05).There were significant correlation between the location of positive Ki67 staining in epidermis and sex with lesion type (P< 0.05), but no significant correlation between age and lesion type statistically (P>0.05). It can be concluded when the histopathological findings fail to differentiate between Keratoacanthoma and squamous cell carcinoma, Ki67 marker could be helpful in distinguishing these lesion types.

Keywords: *Keratoacanthoma, Squamous cell carcinoma, Immunohistochemistry, Ki67*

Received 01.03.2017 Revised 10.03.2017 Accepted 20.04.2017

How to cite this article:
N Ranjbari, A Alahdin, P Kheradmand, R Yaghoubi, S Zaheri. Value of Ki67 in Differentiating between Keratoacantoma and squamous cell carcinoma. Adv. Biores., Vol 8 [3] May 2017.91-95

INTRODUCTION

Squamous cell carcinoma (SCC) is among the 10 most common cancers worldwide, and its high mortality rate gives rise to a considerable global public health burden [8, 14]. Despite the currently available therapeutic strategies, comprising the surgical excision of malignant tissue and a combination of radiotherapy and chemotherapy, the 5-year survival rate is still poor [13]. The high mortality rate is usually attributed to late diagnosis, but some cases of SCC surgically treated at an early stage still present with aggressive behavior and disease progression [6, 20]. The well-differentiated type is characterized by its similarity with normal squamous epithelium, high power of keratinization, and very mild cell atypia; the moderately differentiated type contains distinct nuclear pleomorphism and mitotic activity including atypical figures. In this type, keratinization is much lower [1].

In the poorly differentiated type, there is predominance of immature cells, with numerous mitotic figures and virtually absent keratinization. The level of invasion may be limited to the epithelium respecting the basal lamina, which is called in situ. When it affects only the lamina propria, it is considered micro invasive or superficially invasive. As for the frankly invasive carcinoma, it manifests as the destruction of the basal lamina, clearly extending to the underlying tissues, possibly accompanied by stromal reaction.

Perineural invasion and angiolymphatic invasion are signs of increased aggressiveness and change in staging respectively [3].
Keratoacanthoma (KA) is a self-limiting, benign epidermal keratinocytic neoplasm often associated with sun exposure and with occurrence in older individuals [21]. Particularly during the short proliferative phase, KA harbors histologic features that resemble well-differentiated SCC [21]. KA can be diagnostically challenging for clinicians and pathologists because of its rapid growth and histologic pattern [12]. Nevertheless, KA has distinctive genetic aberrations compared with squamous cell carcinoma. KA is often not diagnosed and treated because it can regress spontaneously, sometimes in a matter of months [22].
The behavior of the squamous cell carcinoma is marked by the degree of cell proliferation and differentiation, and this index can be derived by measuring Ki 67 [1]. The Ki-67 (MKI67) is a protein present in the nucleus, whose function is related to cell proliferation. This protein is only expressed in the cell division cycle: interphase, prophase, metaphase, anaphase and telophase, and is absent in the G0 phase, when the cell is quiescent; therefore, it is an excellent marker of cell division [4]. Based on the literature there was no report on role of the Ki67 differentiating between KA and SCC as well as the appearance of the marker. So, this study assessed value of Ki67 in differentiating between KA and SCC.

MATERIAL AND METHODS

Sampling

This prospective study includes 15 cases of KA and 15 cases of SCC. Cases were collected (21 male and 9 female) randomly from archive of pathology department of Imam Khomeini hospital, Ahvaz University of Medical Sciences, Ahvaz, Iran in a one year period from 2015- 2016. Patient's information such as age, sex and diagnosis were recorded.

Staining

Tissue slide sampling was done using H&E staining from all patients (n=15 in each group). Then all slides studied for expression of KA or SCC pattern. Then a 4 µm thickness of each sample was put on the poly-L-lysine covered slide for Ki67 immunohistochemical staining [7]. Then, slides allocated into 60 ºC oven for 60 minutes. After deparaphenirezation and rehydration and inactivation of endo-peroxidase the antibody staining for Ki67 was done. The brownish cytoplasm or cell membrane was an indicator Ki67 [7]. Then slides studied for KA or SCC expression using 10HPF microscope. The mean expression for positive detection in tumoral (epithelial) cells was mentioned as described below:
1. Negative expression: <10%
2. Low expression: 10-50%
3. High expression: >50% [7].

Statistical analysis

Data was processed in excel and analyzed using SPSS 21.0 for Windows (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered as significant differences between treatments.

RESULTS

The results for value of Ki67 in differentiating between keratoacantoma and squamous cell carcinoma are presented in tables 1-3 and figure 1. According to the results, the average age of the patinas in SCC and KA groups was 63.2 ± 22.4 and 60.3 ± 11. 8 and there was no significant difference among them ($P>0.05$).

Table 1. the average age of the patinas included into study

	Average ± sd	min	max
SCC	63.2 ± 22.4	13	86
KA	60.3 ± 11.8	11	90
Total	61.7 ± 20. 2		

SCC: Squamous cell carcinoma, KA: Keratoacanthoma

The frequency of the sex for SCC and KA is presented in table 2. According to the results, the frequency of the SCC was higher in males compare d to the female ($P<0.05$). Also, there was no difference for frequency of the KA between men and women included into study ($P>0.05$). Totally, the frequency of the carcinoma was higher in male compared to the women ($P<0.05$).

Table 2. Frequency of the sex for SCC and KA

		Female	Male
SCC	N	2	12
	%	13.3	86.7
KA	N	7	8
	%	46.7	53.3
Total	N	9	21
	%	30	70

SCC: Squamous cell carcinoma, KA: Keratoacanthoma

The frequency of the appearance of the marker in detection of tumoral cells using Ki67 is shown in table 3. According to the results, most of the SCC samples expressed Ki67 marker more than 50% ($P<0.05$), but Ki67 marker expression were mostly 5-50% ($P<0.05$).

Table 3. Frequency of the appearance of the marker in detection of tumoral or stromal cells using Ki67

Lesion		Appearance of the marker			Total
		<%5	%5-50	>%50	
SCC	N	0	5	10	15
	%	0	33.33	66.66	100
KA	N	5	9	1	15
	%	33.33	60	6.66	100

SCC: Squamous cell carcinoma, KA: Keratoacanthoma

The frequency of the position for the tumoral is shown in figure 1. According to the results, distribution of the Ki67 marker in the KA was frequently detected in basal section while SCC was diffuse.

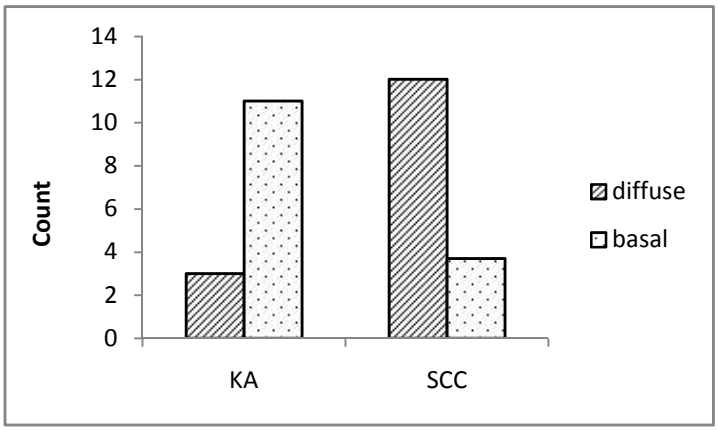

Figure 1. frequency of the position for the tumoral

DISCUSSION

Based on the results, Ki67 marker is a valuable indicator for detecting AK and SCC in distinguishing these lesion types. Numerous studies have analyzed the possible mechanisms underlying the oncogenesis of AK and SCC or focusing on how to differentiate these lesions using histologic criteria, immunohistochemical markers and chromosomal abnormalities [10]. Previously it is reported, AKs were consistently positive for K6, K10 and K14. K10 and K14 were observed in specialized cells within the hair follicle and in the interfollicular epithelium [10]. Ki67 is one of the mitotic indicators in proliferative activity of tumors. Expression of Ki-67 in mean of proliferative activity of tumor cells is one of the indicators for tumor invasion potential and invasive activity of cancers related to degree of malignant neoplastic cells [16].

Many studies have shown that Ki-67 monoclonal antibody staining is the best method for measuring cell proliferation [2]. Wangsa *et al* [21] in their study have shown that Ki-67 expression level is also a potentially useful clinical marker for predicting recurrence in surgically treated stage I oral tongue SCC. Also, Motta *et al* [11] have proven Ki-67 expression is significantly higher in oral epidermoid carcinoma

patients with neck lymph node metastasis. In a study on combine application of the p53 and Ki-67, it is reported, combination of p53 and Ki-67 over expressions can be used as a specific marker for oral lesions that are probably at high risk for malignant transformation, their immunohistochemistry emerges as a clinically useful supplement for histopathological assessment of grading of oral SCC and intraepithelial lesions [15].

The low Ki-67 expression in epithelial cells in normal and non-neoplastic oral mucosa in this study, suggests that most epithelial cells in these mucosae are in the G0 and G0–G1 transition phases. Only about 23% of cells of non-neoplastic oral mucosa are actually in the cell cycle (demonstrated by Ki-67) suggesting that these tissue compartments have a low and controlled proliferation rate but with a continuous proliferative capacity [9]. Immunohistochemical expression of Ki-67 reflects the cell proliferation status of a neoplasia. When it is present in more than 50% of tumor cells, it is usually associated with increased aggressiveness. However, findings are somewhat controversial [5].

Among the biomarkers with predictable potential for the prognosis of oral squamous carcinoma, p53, p16 and Ki67 were intensively investigated in specialty literature with no consensus in this direction [23]. In this regard, Sharma et al. [18] on Ki-67 expression in cytologic scrapes from oral squamous cell carcinoma before and after radiotherapy reported, Ki-67 expression was seen in an extremely small number of cells. Only 10 tumors showed positive cells, and the labeling index in them varied from 0.1 % to 0.01 %. It is revealed high-risk dysplasia and high Ki67 PI of the adjacent non-malignant mucosa are parameters which are indicative of tumor recurrence. Furthermore, T3/T4 tumor sizes and high Ki67 PI in the invasive front appear to be important prognostic tools for squamous cell carcinoma [26].

Limitations of the study should be noted. This study was performed on only 30 samples. Therefore it should be repeated on a large number of various skin tumor specimens in order to compare different age ranges and achieve more accurate results. The knowledge of the mechanism responsible for neoplastic transformation of keratinocytes gives an opportunity to create new diagnostic and therapeutic methods [24]. Ki-67 antigen in AK and SCC as well, while MT-expression are higher in SCC in comparison to its in AK lesions. A confrontation of those two values could serve as a helpful diagnostic method for differentiation of SCC from AK in doubtful cases [24]. Because the cell cycle-regulating protein Ki-67 is expressed during all phases of the cell cycle except G_0, cellular expression of Ki-67 provides a measure of the growth fraction of a tumor [25]. In conclusion it can be concluded that when the histopathological findings fail to differentiate between KA and SCC, using Ki67 marker based on the above model, could be helpful in distinguishing these lesion types.

REFERENCES

1. Altemani AMAM, (2009). Amstalden EMI. Bogliolo. Patologia. 8th ed.Guanabara Koogan.
2. Baghaei F, Eslami M and Sadri D. (2004). Evaluation of Ki-67 antigen and protein P53 expression in orthokeratinized and parakeratinized odontogenic keratocyst. Journal of Dentistry ; 1 (2): 53-58
3. Carvalho GM, Chone CT, Guimarães AG, Kohler HF, Gripp FM,Crespo AN, et al. (2011). Planned neck dissection: evaluation of viablemetastases. Rev Bras Cir Cabec¸a Pescoc¸o. 40:133-7.
4. Castilho RM, Squarize CH, Chodosh LA, Williams BO, Gutkind JS. (2009). mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging. Cell Stem Cell. 5:279-289.
5. Cernea CR, Ferraz AR, de Castro IV, Sotto MN, Logullo ÂF, Potenza AS, Bacchi CE. 2005. Ki-67 index and skin carcinomas with skull base invasion: a case–control study. Cancer Therapy, 3, 471-476.
6. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (eds), AJCC cancer staging handbook, 7th ed. New York, NY: Springer, 2010
7. Gray DT, Suman VJ, Su WP, et al. (1997). Trends in the population-based incidence of squamous cell carcinoma of the skin first diagnosed between 1984 and 1992. Arch Dermatol; 133:735.
8. Kademani D: (2007). Oral cancer. Mayo Clin Proc 82(7): 878e887.
9. Klieb HBE and Raphael SJ. (2007). Comparative study of the expression of p53, Ki67, E-cadherin and MMP-1 in verrucous hyperplasia and verrucous carcinoma of the oral cavity. Head Neck Pathol. 1(2):118-22.
10. Li J, Wang K, Gao F, et al. (2012). Array comparative genomic hybridization of keratoacanthomas and squamous cell carcinomas: different patterns of genetic aberrations suggest two distinct entities. J Invest Dermatol.;132:2060-2066.
11. Motta RDR, Galeano ZC, Cambruzzi E, Jotz GP, Berni RB. Ki-67 and p53 correlation prognostic value in squamous cell carcinomas of the oral cavity and tongue. Braz J Otorhinolaryngol 2009; 75(4):544-49.
12. Omar E. Recurrent facial keratoacanthoma in a patient with diabetes: a case report. BMC Res Notes. 2014;7:257.
13. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 55(2): 74e108, 2005.
14. Petersen PE: Oral cancer prevention and controlethe approach of the World Health Organization. Oral Oncol 45(4e5): 454e460, 2009.
15. Pity IS and Jalal JA.(2013). Expression of Ki-67 and p53 in Oral Squamous Epithelial Abnormalities. Medical Journal of Babylon-Vol. 10- No. 1 -20-33.

16. Patel SM, Patel KA, Patel PR, Gamit B, Hathila RN, Gupta S. (2014). Expression of p53 and Ki-67 in oral dysplasia and squamous cell carcinoma: An immunohistochemical study. Int J Med Sci Public Health;3:1201-1204.
17. Rodrigo Gonzalez Bonhin, Guilherme Machado de Carvalho, Alexandre Caixeta Guimarães, Carlos Takahiro Chone, Agrício Nubiato Crespo, Albina Messias de Almeida Milani Altemani, Eliane M.I. Amstalden.(2014). Histologic correlation of expression of Ki-67 in squamous cell carcinoma of the glottis according to the degree of cell differentiation. Braz J Otorhinolaryngol.;80(4):290-295.
18. Sharma P, Kumar N, Bahadur AK, Mandal AK. (2005). Ki-67 expression in cytologic scrapes from oral squamous cell carcinoma before and after 24 gray radiotherapy- a study on 43 patients. Med Oral Patol Oral Cir Bucal;10:E15-E17.
19. Vivian Petersen Wagner, Manoela Domingues Martins, Caroline Siviero Dillenburg, Luise Meurer, MD, PhD,b Rogerio Moraes Castilho, Cristiane Helena Squarize. (2015). Histogenesis of keratoacanthoma: histochemical and immunohistochemical study. Oral Surg Oral Med Oral Pathol Oral Radiol;119:310-317
20. Walker DM, Boey G, McDonald LA: The pathology of oral cancer. Pathology 35(5): 376e383, 2003
21. Wangsa D, Ryott M, vall-Lundqvist E A, Petersson F, Elmberger G, Luo J et al. (2008). Ki-67 expression predicts locoregional recurrence in stage I oral tongue carcinoma. British Journal of Cancer; 99: 1121 –1128
22. Zito G, Saotome I, Liu Z, et al. (2014). Spontaneous tumour regression in keratoacanthomas is driven by Wnt/retinoic acid signalling cross talk. Nat Commun.5:3543.
23. Dragomir LP, Simionescu C, Mărgăritescu CL, Stepan A, Dragomir IM, Popescu MR. (2012). P53, p16 and Ki67 immunoexpression in oral squamous carcinomas. Rom J Morphol Embryol. 53(1):89–93
24. Zamirska A, Matusiak Ł, Dziegiel P, Szybejko-Machaj G, Szepietowski JC. (2012). Expression of metallothioneins in cutaneous squamous cell carcinoma and actinic keratosis. Pathol. Oncol. Res. 18:849–855
25. Brown DC, Gatter KC. Ki67 protein: the immaculate deception? Histopathology 2002; 40: 2–11.
26. Pereira CS, de Oliveira MVM, de Carvalho Fraga CA, Barros LO, Domingos PLB, Roy A, De-Paula AMB, Guimarãe ALS. (2012). Impact of the epithelial dysplasia grading and Ki67 proliferation index in the adjacent non-malignant mucosa on recurrence and survival in head and neck squamous cell carcinoma. Pathology – Research and Practice 208, 651– 656