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Cut-off Point of Ki-67 Proliferation Marker in Differentiating Premalignant and Malignant Prostatic Lesions

Punto de corte del marcador de proliferación Ki-67 en la diferenciación de lesiones prostáticas premalignas y malignas

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SUMMARY

Immunohistochemistry Ki-67 is a marker of cell proliferation. Using immunohistochemical markers can be helpful to determine and diagnose prostate intraepithelial neoplasia (PIN) and carcinoma prostate. There are many studies about Ki67 as a prognostic tool, a diagnostic tool, and a potential therapeutic target for cancer therapy, but there are no standard criteria to define the cut-off point for the value of Ki-67 in premalignant and malignant prostate lesions. We conducted a diagnostic test study on 55 specimen transurethral resections of the prostate (TUR-P) to evaluate the cut-off point Ki-67 in the prostate lesions with the potential for malignant transformation and prostate carcinoma. These results showed a significant difference in Ki-67 value between premalignant and malignant lesions (p<0.005). The cut-off point of Ki-67 value was ≥27.5 % for malignant lesions with a sensitivity of 97.4 %; specificity of 82.4 %; PPV of 86.2 %; NPV of 52 % and accuracy of 70.3 %. Meanwhile, a Ki-67 value cut-off point of ≥4.5 % for premalignant lesions would assign a sensitivity of 94.1 %; specificity of 48.4 %; PPV of 42.3 %; NPV of 72.7 %, and accuracy of 56.3 %. The area under the curve values of 0.932 indicated an excellent ability of a diagnostic test to differentiate premalignant and malignant lesions.

Keywords: *Cut-Off point, Ki-67 immunohistochemistry, malignant, premalignant, prostate lesions.*

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RESUMEN

La inmunohistoquímica Ki-67 es un marcador de proliferación celular. El uso de marcadores inmunohistoquímicos puede ser útil para determinar y diagnosticar la neoplasia intraepitelial de próstata (PIN) y el carcinoma de próstata. Hay muchos estudios sobre Ki67 como herramienta de pronóstico, herramienta de diagnóstico y como posible diana terapéutica para la terapia del cáncer, pero no existen criterios estándar para definir el punto de corte para el valor de Ki-67 en lesiones de próstata premalignas y malignas. Realizamos un estudio de pruebas diagnósticas sobre 55 muestras de resecciones transuretrales de próstata (RTU-P) para evaluar el punto de corte Ki-67 en las lesiones prostáticas con potencial de transformación maligna y carcinoma de

próstata. Estos resultados mostraron una diferencia significativa en el valor de Ki-67 entre lesiones premalignas y malignas (p<0,005). El punto de corte del valor de Ki-67 fue ≥27,5 % para lesiones malignas con una sensibilidad del 97,4 %; especificidad del 82,4 %; VPP de 86,2 %; VAN del 52 % y precisión del 70,3 %. Por su parte, el punto de corte del valor Ki-67 de ≥4,5 % para lesiones premalignas asignaría una sensibilidad del 94,1 %; especificidad del 48,4 %; VPP de 42,3 %; VAN del 72,7 % y precisión del 56,3 %. Los valores del área bajo la curva de 0,932 indicaron una excelente capacidad de la prueba diagnóstica para diferenciar lesiones premalignas y malignas.

Palabras clave: *Punto de corte, inmunohistoquímica Ki-67, lesiones malignas, premalignas, prostáticas.*

INTRODUCTION

The incidence rate of prostate cancer in three teaching hospitals in Indonesia (Jakarta, Surabaya, and Bandung), in the last eight years, was 1 102 with the average age being 67.18 years. In Rumah Sakit Cipto Mangunkusomo and Rumah Sakit Dharmais the incidence rate has increased twice in the years 2001-2006 compared to 1995-2000; the incidence rate each year is 70-80 new cases. Almost all the patients are more than 60 years old and rarely under 40 years.

Prostatic intraepithelial neoplasia (PIN) increases the risk of prostate cancer. On transrectal ultrasound, the PIN may be hypoechoic, like carcinoma but this declaration had not been confirmed (1-3). Most urologists and radiologists do not believe that PIN can be detected by using transrectal ultrasound and magnetic resonance imaging (MRI) because the PIN is a microscopic finding (4).

Using immunohistochemical markers can be helpful to determine the diagnosis of PIN and prostate carcinoma. Selected antibodies such as antikeratin 34βE12 (high molecular weight cytokeratin) and p63 are used to stain tissue sections for the presence of basal cells. The interpretation is, that PIN showed the intact or discontinued basal cell layer, whereas in malignant lesions it does not.

Immunohistochemistry Ki67 is a marker of cell proliferation. It is a protein that is involved in cell cycle regulation and expressed in proliferating cells during all active phases of the cell cycle (G1, S, G2, and mitosis) and is absent from the G0 phase, which makes it the best marker for determining the growth fraction of a determined cell population (normal or tumoral) (5,6). Ki67 confirmation can be promising for routine practical application to evaluate the biological behavior and malignant potential of prostatic carcinoma.

There are many studies about Ki67 as a prognostic tool, a diagnostic tool, and a potential therapeutic target for cancer therapy (7,8). However, there are no standard criteria to define the cut-off point for a value Ki67 in premalignant and malignant prostate lesions.

The purpose of this study is to evaluate a cut-off point for Ki67 in prostate lesions with the potential for malignant transformation and prostate carcinoma.

METHODS

This is a diagnostic test study. Paraffin blocks prepared from 55 transurethral resections of the prostate (TUR-P) were collected in this study. The specimens were obtained from the Department of Pathology, Universitas Sumatera Utara, Medan, Indonesia. The specimens were divided into two types of lesions, 17 cases of high-grade PIN and 38 cases of diagnosed prostatic carcinoma. Each paraffin block was recut into serial sections and stained with Haematoxylin and Eosin, immunohistochemistry p63, and Ki67. The block paraffin was sectioned into 2 to 3 μ, then prepared and stained with p63 immunohistochemistry to make sure that the lesion was diagnosed as premalignant (PIN) or a malignant lesion. We used the REAL EnVision method for p63 immunohistochemistry staining with 1:100 dilution and Ki67 1:200. The analysis used continuous expression from p63 as a benign lesion, discontinuous as a premalignant lesion (PIN), and no expression from basal cell glandular as a malignant lesion (adenocarcinoma). Ki67 positive staining was identified by the presence of brown nuclear (DAB) staining in 100 tumor cells.

The statistical analyses were carried out using SPSS 22 version. The difference between two continuous variables was determined by using the Mann-Whitney U test. The cut-off point value was determined by using the receiver operating

characteristic (ROC) curve analysis and the subsequent diagnostic test would be used to assess sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy.

RESULTS

A total of 55 cases were used in this study. We divided them into two types of lesions: (1). premalignant lesions, about 17 cases (30.9 %) and (2). malignant lesions in about 38 cases (69.1 %). All specimens were collected by transurethral resection of the prostate (100 %). The mean Ki-67 value was 14.71 % for premalignant lesions and 57.53 % for malignant lesions. There was a significant difference in Ki-67 value between premalignant and malignant lesions ($p<0.005$). The cut-off point of Ki67 value was ≥27.5 % for malignant lesions with a sensitivity of 97.4 %; specificity of 82.4 %; PPV of 86.2 %; NPV of 52 % and accuracy of 70.3 %. Meanwhile, a Ki-67 value cut-off point of ≥4.5 % for premalignant lesions would assign a sensitivity of 94.1 %; specificity of 48.4 %; PPV of 42.3 %; NPV of 72.7 %, and accuracy of 56.3 %. The area under the curve values of 0.932 indicated an excellent ability of the diagnostic test to differentiate premalignant and malignant lesions.

DISCUSSION

The Ki67 antigen which encodes two protein isoforms with a molecular weight of 345 kDa and 395 kDa, was originally identified by Scholzen and Gerdes in the early 1980s (5). The Ki67 protein has a half-life of only ~1-1.5 hours. It is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent in resting cells (G0) (2,9). In later phases of mitosis (during anaphase and telophase), a sharp decrease in Ki67 levels occurs (10). Expression of the Ki67 protein (pKi67) is associated with the proliferative activity of intrinsic cell populations in malignant tumors; it means Ki67 can be used as a marker of tumor aggressiveness (11,12).

Immunohistochemical markers are mainly used as an aid in examination in the diagnosis of prostatic lesions, especially if the lesions can make confused for high-grade PIN and adenocarcinoma (13). The diagnosis of prostate carcinoma is based on the absence of basal cells in the glandular prostate tissue. The method for identification of basal cells was using immunohistochemistry p63 and high molecular weight cytokeratin. P63 immunohistochemistry has more reliability to identify basal cells than high molecular cytokeratin (14,15). Because of these reasons, in this study, we used p63 immunohistochemistry before we did Ki67 staining to define premalignant and malignant lesions to make sure of the diagnosis, so we could define an accurate cut-off point for the premalignant and malignant lesions.

Ki67 is frequently used as an indicator of cell proliferation (9,16). A number of diagnostic applications for pKi67 have been described, where Ki67 was significantly more highly expressed in malignant than in normal tissues (17,18). Uncontrolled proliferation is a hallmark of malignancy and the measurement of Ki67 antigen by using IHC is the most widely performed assessment of a tumor's proliferation potential. In the present study, our result showed that there was a significant difference in the expression of Ki67 in malignant lesions than in premalignant lesions ($p<0.001$). This means that the data showed that the expression of Ki67 is higher in malignant lesions than in premalignant lesions.

The accuracy of the test can be shown from the area under the ROC curve. This study showed an AUC value of 0.932; it represents a perfect test (excellent). A guide to classifying accuracy of the diagnostic test can be seen in Table 1 (11).

Table 1

The classifying area under the curve (AUC)

AUC	Classification
0.90-1	Excellent
0.80-.90	Good
0.70-.80	Fair
0.60-.70	Poor

This study showed that the Ki-67 immunohistochemistry is a good method to identify premalignant and malignant prostate lesions, with a sensitivity in the range of 94.1 %-97.4 %.

Ki-67 measurement can be a predictor of prostate cancer outcome. However, a common consensus in Ki-67 cut-off points is needed to determine benign, premalignant, and malignant prostate lesions. Thus, we think this marker might be studied in larger samples for its further validation for cell proliferation.

CONCLUSIONS

This study concluded that in prostate tissue examination, immunohistochemical expression of Ki-67 may help in determining cell proliferation in terms of whether the lesions are premalignant or malignant lesions and can be a potential application to guide pathologists in diagnosis.

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