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Clinical characteristics of actinic keratoses and their histological correlations: suggestion for a clinical severity scale

Características clínicas das queratoses actínicas e suas correlações histológicas: sugestão de escala de gravidade clínica

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ABSTRACT

Introduction: Actinic keratoses are premalignant lesions with a risk of transformation to invasive squamous cell carcinoma. There is no identified correlation between clinical classification and histological grade of these lesions.

Objectives: To correlate the clinical characteristics of actinic keratoses of the forearms and back of the hands with the degree of histological atypia (Keratinocyte Intraepidermal Neoplasia); to develop and validate a clinical severity scale correlated with the histological grade of actinic keratoses.

Methods: Cross-sectional study with 162 actinic keratoses clinically evaluated for diameter, erythema, infiltration, hyperkeratosis, and exulceration and 34 lesions with different patterns were biopsied. Clinical features were correlated with the degree of histological atypia and p53 and Ki-67 expression.

Results: Only the diameter of the lesions was significantly correlated with the degree of atypia (p=0.04), and only the erythema, hyperkeratosis, and the diameter linked with the immunohistochemical markings. A clinical score including diameter, hyperkeratosis, and exulceration was developed, which associated significantly with the degree of atypia (Spearman's Rho=0.43; p=0.01).

Conclusions: A score composed of diameter, hyperkeratosis, and exulceration correlated with the histological grade of actinic keratoses of the upper limbs was developed.

Keywords: Carcinoma in situ; Carcinoma squamous cell; Keratosis actinic; Skin diseases

RESUMO

Introdução*: as queratoses actínicas são lesões pré-malignas com risco de transformação para carcinoma espinocelular invasivo. Não há correlação identificada entre classificação clínica e grau histológico destas lesões.*

Objetivos: *correlacionar as características clínicas das queratoses actínicas dos antebraços e dorso das mãos com o grau de atipia histológica (Keratinocyte Intraepidermal Neoplasia); desenvolver e validar uma escala de gravidade clínica correlacionada ao grau histológico das queratoses actínicas.*

Métodos: *estudo transversal com 162 queratoses actínicas avaliadas clinicamente quanto a diâmetro, eritema, infiltração, hiperqueratose e exulceração; biopsiadas 34 lesões com diferentes padrões. As características clínicas foram correlacionadas com o grau de atipia histológica e a expressão de p53 e Ki-67.*

Resultados: *apenas o diâmetro das lesões correlacionou-se significativamente com o grau de atipia (p=0,04), e apenas o eritema, a hiperqueratose e o diâmetro correlacionaram-se com as marcações imuno-histoquímicas. Foi desenvolvido um escore clínico incluindo o diâmetro, a hiperqueratose e a exulceração, o qual se correlacionou significativamente com o grau de atipia (Rho de Spearman=0,43; p=0,01).*

Conclusões*: desenvolveu-se um escore composto por diâmetro, hiperqueratose e exulceração correlacionado com o grau histológico das queratoses actínicas dos membros superiores.*

Palavras-chave: *Carcinoma de Células Escamosas; Carcinoma In Situ; Queratose Actínica; Neoplasias*

Original article

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INTRODUCTION

Actinic keratoses (AKs) are the most frequent pre-malignant lesions in the human race. They are clinically described as slow-growing, dry, erythematous-scaly plaques, with mild or no infiltration of the base, located mainly in photoexposed areas, such as the scalp, face, cervical region, upper torso, and extremities.[1]

In 1999, Yantsos proposed a histological classification for AKs, based on the proportion of intraepithelial cell atypia observed. Due to the continuous progression of keratinocyte AKs atypia, similar to the progression of cervical intraepithelial neoplasia (CIN) associated with human papillomavirus (HPV), an analogous term was proposed to classify the histological grade of AKs - keratinocyte intraepidermal neoplasia (KIN).[2]

KIN 1 lesions, considered of low histological grade, present atypia only in the lower third of the epidermis and, theoretically, have a low probability of malignant transformation. On the other hand, KIN 2 and KIN 3 lesions, considered to be of high histological grade, have, respectively, atypia in the lower two-thirds and throughout the epidermis and would have a greater chance of malignancy.[2]

Regarding the clinical classification of AKs, the best known is the Olsen classification, which divides the lesions into three different grades according to their thickness and hyperkeratosis. However, there is still no clinical classification that sought to correlate the histological grade of AKs according to KIN.[3,4]

The present study assessed the correlation between the clinical AKs characteristics and the atypia degree in the histopathological examination, as well as the combined effect of the clinical features in the development of a clinical classification with the better correlation between clinical and the degree of histological atypia of the AKs of the superiors limbs.

METHODS

It is a cross-sectional study conducted from February 2019 to May 2019 at the Oncological Dermatology Outpatient Clinic. Nine patients diagnosed with AKs on the forearms and back of the hands were evaluated, and 34 lesions with distinct clinical characteristics were biopsied. Biopsies were performed by elliptical excision with 2 mm margins after marking their clinical limits. The Research Ethics Committee of the Faculty of Medicine of Botucatu - UNESP approved this study (N. 1,874,384).

We included patients aged over 18 years; lesions clinically compatible with AKs on the forearms and back of the hands; absence of lesions that generate diagnostic doubt with squamous cell carcinoma (SCC) in situ (Bowen's disease) or with invasive carcinomas; and the presence of up to 20 AKs per anatomical region examined. Also, we excluded individuals with any genodermatosis, immunosuppressed patients, previous users of systemic retinoids, those previously submitted to radiotherapy or any clinical treatment for AKs within less than six months.

Two dermatologists independently evaluated five clinical features: diameter in millimeters, erythema, infiltration, hyperkeratosis, and exulceration. Table 1 details the clinical grading used, and Figure 1 illustrates it with photographs.

A total of 162 lesions were clinically examined. Of these, 34 lesions with different clinical characteristics underwent excision.

Biopsies were fixed in 10% buffered formalin, embedded in paraffin blocks, submitted to 4 μm cross-sectional histological sections in the center of the lesion, and subsequently stained with hematoxylin & eosin. After confirming the histopathological diagnosis of the AKs, two qualified dermatologists graded them all according to the KIN score.

Table 1: Clinical characteristics evaluated in each actinic keratosis lesion

Clinical characteristic	Clinical grading
Diameter	Assessed in millimeters, with ruler. The largest diameter of each lesion was considered
Erythema	**Grade 1.** Absent or light pink **Grade 2.** Live erythema
Infiltration	**Grade 1.** Flat lesion, without signs of infiltration **Grade 2.** Raised plaque or papule
Hyperkeratosis	**Grade 1.** Absent or slightly rough lesion on palpation (peeling is more palpable than visible) **Grade 2.** Evident scaly appearance **Grade 3.** Compact keratin adhered to the surface of the lesion
Exulceration	**Grade 1.** Absent **Grade 2.** Present (including areas with blood crust)

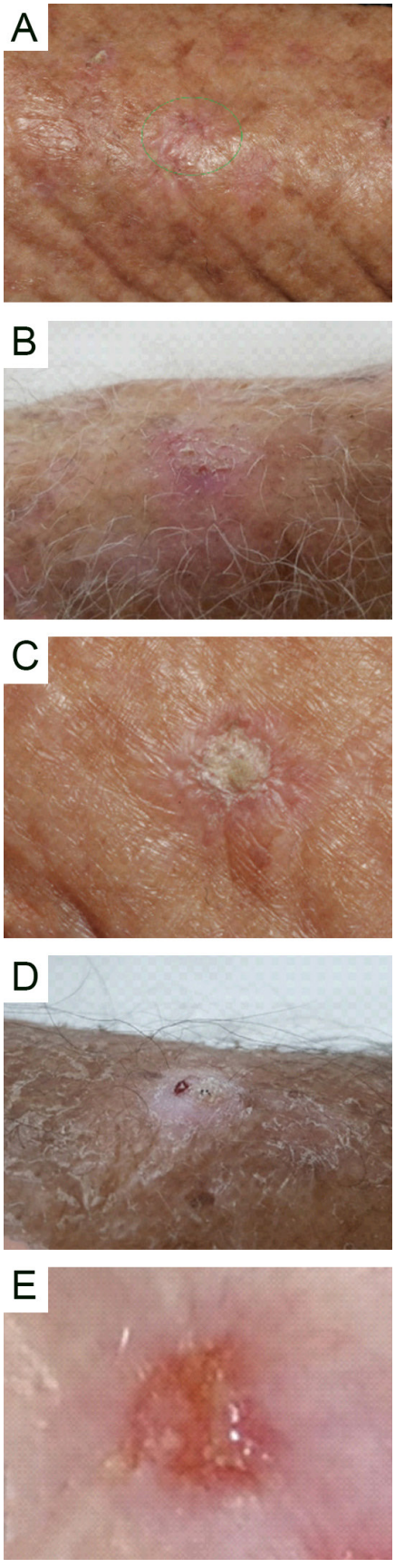

Figure 1: * A - Grade 1 erythema, Grade 1 infiltration, Grade 1 hyperkeratosis, Grade 1 exulceration. **B -** Grade 1 erythema, Grade 1 infiltration, Grade 2 hyperkeratosis, Grade 1 ulceration. **C -** Grade 1 erythema, Grade 2 infiltration, Grade 3 hyperkeratosis, Grade 1 exulceration. **D -** Grade 1 erythema, Grade 2 infiltration, Grade 3 hyperkeratosis, Grade 1 exulceration (bleeding area is due to anesthetic injection). **E -** Grade 2 erythema, grade 1 infiltration, grade 1 hyperkeratosis, grade 2 exulceration.

For immunohistochemistry, 4µn-thick histological sections were mounted on salinized slides (Sigma Chemical Corporation, Saint Louis, MO, USA) and stained for Ki-67 and p53 detection using the immunoperoxidase technique and avidin-biotin-peroxidase. For the p53 protein, the murine anti-human p53 monoclonal antibody, clone D0-7 (Dako, code no M7001, CA, USA), was used at a 1/30 dilution. For Ki-67, the murine anti-human Ki-67 monoclonal antibody, clone MIB-1 (Dako, code F7268, CA, USA), was used at a titer of 1/30. Immunohistochemical expression was considered positive when labeled nuclei stained brown. In the absence of the primary antibody, the negative control was tested, and histological sections of mammary carcinoma were used as a positive control.

Two qualified dermatologists assessed the nuclear expression of protein p53 and Ki-67 of keratinocytes in the lesional and perilesional epithelium in a semi-quantitative manner. Numbers from zero to three are assigned, according to the percentage of nuclei labeled as follows: (0) negative immunohistochemical reaction; (1) <30% labeled nuclei; (2) 30-60% labeled nuclei; (3) >60% labeled nuclei; and according to the intensity of the reaction: (0) negative immunohistochemical reaction; (1) weak reaction; (2) moderate reaction; (3) strong reaction. The final immunohistochemistry score was calculated by adding the number assigned to the percentage of labeled nuclei and the intensity of the reaction, with a minimum value of zero and a maximum of six.[5,6]

Categorical variables were represented by absolute, proportional, or percentage values. Continuous variables were described regarding central tendency by the mean and standard deviation or median and first and third quartiles, depending on the normality of the distributions analyzed by the Shapiro-Wilk test.

Inter-rater agreement regarding clinical characteristics was assessed using the intraclass correlation coefficient, single measures.

For statistical analysis, the diameter was also classified into three categories: up to 5 mm (inclusive); between 5 and 10 mm (inclusive); and above 10 mm.

The statistical correlation between the clinical characteristics, the histological grade, and the nuclear expression of lesional and perilesional p53 and Ki-67 was estimated by the Spearman correlation coefficient and chi-square test for trend analysis.

The coefficients of generalized linear models estimated the weight of each clinical characteristic to create the final severity scale.

The sample size was calculated expecting a correlation greater than 0.5 (alpha 0.05 and power of 90%) between the severity score and the histological and immunohistochemical indices.

Data were tabulated in MS Excel, and all analyzes were performed in IBM SPSS 24.0 software. A $p \leq 0.05$, the two-tailed value was considered significant.

RESULTS

In total, 162 lesions distributed on the forearms and dorsum of the hands of nine patients were examined: five women and four men, aged between 44 and 89 years, mean age of 67.6 years (standard deviation: 13 years). Each patient had, on average, 18 AKs. According to the Fitzpatrick classification, eight patients were skin phototype II, and one patient was skin phototype III.

Table 2 shows the frequency of the clinical characteristics of the lesions examined and biopsied, where there is a predominance of small lesions (median diameter = 5 [p25-p75: 4-8] millimeters), absent or mild erythema (grade 1), flat lesion (grade 1), evident desquamation (grade 2), and no exulceration (grade 1).

Histological evaluation of 34 lesions identified five KIN 1 lesions (14.7%), 21 KIN 2 lesions (61.7%), and eight KIN 3 lesions (23.6%). Regarding the predominant histological type, 23 were hypertrophic, 6 were atrophic, 1 was acantholytic, and 4 were lichenoid lesions. Inflammatory infiltrate was absent or mild in 14 lesions, moderate in 16 lesions, and intense in 4 lesions.

Table 3 illustrates the correlation between clinical variables and the KIN score, where a significant correlation is observed only with the categorized diameter (Spearman's Rho = 0.31; p=0.04). The inflammatory infiltrate intensity did not correlate significantly with the clinical features of erythema, hyperkeratosis, infiltration, diameter, and exulceration (Spearman's Rho = 0.31; p>0.4).

Table 4 illustrates the association between clinical features and immunohistochemical markers. There was a significant correlation between hyperkeratosis and the lesional and perilesional expression of Ki-67 (p<0.01); an inverse correlation between erythema and perilesional expression of Ki-67 (p=0.05); and an inverse correlation between diameter and perilesional p53 expression (p=0.04).

Table 5 shows an inverse correlation between histological grade (KIN) and perilesional p53 expression.

Table 2: Frequency of each clinical characteristic of actinic keratoses examined and biopsied

Clinical characteristic	Clinical grading	Total CAs examined	Total CAs biopsied
		N=162(%)	N=34(%)
Diameter in millimeters	Up to 5	89 (55)	14 (41)
	6 to 10	59 (36)	17 (50)
	Higher to 10	14 (9)	3 (9)
Erythema	Grade 1	143 (88)	27 (80)
	Grade 2	19 (12)	7 (20)
Infiltration	Grade 1	135 (83)	19 (56)
	Grade 2	27 (17)	15 (54)
Hyperkeratosis	Grade 1	39 (24)	5 (15)
	Grade 2	90 (56)	15 (44)
	Grade 3	33 (20)	14 (41)
Exulceration	Grade 1	154 (95)	31 (91)
	Grade 2	8 (5)	3 (9)

Table 3: Correlation between clinical features of actinic keratoses and grade of keratinocyte intraepithelial neoplasia (KIN)

Characteristic	Coefficient	p
Erythema ⋆	0.12	0.73
Hyperkeratosis ⋆	2.96	0.09
Infiltration ⋆	0.18	0.67
Exulceration ⋆	0.19	0.66
Diameter (millimeters)⋆⋆	0.31	0.08
Diameter (categorized)⋆	4.1	0.04

⋆ *Chi-square test trend analysis*

⋆⋆ *Spearman's Rho*

Table 4: Correlation between the clinical features of actinic keratoses and the epithelial expression of p53 and Ki-67

	Lesional				Perilesional			
	p53*	p	Ki-67*	p	p53*	p	Ki-67*	p
Erythema	0.01	0.96	-0.01	0.94	-0.12	0.52	-0.34	0.05
Hyperkeratosis	-0.15	0.42	0.47	<0.01	0.13	0.46	0.38	0.03
Infiltration	0.08	0.66	-0.21	0.24	-0.08	0.67	-0.02	0.93
Exulceration	0.12	0.51	-0.09	0.6	-0.01	0.98	-0.05	0.77
Diameter (mm)	0.08	0.66	0.12	0.49	-0.37	0.04	-0.07	0.69
Diameter (cat.)	0.02	0.90	-0.02	0.91	-0.34	0.06	-0.18	0.32

* *Spearman's Rho*
Significance level: p≤ 0.05

Table 5: Correlation between the grade of keratinocyte intraepithelial neoplasia (KIN) and the epithelial expression of p53 and Ki-67

Characteristic	Spearman' Rho	p
p53 lesional	-0.26	0.15
p53 perilesional	-0.36	0.04
Ki-67 lesional	0.18	0.31
Ki-67 perilesional	-0.08	0.66

Table 6: Final generalized linear model regarding the relationship between clinical features and grade of keratinocyte intraepithelial neoplasia (KIN)

Characteristic	Coefficient	CI 95%	p
Hyperkeratosis	0.28	0.03 a 0.53	0.03
Diameter (categorized)	0.26	-0.01 a 0.52	0.06
Ulceration	0.25	-0.22 a 0.72	0.3

P (final model) = 0.05; deviance = 0.34.

For the combined assessment of the correlation of clinical characteristics with the KIN, they were submitted to a regression model with adjustment through the "backward-stepwise" process, including all variables in the initial model and, later, leaving only those with p≤0.3 in the final model (Table 6).

We defined a score with the sum of the characteristics of diameter, hyperkeratosis, and exulceration, with the score attributed to each grade (Table 7). The intraclass correlation coefficient (ICC) for the clinical score between the two evaluators was 0.71 (CI 95 %: 0.59 to 0.79).

From the ROC curve developed for the identification of lesions with KIN 3, we obtained a sensitivity of 75%, a specificity of 77%, and an accuracy of 76% for the identification of these lesions when using a cut-off value ≥3 points. The correlation between the clinical score and the KIN was 0.43 (Spearman's Rho p=0.01). The agreement between the evaluators regarding the identification of lesions with a score ≥3 (indicative of KIN 3) was 0.69 (Cohen's Kappa).

DISCUSSION

In the present study, we found, in general, a low correlation between the clinical characteristics and the histological grade of the AKs, with a significant association only with the diameter of the lesions.

Jiyad *et al.* already demonstrated the importance of lesion size in developing SCCs in 2017. The author identified a

Table 7: Clinical actinic keratoses severity scale - AKSS

Characteristic	Description	Points
Diameter	0 a 5mm	0
	6 a 10mm	1
	Higher than 10mm	2
Hyperkeratosis	Absent or slightly rough lesion on palpation (scaling is more palpable than visible)	0
	Evident scaly appearance	1
	Compact keratin adhered to the surface of the lesion	2
Exulceration	Absent	0
	Present	1

fourfold increased risk of kidney transplant recipients developing SCCs in areas of the skin that had at least one AK with an area of 1cm2.[7,8,9]

On the other hand, the KIN assessment proposed by Yantsos *et al.* in 1999 recommends considering the area of greatest atypia as the one that represents the entire lesion. Thus, due to a probabilistic factor, lesions with a larger diameter would be more likely to present higher degrees of KIN.[2]

We found an inverse relationship between lesion size and KIN grade and perilesional p53 expression (p=0.04). The reduction of the p53 protein around the larger diameter lesions suggests the high activity of the adjacent cancerization field due to the lower antitumor effect of this protein. Loss of p53 function in animal studies has led to aneuploidy and accumulation of mutations in tumors.[5,9,10]

Corroborating the findings of Marinescu *et al.* (2016), we did not identify a correlation between the degrees of KIN and the expression of lesional p53 or Ki-67 (p=0.15; p=0.31, respectively). In a 2016 study, Herfordt *et al.* also demonstrated no relationship between the degree of epidermis atypia in AKs and the expression of the p53 protein.[11,12]

There was a significant association between clinically evaluated hyperkeratosis and lesional and perilesional Ki-67 expression (p<0.01 and p=0.03, respectively), indicating high proliferative activity of the hyperkeratotic lesion. Consistently, in a 2013 study, Pimentel *et al.* identified invasive SCCs associated only with AKs histologically classified as common or hypertrophic, suggesting hyperkeratosis as a marker for the invasive evolution of the lesions.[5,13]

Despite the low clinical-pathological correlation, the study allowed the definition of a clinical score of the AKs of the forearms with the categorization of the diameter, the hyperkeratosis in three degrees, and the presence or absence of exulceration moderately correlated with the histological degree. As in the analysis of pre-malignant cervical lesions, clinical prediction of the atypia level of AKs can guide more or less aggressive therapies and, when analyzed together, scale the activity of the field cancerization in the region. ●

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