Original article

Matricectomy of the hallux's ingrowing nail: immunohistochemical study of the removed matrix

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ABSTRACT

Introduction: The lateral extent of the germinal matrix area of the nail is not demonstrated in the literature. **Objective**: To identify the lateral limits of the ungueal matrix. **Methods**: We evaluated the proliferation area of the lateral extent of the nail matrix on surgical samples resulting from the technique of canthotomy for the treatment of ingrowing toenail of the hallux with the Ki-67 immunohistochemical marker. We obtained 21 samples and observed the epidermis, ventral and dorsal region of the matrix, as well as ventral and dorsal matrix of the lateral/medial angle. **Results**: The test of multiple comparisons demonstrated that the number of cells in the ventral matrix is higher than the number found in the skin, in the dorsal matrix, in the angle of the dorsal matrix and in the angle of the ventral matrix. The number of cells in the skin and in the angle of the ventral matrix is higher than the number found in the angle of the dorsal matrix. **Conclusion**: The lateral limit of the ungueal matrix has proliferation activities. The surgery performed without the complete removal of the lateral/medial proliferation region of the nail matrix may influence the recurrence rate of matricectomies.

Keywords: hallux, nail, immunohistocytochemistry, ambulatory surgery procedure.

INTRODUCTION

The ingrowing nail[1-3] or onychocryptosis[1,2,4,5] is a chronic and painful disease, on the edge or on both sides of the nail, usually on the hallux. A spicule on the lateral border of the nail, continuously growing, causes ulceration of the skin on the distal phalanx.[6,7] Infection may start shortly thereafter. The inadequate care of this region, the chronic pressure that causes irritation, as well as the excessive sweating of the feet inside tight shoes stimulate the growth of a granulation tissue and the nail growth inwards the toe skin.[6]

Some factors can contribute to the determination and to the evolution of the disease, such as anatomical causes,[1,2,8-11] variations due to external pressure,[8,9] variations due to internal pressure because of associated diseases and nail diseases,[12-15] drugs like the aromatic retinoids, anatomical congenital malformations of the hallux and others: emotional disorders, geriatric alterations (onychogryphosis, onychauxis, subungual hyperkeratosis), infections and intoxications.[10,11]

It affects men (68.5%), and women (31.5%), at the ratio of 2:1, particularly between their second and third decades (15 to 40 years old, of which 73% between the ages of 12 and 30, exactly at their productive age).[16-18] The embedment of the lateral nail fold occurs in 72% to 84% of the patients, according to Fowler.[4,15,16,19] There is no predominance of the right or left side of the member.[10,11] The ingrowing toenail is twice as frequent among relatives in first and second degrees, thus suggesting a family predisposition, possibly due to inherited nail deformities.[16]

The alterations seen at the lateral nail fold in this condition are divided in three types: I- presence of slight erythema and edema, II- presence of infection and suppuration, and III- chronic presence of granuloma tissue and hypertrophy.[8]

The first two may be treated by means of conservative procedures, while the third one, and sometimes even the second one, requires a surgical treatment, under local or general anaesthesia.[20,21] Surgery should be avoided during the inflammatory and infectious acute

phase, but the granulation tissue on the chronic phase is not a contraindication against the surgical procedure,[20,22] although it is not necessary to evaluate the patients at risk, such as, those suffering from diabetes, vasculopathy caused by collagen disease, atherosclerosis, and other ischemic diseases.[23-25]

The conservative treatment presents a high cure rate (96%) for types I and II although, in the long run, there is a greater recurrence of type II and a failure of 62% at type III. The average cure rate is 50%.[26]

As concerns the surgical treatment, the recurrence rate varies considerably. Several studies held on teaching hospitals attribute this factor to its practice by inexperienced doctors: the avulsion of part of the nail has a very high recurrence rate, ranging from 50% to 83%; therefore, it should not be practiced.

By using phenol, the recurrence rate ranges from 1% to 53%. As concerns the cryotherapy with liquid nitrogen, the recurrence rate varies from 16% to 35%. By the use of plastic tubes and grooving devices, the rate of recurrence is calculated to be 33-48% in one year, and using dental material and clips, 23%.

Most of the recurrences are due to the preservation of the nail matrix with the keratinization under different forms: small focuses of accumulated keratin (removed with keratolytic agents); small painful spicules that, when traumatized, present secretion or require a local treatment; multiple spicules or the growth of a nail separate plate, always having a dystrophic appearance; or the growth of the nail plate to its original size (insufficient removal of the matrix); or even the nail plate repositioning, causing the development of ingrowing nail on the opposite side of the plate. Such spicules must be resected again and the area may be curetted with phenol or even undergo a surgical removal.

The histology of the ungueal matrix and its localization were described by Lewin (1965), Achten (1968), Ackerman (1997), Cameli (1994) and Reardon (1999).[3,15,27,28] The ungueal matrix is divided in dorsal, intermediate and ventral matrix. The proliferative area of the matrix responsible for the nail is formed by the intermediate matrix and the ventral matrix and not by the dorsal matrix. The epidermal ridges have a raising appearance and the epithelium suffers a thickness reduction at the nail bed.[27,28]

The Ki-67 is a mouse monoclonal antibody (IgG class 1) described in 1983 by Gerdes and his collaborators. It can be used for the evaluation of the growth ratio of normal, reactionary and neoplastic tissues, such as the number of cells on a cell cycle; evaluation of the cellular proliferative index on histological incisions; evaluation of the cell kinetics, and of the effectiveness of several topical drugs.[29-31]

The objective of our research was to identify the lateral limits of the ungueal matrix after the excision of the lateral edges of de ingrowing nail.

MATERIAL AND METHODS

Twenty one patients from the Dermatology Clinic of the Hospital das Clínicas at the Medical School of Universidade de São Paulo were included, after the approval of this research project by the Ethical Committee of the institution. The ages varied from 8 to 57 years, and ten were female and eleven male. The patients presented a typical clinical picture of ingrowing toenail, with indication for surgical treatment.

Patients suffering from systemic disease, such as diabetes, immunodepression (caused by drug or infection), peripheral vascular diseases, skin diseases, congenital defects or any other disease that could somehow alter the evaluation results of this research were excluded from the study. The patients in our practice where always treated with matricectomy after all conservative treatments failed or in types II and III.

The surgical specimens excised were studied prospectively considering that they were immediately submitted to histological methods for study, not randomized and open without control specimens concerning the nature of the procedure and ethical considerations. For this study, we have used the matricectomy surgical technique as follows:

1) Asepsis of the foot with povidone-iodine;
2) Regional toenail nerves anesthetized with local injection of 2% lidocaine without epinephrine;
3) Exsanguination and for the tourniquet we used Penrose drain;
4) Excision of a wedge of tissue of the affected lateral fold, from the matrix (1.5 cm to the proximal nail fold), 0.3-0.5 medial or lateral to the lateral fold over the nail plate, incision of the nail plate and bed, being extended until 0.5 cm of the hyponychium, and deep enough to reach the cartilage. The fusiform excision was completed at an angle of 45° on the edges, encompassing the lateral border of the nail plate with the offending spicule, the granuloma and the lateral fold of the affected edge;
5) Primary suture of the fusiform excision by using a 3-0 mononylon suture, keeping the nail border underneath the nail plate;
6) Compressive dressing placed over the toenail;
7) Macroscopic evaluation of the surgical sample and bloody area, thus confirming the removal of the matrix proximal and lateral region.

As concerns the histopathological and the immunohistochemical evaluation:

1) The usually disregarded surgical sample was preserved in an aqueous solution with formol 10%, with monobasic and dibasic sodium phosphate;
2) For the immunohistochemical evaluation, we waited for the 24-hour period after the procedure;[31]

3) As preparation, the surgical samples needed to be severed as follows: the first incision was made at the proximal angle of the matrix (A), the second one was at the proximal fold (B), and the third one at mid distance between the proximal fold and the end of the nail bed (C). When the sample was too big, a fourth incision was made, dividing the nail bed into medium and distal proximal third (D). The nail plate was carefully removed before the incision;
4) The sets of matrix areas were separated (A, B and C) and all the samples were evaluated, the ones prepared with the hematoxylin and eosin staining, as well as the ones prepared with the monoclonal immunohistochemical marker. The marker chosen for this study was the Ki-67 antigen by Dako, Clone Ki-S5, code number M7187 and lot number 059. The indirect method SABC (Streptavidin Biotin Complex conjugated to peroxidase enzyme) was used.

The counting of the proliferative index chosen for the evaluation of the nail matrix in our study was based upon a paper written by De Berker (1996),[25] in which three epithelial sites had been examined: the matrix, the nail bed and the digital pulp. The number of marked cells was counted in a compartment of 100 cells. It consisted of 25 basal cells and other three upper layers of cells, resulting in a set composed of four layers of 25 cells each, in an attempt to recognize the proliferative compartment.

The proliferation index was determined as follows:

Ki-67 Index = number of marked nuclei / 100 cells, in which the index of marked cells is the number of positive cell nuclei divided by the total of 100 cells counted per area.

In the skin, the counting was triplicated; however, in the ventral and dorsal region of the matrix, as well as in the ventral and dorsal of the lateral/medial angle, the extent of the tissue was small, and it was not possible to count similarly. We have managed to double count in the ventral and dorsal region of the matrix, but the count was single in the angle region. Beginning at the angle bisector, the 25 cells corresponding to the area of the ventral matrix and the 25 cells corresponding to the area of the dorsal matrix were counted, followed by the three upper layers of cells, adding up to 100 cells, as proposed in this paper.

Nonparametric statistics were used for analyzing the results, taking into consideration the nature of the variables studied. The variance analysis proposed by Friedman[32] was applied, for the purpose of comparing the five regions subject to study (skin, ventral matrix, dorsal matrix, angle of the ventral matrix, and angle of the dorsal matrix), in relation to the percentage of cells marked with the Ki-67. When a significant differentiation was visible, such analysis was complemented by the test of multiple comparisons.[33]

RESULTS

With regard to the proliferative index, it expressed an average of 3.8 cells per 100 cells in the epidermis. The minimum and maximum amounts are 2 and 8 cells, respectively (Figure 1).

In the ventral matrix region, from 3 to 17 cells were detected (Figure 2), with an average of 7 proliferative cells per 100 cells; and in the dorsal matrix region (Figure 3), the number ranged from 0 to 8 cells, with an average of 3.4 proliferative cells per 100 cells.

In the ventral matrix region of the lateral/medial angle, from 1 to 12 cells were detected (Figure 4), with an average of 4.9/100 cells; and in the dorsal matrix of the lateral/medial angle (Figure 5), the number ranged from 0 to 8 cells, with an average of 2.6/100 cells.

The demonstration of the patients suffering from ingrowing toenail is expressed in Table 1, according to the percentage of the Ki-67 cellular proliferative labeling index in the skin, ventral matrix, dorsal matrix, ventral matrix of the lateral/medial angle, and dorsal matrix of the lateral/medial angle, observed in the postcanthotomy samples.

The variance analysis proposed by Friedman shows that the X^2r calculated = 56.16 ($p < 0.001$) X^2 critical = 9.49.

By the test of multiple comparisons, the number of cells in the ventral matrix was higher than the number found in the skin, in the dorsal matrix, in the angle of the dorsal matrix and in the angle of the ventral matrix; and also the number of cells

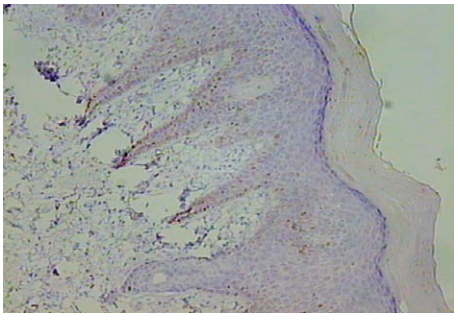

Figure 1 – Visualization of the skin marked with ki-67. 100x.

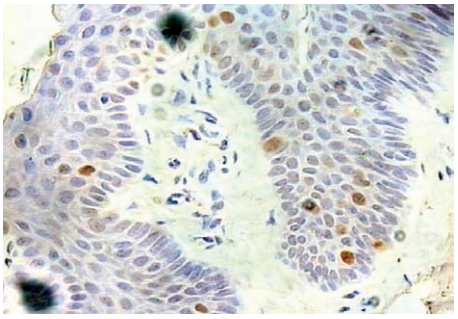

Figure 2 – Ventral matrix region. 400x.

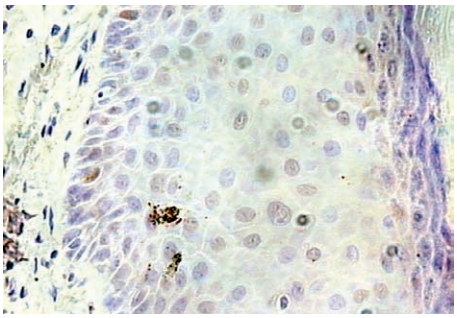

Figure 3 – Dorsal matrix region. 400x

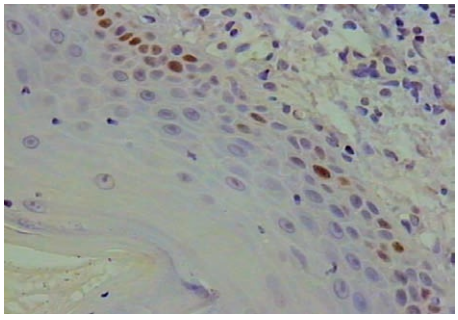

Figure 4 – Ventral matrix region at the angle. 400x

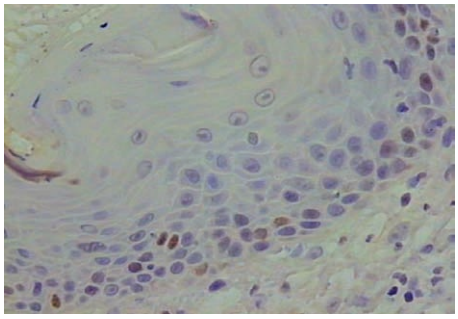

Figure 5 – Dorsal matrix region at the angle. 400x.

in the skin and in the angle of the ventral matrix was higher than the number found in the angle of the dorsal matrix. With regard to the casuistics, no one of the cases recurred.

The ventral region epithelium with an elongated epidermal ridges was thicker, and, in hematoxilin eosin coloration, the superficial area had an eosinophilic aspect due to the presence of the keratin and absence of the granular area. The dorsal region, on the other hand, demonstrated poorly defined or absent crystals and dermal papillae with a thinner epithelium.

DISCUSSION

We have obtained the expected results, that is, a higher number of proliferative cells in the areas corresponding to the production of the nail plate in the ventral matrix region. We have also evidenced that the proliferative cells of this same

Tabele 1 – Representation of Ki-67 marked cells at the skin, ventral and dorsal matrix area and ventral and dorsal matrix at the angle

Patient	Skin	Ventral matrix	Dorsal matrix	Ventral matrix of the angle	Dorsal matrix of the angle
1	2	4	2	2	2
2	6	6	5	6	4
3	6	3	0	2	1
4	4	7	3	7	3
5	7	17	8	12	8
6	4	3	2	3	2
7	3	10	4	6	3
8	3	5	3	5	3
9	3	14	8	12	6
10	3	8	4	8	3
11	4	7	4	2	0
12	3	3	2	3	1
13	3	5	2	2	1
14	3	4	2	2	1
15	4	4	1	1	0
16	2	10	4	4	1
17	3	6	3	4	3
18	4	8	3	5	3
19	4	7	5	6	4
20	4	9	3	6	4
21	4	8	3	5	2
Mean rate	**3,8**	**7,0**	**3,4**	**4,9**	**2,6**

matrix are diffused until the lateral/medial angle of the ungueal embedding. There were no studies describing the lateral aspect of the ungueal matrix until now.

Based on such results, we can explain the reason why some cases of canthotomy evolve, after a few months, showing a cutaneous cornu with characteristics of a newformed nail located laterally to the previous surgical scar. In such circumstances, the professional has probably removed just the lateral nail matrix, not including the region of the angle of the matrix up to its proximal edge. The residual proliferative tissue keeps producing that portion of the nail plate, although now separated by a postoperative scar. We have also observed that the lateral/medial extent of the nail matrix follows the border of the nail plate, in opposition to the schematic drawing presented by Mogensen.[34]

Ackerman[35] says that the lateral/medial matrix is along the ungueal plaque, and Reardon[28] also reports that the surgery excision must be done within a larger area at the proximal matrix region.

A few doubts still remain about the epidemiology and other causal agents. However, we face great difficulties, when researching the best therapeutic method, since the statistic data register lower recurrence rates concerning conservative procedures, such as the chemical cauterization, and higher recurrence rates concerning slightly more aggressive surgeries, such as the matricectomy.

This paper shall bring an additional contribution concerning the surgical therapeutics for ingrowing nail, emphasizing the need to have a thorough understanding of the normal histology of the nail.

CONCLUSION

The clinical study of our cases, the macroscopic and immunohistochemical evaluation of the nail, as well as the estimation of the dorsal and ventral lateral matrix of ingrowing nails have led us to the following conclusions:

The number of cells in the ventral matrix does not significantly differ from the number of cells in the angle of the ventral matrix.

The surgery performed without the complete removal of the lateral/medial proliferative region of the nail matrix may influence the recurrence rate of matricectomies.

The lack of knowledge respecting the normal histology of the nail probably affects the ineffectiveness rate of the surgical procedures recommended for the treatment of ingrowing toenail.

Ki-67 allows a secure determination of the removed matrix area and the efficacy and prognosis of the surgical treatment preventing a greater recurrence index. The lateral limit of the ungueal matrix has proliferation activities, and if the surgery is performed without the complete removal of the lateral/medial proliferative region of the nail matrix it might influence the recurrence rate of matricectomies.

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