

Ki-67 Staining as a Tool to Differentiate Verrucous Carcinoma from Condyloma Acuminatum on Skin Biopsy

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Abstract: Verrucous carcinoma is a variant of well differentiated squamous cell carcinoma that slowly growing and rarely metastasize. It grows as polypoid mass with verrucous surface. Macroscopically can produce exophytic appearance that mimic condyloma acuminatum. On skin biopsy specimen that usually tiny tissue, the microscopic appearance can be similar with condyloma acuminatum because it composed of well differentiated squamous epithelial cells. Ki-67 staining can label proliferating cell even they are not in mitotic state. Verrucous carcinoma has high proliferation index while condyloma acuminatum has low index. This study want to prove whether Ki-67 staining can serve as reliable marker to differentiated verrucous carcinoma from condyloma acuminatum. Sixteen case are retrieve from archive of Pathologic Department Dr. Soetomo Hospital consist eight case verrucous carcinoma and condyloma acuminatum respectively. Immunohistochemical staining with antibody Ki-67 was performed on each case. Squamous epithelial cells that stain positive were count from each case, and the results were statistically compare between verrucous carcinoma and condyloma acuminatum. The result of this study shows significant difference in number of Ki-67 positive cells in verrucous carcinoma compare to condyloma acuminatum. There is also different in squamous cell layer that expressed Ki-67. It is concluded that Ki-67 staining is a reliable tool that can differentiate verrucous carcinoma from condyloma acuminatum on skin biopsy specimen.

1 INTRODUCTION

Verrucous carcinoma is a variant of well differentiated squamous cell carcinoma. It is grow exophytic with verrucous surface. Unlike squamous cell carcinoma, it grows slowly with no metastatic potential and behave as low grade malignancy. Verrucous carcinoma can arise in anogenital region and should be distinguished from condyloma acuminatum (Liu, G., Li, Q., Shang, X., Qi, Z., Han, C., Wang, Y., Xue, F., 2016). Verrucous carcinoma at anogenital region can give clinical features mimicking giant condyloma acuminatum which is benign proliferative lesion caused by human papilloma virus (HPV). On microscopic examination, both disease can give similar features such as acanthosis with papillomatosis with minimal nuclear atypia. On the other hand, warty squamous cell carcinoma can arise in giant condyloma acuminatum making it more complicated. Therefore, it takes a reliable test to differentiate verrucous carcinoma from condyloma acuminatum especially in giant form (Bambao, C., Nofech-Mozes, S., Shier, M., 2010).

Ki-67 protein are expressed in all proliferating cells. Since the nature of malignancy is uncontrolled excessive proliferation, examination of tumor tissue with Ki-67 theoretically can be used as reliable tool to detect malignant transformation in condyloma acuminatum (Li, L. T., Jiang, G., Chen, Q., Zheng, J. N, 2015). The aims of this study was to prove whether Ki-67 stain can distinguish verrucous carcinoma from condyloma acuminatum.

2 METHODS

Blok paraffin from verrucous carcinoma and condyloma acuminatum each eight case (totally sixteen case) were retrieved from archive of Pathology department Dr. Soetomo Hospital Surabaya. These paraffin blocks were sliced 6 µm thick and were placed in object glass to perform immunohistochemistry stain with Ki-67 antibody. Microscopic analysis were made by light microscope to count Ki-67 positive cells. Only squamous cells

that expressed Ki-67 protein were count as positive. The number of positive cells were count in area $40.000 \mu\text{m}^2$ using microscopic graticulae at 400 times magnification. The number of squamous epithelia cells that expressed Ki-67 protein were tabulated and statistically analyzed to assessed is there significance difference in number from verrucous carcinoma compare to condyloma acuminatum.

3 RESULTS

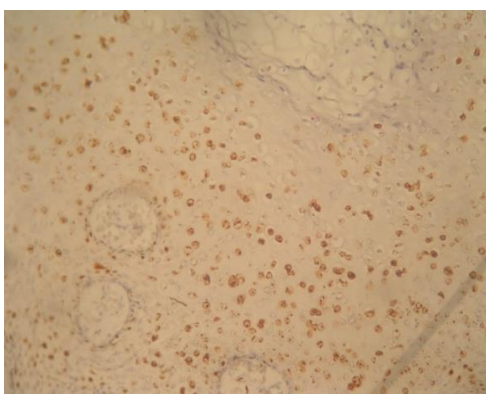


Figure 1: Expression of Ki-67 protein in verrucous carcinoma specimen. Ki-67 were expressed in all layer of squamous epithelia cells. (Ki-67 immunohistochemistry staining 400X magnification).

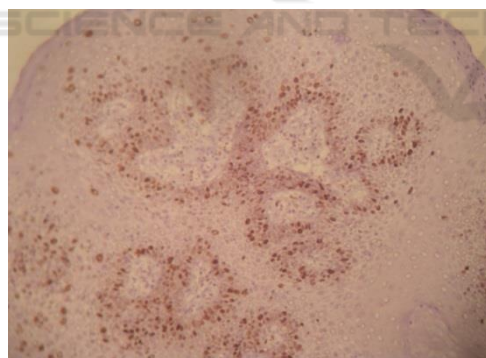


Figure 2: Expression of Ki-67 protein in condyloma acuminatum specimen. Ki-67 were expressed mainly at basal layer of squamous epithelia cells, only few scattered cell in spinous layer expressed Ki-67. (Ki-67 immunohistochemistry staining, 400X magnification).

On microscopic examination there is significant difference in Ki-67 positive cell from verrucous carcinoma ($56,75 \pm 9,00$) compare to condyloma acuminatum ($22,25 \pm 4,40$) ($p=0.000$). At cut off value 40 cells per $40.000 \mu\text{m}^2$ area, Ki-67 staining can differentiate all case of verrucous carcinoma from

condyloma acuminatum which means 100 percent of accuracy.

The pattern of Ki-67 positive cells were also differ on verrucous carcinoma compare to condyloma acuminatum. On verrucous carcinoma, Ki-67 were expressed in all layer of squamous epithelia (figure 1) while in condyloma acuminatum, the Ki-67 were expressed only in basal layer (figure 2).

4 DISCUSSION

Ki-67 protein were expressed in proliferating cells. Ki-67 protein can be detected within the nucleus of proliferating cells during all active phases of the cell cycle i.e. G_1 , S, G_2 and mitosis, but it is not expressed in resting cells G_0 . Therefore it an excellent marker for determining proliferative activity (Scholzen, T., Gerdes, J., 2000). Assessment of Ki-67 positive cell can give different result from mitotic count since Ki-67 were expressed in all proliferation cycle. Ki-67 index has been used for evaluating prognostic test for disease progression in several cancer (Jonat W. and Arnold N, 2011). In skin tumor, Ki-67 evaluation can be used as a marker indentify a malignant process. Leblebici has performed immunohistochemical staining with Ki-67 on keratoacanthoma compare with squamous cell carcinoma. It gives 81% sensitivity and 100% specificity in detecting malignant squamous cell from benign squamous epithelial cells (Leblebici, C., Pasaoglu, E., Kelten, C., Darakci, S., Dursun, N., 2017).

This study give different result in number of Ki-67 positive cells as well as the pattern of Ki-67 positive cells. In verrucous carcinoma, the number squamous epithelial that expressed Ki-67 is larger significantly compare to that in condyloma acuminatum. As it was count on $40.000 \mu\text{m}$ area, the verrucous carcinoma always have more than 40 Ki-67 positive cells while squamous epithelia in condyloma acuminatum consistently give less than 40 positive cells. It means 100 percent of accuracy. There is also difference in staining pattern of squamous cells that express Ki-67 protein. In condyloma acuminatum, cells that express Ki-67 mostly on basal layer. Only a few scattered cells in upper layer express Ki-67 whereas in verrucous carcinoma, squamous cells from all layer express Ki-67 protein.

Distinguishing condyloma acuminatum from verrucous carcinoma are not always give clear-cut criteria, since giant condyloma acuminatum can give similar feature both macroscopic and microscopically with verrucous carcinoma. Condyloma acuminatum

usually behave as benign proliferative lesion with no malignant potential but in a few circumstances it can undergo malignant transformation. Papapanagiotou has reported malignant transformation of giant condyloma at perianal area (Papapanagiotou, I.K., Migklis, K., Ioannidou, G., Xesfyngi, D., Kalles, V., Mariolis-Sapsakos, T., Terzakis, E., 2017). The coexistence of condyloma with verrucous carcinoma make it more complicated in diagnosis since the benign process can contain focus of some malignant cells making malignant marker was strongly required for benign looking cases (ErmanVlahovic, M., Vlahovic, J., Mrcela, M., Hrgovic, Z., 2017).

Sometimes microscopic examination with hematoxylin-eosin staining cannot detect malignant transformation. Huang has reported a giant condyloma acuminatum with benign microscopic feature give recurrent lesion with malignant feature (Huang SM, Leung WH and Chen BF, 2007). Therefore it need an additional staining to detect microscopic malignancy that involve a few epithelial cells.

Ki-67 protein which expressed in proliferating cells has been used to determine tumor grade in many cancer has potential ability to differentiate malignancy from benign process and also useful for detection focus of malignant transformation that arise in benign lesion (Jonat W. and Arnold N, 2011).

5 CONCLUSION

Ki-67 staining is useful tool for differentiate verrucous carcinoma from condyloma acuminatum with 100 percent accuracy.

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