

Expression profile of Ki67 in palmoplantar melanocytic lesions: a case-control study

Perfil de expresión de Ki67 en lesiones melanocíticas palmoplantares: estudio de casos y controles

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Abstract

Background: Acral lentiginous melanoma is a malignant neoplasm which appears in hands and feet. Acral lentiginous melanoma has an unclear etiology, and usually affects non-Caucasian population. Because it is frequently diagnosed lately, acral melanoma has bad prognosis; however, it is biologically more aggressive than other clinicopathological types of melanoma, even when diagnosed early. **Objective:** To determine the expression of Ki67 in invasive lentiginous acral melanoma and to compare it with acral nevi. **Method:** Cross-sectional, descriptive, observational study. Immunohistochemistry with Ki67 marker was performed on 17 biopsies of invasive lentiginous acral melanoma (cases) and 17 biopsies of palmoplantar nevi (controls). Nuclear expression of Ki-67 was determined and both were compared between both groups. **Results:** The mean expression of Ki67 was 8.5% in the control group, and 34% in the melanoma group, which was statistically significant ($p < 0.0001$). **Discussion:** Ki67 expression in acral lentiginous melanomas is higher than in acral nevi. Prognostic value of Ki67 is still considered controversial. However, there are several studies where, in combination with other markers, their prognostic value is reinforced. **Conclusions:** Due to the wide gap in Ki67 expression between melanomas and nevi showed in this study, Ki67 expression, referred to as a proliferative index, could be considered as a prognostic factor even more objective than the mitotic index.

KEY WORDS: Ki67. Acral lentiginous melanoma. Nevi. Palms. Soles.

Resumen

Introducción: El melanoma acral lentiginoso es una neoplasia maligna que afecta a población predominantemente no caucásica. Debido al diagnóstico tardío suele tener mal pronóstico, además de que se considera una neoplasia biológicamente más agresiva, incluso cuando se detecta tempranamente. **Objetivo:** Determinar la expresión de Ki67 en el melanoma acral lentiginoso invasor y compararla con los nevos acrales. **Método:** Estudio transversal, descriptivo, observacional. Se realizó inmunohistoquímica con marcador Ki67 en 17 biopsias de melanoma acral lentiginoso invasor (casos) y 17 biopsias de nevos palmoplantares (controles). Se determinó la expresión nuclear de Ki-67 y se comparó entre ambos grupos. **Resultados:** La media de expresión de Ki67 fue del 8.5% en el grupo control y del 34% en el grupo de melanomas, siendo esta diferencia estadísticamente significativa ($p < 0.0001$). **Discusión:** La expresión de Ki67 en los melanomas acrales es considerablemen-

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te mayor que en los nevos acrales. El valor pronóstico del marcador Ki67 sigue siendo considerado controversial. Sin embargo, hay estudios en los que en combinación con otros marcadores se refuerza su valor pronóstico. **Conclusiones:** Por la gran diferencia en inmunorreactividad de Ki67 entre melanomas y nevos, la expresión de Ki67, referida como índice proliferativo, podría ser considerada como factor pronóstico incluso más objetivo que el índice mitótico.

PALABRAS CLAVE: Ki67. Melanoma acral lentiginoso. Nevos. Palmas. Plantas.

Introduction

Acral lentiginous melanoma (ALM) is one of the clinicopathological presentations of cutaneous melanoma. In 1976, Reed introduced the concept that ALM is a distinct histopathological subtype, and currently it is well described by pathologists and dermatologists¹. Its definition is both clinical and histopathological: the term “acral” refers to its anatomical location in the volar skin of the limbs, and “lentiginous” describes its distinctive radial growth pattern².

ALM mainly affects non-Caucasian populations; for example, its prevalence in an Asian cohort was around 58% of all cutaneous melanomas³, and it is estimated to range between 60 and 70% in dark-skinned populations⁴.

The frequency of ALM has been reported to vary between 40 and 80% of all melanomas located in the limbs⁵⁻⁷. The lentiginous form is the most common presentation in acral areas².

The pathogenesis of ALM remains unclear. Some studies have associated it with exposure to ultraviolet radiation, chemical agents or even previous trauma, while other authors rule out these factors as its causes^{2,8-11}.

Its unfavorable prognosis is mainly due to late diagnosis. However, there are studies that suggest that ALM is biologically more aggressive than other melanoma presentations¹².

To define the therapeutic approach to cutaneous melanoma, it is important to consider patient prognostic factors, out of which the most widely used is tumor thickness¹³. However, its use as the main prognostic criterion has been shown to be insufficient, which has been demonstrated by studies that have shown that some patients with thin melanomas (0.76 mm) die from melanoma, while there are series where 45 to 50% of patients with thick melanomas (> 4 mm) have survived for 5 years¹⁴⁻¹⁶.

Previous studies suggest that the immunohistochemical biomarker Ki-67 might play a role in the prognosis of the disease. However, these studies combine all different clinicopathological forms of malignant melanoma. The purpose of this study is to describe the degree of expression of Ki-67 in benign

(nevi) and malignant palmoplantar lesions (ALM), in order to determine the value of this marker in the proliferative activity of two groups of lesions with different biological behavior.

Method

This was a cross-sectional, comparative, retrospective, non-blinded observational study. The studied specimens were palmoplantar skin biopsies detected in the database of the Department of Pathologic Anatomy and Cytopathology of the Dr. José Eleuterio González University Hospital of Monterrey, Nuevo León, Mexico. The included samples were formalin-fixed biopsies embedded in paraffin blocks, obtained from 17 patients with tumors diagnosed as ALM and that had been located in volar, either palmar or plantar skin. A control group was also included, composed of 17 formalin-fixed, paraffin-embedded biopsies of lesions diagnosed as nevi, located in palmar or plantar skin. The samples, stained with hematoxylin-eosin, were reevaluated to corroborate the diagnosis of both cases and controls. All the included paraffin blocks were sectioned into 5- μ m thick slices and then immunohistochemical staining was performed with anti-Ki67 monoclonal antibody M7240 (Dako, Carpinteria, CA) at a 1:25 dilution. The same immunohistochemical staining process was applied to the biopsies corresponding to the control group (nevi).

Nuclear immunoreactivity for Ki67, both in the case and in the control groups, was determined based on the percentage of marker-positive cells at the site of highest expression of all the samples^{17,18}. Subsequently, a contrast of Ki67 cell expression means between cases and controls was carried out using Student's-t test, with 95% reliability.

Results

Out of 37 patients diagnosed with melanoma in the limbs found in the clinical records administration system of our hospital between 2010 and 2014, eight had melanomas located in the nail apparatus (matrix and

nail bed), and were therefore excluded; five other patients were excluded because the tumors had other locations in the limbs (back of hands or feet); and seven other patients were excluded because they had been diagnosed with melanomas in situ. Only 17 patients with available tissue samples and confirmatory diagnosis were included in the analysis. In the group of melanoma cases, 12 patients (70%) were females. Average age in the group cases was 59 years. Clinical and demographic information of the patients included in the study, obtained from the clinical records, is shown in table 1.

Immunoreactivity to Ki67

In the nevi group, average Ki67 expression was 8.5% (range: 1-10%) of cells. It should be noted that in all specimens of the control group there was a certain degree of Ki67 expression. In the group of cases, 15 out of 17 melanomas (88.24%) had immunoreactivity to the MIB-1 marker in > 20% of the cell population on the site of highest expression of each sample. On average, 34% of tumor cells were immunoreactive to Ki67 (range: 10-70%). When comparing the obtained averages ($8.5 \pm 0.27\%$ in the control group and $34 \pm 18.1\%$ in the problem group) a statistically higher average was observed in the problem group ($p \leq 0.0001$) (Fig. 1).

Discussion

Immunohistochemistry as a diagnostic technique remains profitable and highly useful in melanoma, as well as in other benign and malignant neoplasms. The use of the Ki67 cell proliferation marker (MIB-1) as an indicator of clinical evolution has been tested in different types of tumors¹⁹⁻²¹. In the case of melanoma, it has been considered an independent prognostic factor¹³. In general, our results are similar to those obtained in studies that included melanomas without distinction by clinicopathological form (Table 1). Vishnevskaya et al.²², in a larger population ($n = 65$), reported that Ki67 expression in the melanoma group cells was 10%, whereas in the control group (nevi), it was 5- 9%; however, when compared to our results, there is no statistically significant difference between the results of their study and ours ($p = 0.7553$). Gimmoty et al.²³, with the largest number of patients included in a similar study ($n = 965$), reported a Ki67 average expression of 23.6% in melanoma cells, as well as Ki67-positivity in the dermis, which was 8.9%

Table 1. Ki67 expression in benign and malignant palmo-plantar lesions: comparison with similar studies

Author	Vishnevskaya, et al. ²²	Current study	p
N	65	17	-
Expression in nevi (prevalence)	5-9%	8.50%	0.7553
Expression in melanoma (prevalence)	>10%	34%	-
Author	Gimmoty, et al. ²³	Current study	p
N	965	17	-
Epidermal Ki67 (mean)	23.60%	34%	0.477
Dermal Ki67 (mean)	8.90%	Not assessed	-
Author	Hazan, et al. ¹³	Current study	p
N	137	17	-
Ki67% > 20%	47.40%	88%	0.0037
Author	Ladstein, et al. ²⁴	Current study	p
N	202*	17	-
Ki67 (mean)	27%	34%	0.7355

*Nodular melanomas.

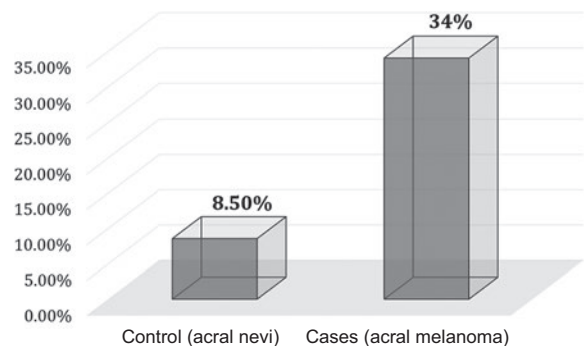


Figure 1. Immunoreactivity to Ki67 in the control group (acral nevi) and in the group of cases (acral melanomas).

Although in our study we did not sub-classify epidermal and dermal Ki67 expression, the prevalence in both studies did not statistically significantly differ ($p = 0.477$). Hazan et al.¹³, in their series of 137 patients with melanoma, reported that a high proliferative index (Ki67 expression > 20%) was observed in 47.45% of cases, while Ki67 positivity was considerably higher (88.24%) in the melanoma cases of our study ($p = 0.0037$).

Ladstein et al.²⁴ also reported Ki67 expression in their study, but they only included nodular melanomas. Ki67 average expression in melanoma cells was 27%. In spite of being a tumor with a higher growth

rate, nodular melanomas showed lower Ki67 expression when compared with our results obtained in ALM cases (34%). Despite these results, the difference is not statistically significant ($p = 0.7355$).

The Ki67 antigen can be expressed in less than 5% of nevi cells, and in 13-30% of melanoma cells; however, in individual cases, expression can be higher. In spitzoid neoplasms, Ki67 expression can also be higher²⁵⁻³¹. Chorny et al.²⁵ demonstrated that Ki67 can be of crucial significance in cases of so-called minimal deviation melanomas or melanocytic tumors of uncertain malignant potential. This specific group of lesions showed a Ki67 cell expression average of 13%, in comparison with 3% in banal lesions and 25% in superficial spreading melanomas.

In the study by Vishnevskaya et al.²², conducted in a Russian population, all clinicopathological melanoma forms were included. In addition, although they showed that the group of melanomas had an expression > 10% of tumor cell population, average expression is not specified, leaving a considerably wide expression margin. This contrasts with other studies that consider a cutoff value of 20%, and above that percentage of expression, lesions had a more ominous prognosis^{13,32-35}. Gimnoty et al.²³, in a large study of biopsies of thin invasive melanomas, determined that melanoma cells expressivity to Ki67 varies according to the growth pattern, which is predominantly epidermal in the horizontal –radial– growth phase, and as vertical (invasive) growth appears, epidermal expression decreases and dermal expression increases. They concluded that a mitotic index > 0, in addition to Ki67 expression > 20% in the dermis, are independent factors for poor prognosis, with 10-year metastasis rates ranging from 20 to 39%.

The most important limitation in our study is the sample size, given that it was a retrospective study with very strict selection criteria, since we only included ALM and benign melanocytic palmoplantar lesions. Notwithstanding, we decided to include only palmar and plantar tumors due to the lack of studies involving these locations, and also because acral melanoma has a high incidence in non-Caucasian populations, specifically in Hispanics, and our population is therefore particularly susceptible to ALM³⁶⁻³⁸.

The prognostic value of the Ki67 marker remains controversial, since there are conflicting studies on whether or not it acts as an independent risk factor for an unfavorable evolution of the patient with melanoma. However, there are studies where, in combination with other markers, its prognostic value is

reinforced, such as the study by Väisänen et al.³⁹, who showed that an elevated Ki67 expression in melanomas, in combination with a high expression of other markers such as p53 and matrix metalloproteinase-2, is associated with high metastatic potential and a 10-year survival of around 28%.

Conclusions

According to the results obtained in our study, and combined with other similar studies, Ki67 immunoreactivity, expressed as the proliferative index, should be taken into account as an even more objective factor than mitotic index, which was included in the American Joint Committee on Cancer latest staging proposal in 2009⁴⁰, considering that the mitotic index quantification in hematoxylin & eosin stains is highly criticized for being inaccurate, poorly reproducible and time-consuming⁴¹⁻⁴³. Moreover, Garbe et al.⁴⁴ reported in their study, where 17 dermatopathologists and pathologists determined the mitotic index of melanoma biopsies, that inter-observer reliability was very low (kappa value: 0.345) and that intra-observer reliability (the same lesions were assessed 1 year later) was also very low (kappa value: 0.18-0.348). Our study suggests that Ki67, as an immunohistochemical marker, has a direct correlation with other prognostic factors already included in clinical practice guidelines, and that its use as an added prognostic factor should therefore be considered and that, on the contrary, it should no longer be a matter of debate between experts.

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