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Dermatopathology

Histopathological and immunohistochemical assessment of acquired ichthyosis in patients with human T-cell lymphotropic virus type I-associated myelopathy

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Summary

Background Patients with human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy frequently display cutaneous alterations such as acquired ichthyosis.

Objectives Elucidation of the pattern of acquired ichthyosis in HTLV-I-associated myelopathy.

Methods Skin fragments from 10 patients with HTLV-I-associated myelopathy presenting with acquired ichthyosis were assessed by histopathological and immunohistochemical tests. We used anticytokeratin antibodies related to normal keratinization (K1/K10), and others related to cutaneous conditions such as activation, migration and hyperproliferation of keratinocytes (K6/K16), and involucrin, a precursor protein in the formation of the protein envelope in keratinocytes. For quantification of the proliferating basal and parabasal cells the anti-Ki-67 antibody was employed.

Results On light microscopy, all skin specimens displayed orthokeratotic hyperkeratosis and hypogranulosis. Three of them presented focal parakeratosis. A slight to moderate perivascular infiltrate of mononuclear lymphocytes was observed in seven cases, three of which showed discrete spongiosis with epidermotropism of lymphocytes. All fragments displayed coexpression of K1, K10 and K16 in the suprabasal layers. Expression of involucrin was also observed in all cases, in the upper spinous and granular layers. Focal expression of K6 was observed in three cases, under a parakeratotic area. The mean number of Ki-67+ basal and parabasal cells was 3.5 cells per mm, similar to that in control skin.

Conclusions In acquired ichthyosis related to HTLV-I-associated myelopathy, histopathology revealed orthokeratotic hyperkeratosis and a perivascular inflammatory infiltrate of mononuclear lymphocytes, with areas of parakeratosis and foci of epidermotropism in rare cases. The expression profiles of K1, K10 and involucrin were similar to those in normal skin. The diffuse coexpression of K16 with K1 and K10 throughout the analysed epidermis, as well as the occurrence of restricted areas of parakeratosis expressing K6, indicate the presence of keratinocyte activation with induction of the alternative keratinization pathway, probably dependent on the cytokines liberated by the mononuclear cells of the dermal inflammatory infiltrate infected with HTLV-I. The absence of acanthosis and of increased cellular kinetics, as shown by the low rate of Ki-67 antigen expression, allow the inference that the pattern of acquired ichthyosis related to HTLV-I-associated myelopathy may be retentional. The observation of foci of parakeratosis expressing K6 in three specimens suggests that, at least in certain areas and in some cases, interference with epidermal differentiation and maturation occurs.

Key words: acquired ichthyosis, human T-cell lymphotropic virus type I-associated myelopathy, immunohistochemistry

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Acquired ichthyosis appears during adulthood, being characterized clinically by cutaneous xerosis with the formation of polygonal thin flat squamae of varied sizes and generalized localization, with predominance in the extremities. The main microscopic alteration is hyperkeratosis, which has been reported to occur as a result of the use of certain drugs or in association with metabolic, degenerative, neoplastic and infectious diseases. Its incidence is unknown.

In the first reports, acquired ichthyosis was related to the use of drugs such as hypcholesterolaemic agents, triparanol, nicotinic acid, fenofibrate, cimetidine, clofazimine, allopurinol, hydroxyurea and retinoids. The largest number of cases is represented by an association with Hodgkin’s disease. Association with non-Hodgkin lymphomas and solid tumours is uncommon. Acquired ichthyosis has been considered to be an autoimmunity marker as it appears in lupus erythematosus, dermatomyositis and graft-versus-host disease. Among the metabolic diseases, acquired ichthyosis is found mainly related to hypothyroidism, hyperparathyroidism and insulin-dependent juvenile diabetes. Malnutrition, protein loss due to enteropathies (Crohn’s disease), coeliac disease and nephrotic syndrome are also associated with acquired ichthyosis. Xerosis is frequently encountered in patients undergoing haemodialysis. It is also commonly observed in the skin of patients with infectious diseases such as leprosy and acquired immunodeficiency syndrome (AIDS).

Xerosis and acquired ichthyosis have been described as the main dermatological manifestations associated with human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy, occurring in up to 66.7% of cases, and are more intense in patients with advanced neuropathy. In addition, they have been observed in asymptomatic HTLV-I-seropositive individuals (I.A.S., unpublished data), and have been described in adult T-cell leukaemia/lymphoma.

HTLV-I-associated myelopathy is a neuropathy characterized clinically by pyramidal liberation with exclusive weakness of the lower limbs. It develops insidiously in an asymmetrical way, with the possibility of being accompanied by disturbances such as urinary incontinence or retention, intestinal obstruction, impotence and libido reduction. The sensitivity may be endangered. It is associated with infection by the lymphotropic retrovirus for human T cells. It occurs in the Caribbean, some areas of the U.S.A., Central and South America, India, Africa, and south-east and northern Japan. Its prevalence ranges from 12 to 128 cases per 100,000 inhabitants, depending on the region. It appears in adults, is rare in children, and occurs mainly in women between the ages of 40 and 59 years.

HTLV-I was the first human retrovirus to be isolated and its participation in the pathogenesis of adult T-cell leukaemia/lymphoma and HTLV-I-associated myelopathy is well documented. It has RNA as genetic material and contains the reverse transcriptase enzyme. After integrating to T lymphocytes, the virus transcribes the viral RNA into DNA. In turn, the DNA, after migrating to the nucleus, by enzymatic action randomly integrates with the host’s genome, initiating a replication cycle, or remaining latent. The virus is morphologically classified as a type C retrovirus belonging to the subfamily Oncovirinae.

Studies on acquired ichthyosis have been done in sporadic and heterogeneous cases. HTLV-I-associated myelopathy seems to be the most common disease associated with acquired ichthyosis, allowing studies in homogeneous groups of patients. The primary objective of this study was to assess the pattern of acquired ichthyosis in patients with HTLV-I-associated myelopathy in terms of its behaviour (whether retentive or hyperproliferative, by acceleration of cell kinetics) through histopathological and immunohistochemical evaluations and verification of the expression and distribution of keratins K1, K10, K6, K16, involucrin, and of the index of cellular proliferation through the expression of the Ki-67 antigen in the nuclei of the basal and parabasal keratinocytes.

**Patients and methods**

Dermatological examination of 35 patients with HTLV-I-associated myelopathy revealed mild to intense ichthyosis, predominating in the lower limbs in 15 (43%) (Fig. 1a, b). Acquired ichthyosis skin fragments obtained by biopsy from 10 patients were assessed. All patients received clear and objective information about the objectives of the research and signed a form of consent upon clarification. The cutaneous biopsy was carried out on the outer lateral part of the leg with a 6-mm disposable punch under infiltrative local anaesthesia with xylocaine without vasoconstrictor.

As a control group, normal skin belonging to patients undergoing orthopaedic surgery was used after informed consent. The route of surgical access was coincident with the anatomical region of the biopsy of the patients with HTLV-I-associated myelopathy selected for the study. The control group patients showed only orthopaedic alterations, with no
cutaneous alterations or systemic diseases, and had negative serology for HTLV-I/II and human immunodeficiency virus.

**Histopathological analysis**

After routine processing for paraffin embedding and histology, 4-μm haematoxylin and eosin-stained sections were examined under a light microscope.

**Immunohistochemistry**

Three-micrometre paraffin-embedded sections of the skin biopsies with ichthyosis, and of normal control skin, were submitted to immunohistochemical processing by the biotin–streptavidin technique. The primary antibodies used were: anticytokeratin antibody CKs1 (clone 34βE12, 1 : 20 dilution), anti-CKs10 (clone LH1, 1 : 50 dilution), anti-CKs6 (clone LH6B, 1 : 20 dilution), anti-CKs16 (clone LL025, 1 : 20 dilution), antininvolutrin (clone SY5, 1 : 100 dilution) and anti-Ki-67 (clone MM1, 1 : 100 dilution). All primary antibodies were obtained from Novocastra (Newcastle upon Tyne, U.K.). Skin fragments from patients with psoriasis, known to be positive for K6, K16 and Ki-67, were used as positive controls. Negative controls included parallel sections from adjacent tissue that were processed as above, omitting the primary antibodies.

**Qualitative analysis**

Cytokeratin was considered to be present in the epidermis when the cytoplasm of keratinocytes showed golden-brown staining in a focal or diffuse pattern. Only the presence or absence of staining was considered, and not its intensity.

**Quantitative analysis**

Fragments immunostained for Ki-67 were quantitatively analysed by light microscopy. Ki-67+ basal and parabasal cells (nuclei with dark brown staining) were counted throughout the whole extent of the fragment at ×400 magnification. The length of the fragment along the basement membrane zone was measured in linear μm (with later conversion to mm) by computational analysis.

**Statistical analysis**

The mean, SD, SEM and 95% confidence interval were calculated for the counting of Ki-67+ basal and parabasal cells per linear μm of skin with acquired ichthyosis (cases), and of normal skin (controls). The t-test was used to assess the difference between groups, with the level of significance set at P < 0.05.

**Results**

The histopathological analysis showed orthokeratotic hyperkeratosis and hypogranulosis without any evidence of acanthosis in all 10 cases of acquired ichthyosis. Flattening of rete ridges was present in seven cases (Fig. 2a). Focal areas of para-keratosis without spongiosis were observed in three cases. A slight to moderate perivascular infiltrate of mononuclear lymphocytes was observed in seven cases, three of which showed epidermoprotein of lymphocytes (Fig. 2b). A periannexal infiltrate was seen in two cases, and a perineural infiltrate in one case. Fibrosis and collagen sclerosis were present in three fragments (Fig. 2a).
The K1 and K10 reactivity pattern in acquired ichthyosis skin fragments was similar to that observed in normal control skin, i.e. positivity only in the spinous and granular layers (Fig. 3a). K6 positivity was observed focally in the spinous layer under a parakeratotic area in three acquired ichthyosis cases (Fig. 3b). K16 positivity was observed in the spinous layer of all acquired ichthyosis skin fragments. Five control skin fragments did not show positivity for K6 or K16. Involutin positivity was found in the upper spinous and granular layers of both acquired ichthyosis and control skin.

The mean number of basal and parabasal cells expressing the Ki-67 antigen was 3.5 ± 1.2 per mm of dermoepidermal junction in the acquired ichthyosis skin fragments, and 3.4 ± 1.5 per mm of dermoepidermal junction in the normal skin fragments. There was no difference between the groups (P > 0.8).

Discussion

Acquired ichthyosis has been described in association with the use of certain drugs, neoplasias, and autoimmune and infectious diseases. Among the infectious diseases, multibacillary leprosy and two retrovirus-induced diseases, AIDS and HTLV-I-associated myelopathy, stand out. The literature reports that about 4-5-20% of AIDS patients show xerosis and acquired ichthyosis, with a direct relationship between the aggravation of the disease and the decrease of CD4+ T-cell counts (< 100 cells mm⁻³), and with co-infection with the HTLV-II virus, of common occurrence among illicit drug users.

A high frequency of xerosis and ichthyosis (66-7%) has been reported in HTLV-I-associated myelopathy. This cutaneous alteration has also been observed in asymptomatic HTLV-I and HTLV-II carriers (J.A.S., unpublished data), and in adult T-cell leukaemia/lymphoma.

To our knowledge, there has been no previous report on the histological pattern of acquired ichthyosis.
related to HTLV-I-associated myelopathy. Acquired ichthyosis has been described as a condition histologically similar to ichthyosis vulgaris. Orthokeratotic hyperkeratosis showing a compact or basket-weave pattern, and hypogranulosis sometimes with epidermal atrophy and flattening of the epidermal rete ridges, have been described. In the present study we observed, besides the histological pattern that suggests prolonged retention of the stratum corneum, three cases with foci of parakeratosis without spongiosis, indicating areas of alteration in keratinocyte differentiation and maturation. There are accounts of slight acanthosis, agranulosis and parakeratosis on light microscopy examination of skin with xerosis and acquired ichthyosis in AIDS patients. There have been descriptions of mild perivascular and periadnexal inflammatory infiltrates in the dermis. In this study, seven patients with acquired ichthyosis showed a perivascular inflammatory infiltrate of mononuclear lymphocytes. In two of them, the infiltrate was also noticed around the adnexa, with a perineural arrangement in one case. Three of these patients presented areas of epidermotropism of lymphoid cells. Epidermotropism in non-specific lymphomatous cutaneous lesions, including acquired ichthyosis, has been observed in patients with HTLV-I-associated myelopathy. Other alterations such as fibrosis and collagen sclerosis were found in four cases, and have been sporadically described in acquired ichthyosis.

Normal skin expresses K1 and K10 in the suprabasal layers, and involucrin in the upper spinous and granular layers, denoting adequate formation of the protein envelope. In normal skin, K6 and K16 are expressed only in the hair follicles and in the palmoplantar regions. In the literature, we found no studies of acquired ichthyosis with respect to the expression of K1, K10, K6, K16, involucrin and Ki-67. In the ichem xerotic skin, independent of age, there are accounts of a significant decrease in the production of K1 and K10 and of premature expression of involucrin, however, in the xerosis and ichthyosis of AIDS, no change in involucrin expression was demonstrated. In ichthyosis vulgaris there is a description of normal or slightly diminished expression of involucrin, with deficiency in filaggrin synthesis correlated with the absence of keratohyaline granules.

K6 and K16 are strongly expressed in lesional skin from non-bullous and bullous congenital ichthyosiform erythroderma and congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD syndrome). This denotes an abnormal pathway of keratinization related to mutations in the K1 and K10 genes. Moreover, in hyperproliferative conditions of the epidermis, such as psoriasis, wound healing, and benign and malignant tumours, K6 and K16 are present in the suprabasal layers, and may be expressed separately or in conjunction with K1 and K10. Usually in these processes there is an increase of cell proliferation, as demonstrated by an increase of germinative cells expressing the nuclear antigen Ki-67 (only manifested in DNA synthesis and in cell division). However, there are circumstances in which the expression of K6 and K16 is dissociated from cell proliferation. It has been demonstrated that the blocking of DNA synthesis in vitro by mitomycin C allows the continuous production of K6 and K16. Our cases showed normal expression of K1 and K10, as well as involucrin, manifesting, from this point of view, a process of apparently normal keratinization up to the beginning of corneal envelope formation; similarly, the study of the expression of Ki-67 did not show differences in cell kinetics between acquired ichthyosis cases and normal skin controls. The observation of K16 coexpression with K1 and K10 in the upper spinous layers in the acquired ichthyosis skin fragments of our cases indicates stimulation of the alternative keratinization pathway, possibly resulting from the presence of substances that induce activation and migration of keratinocytes. The presence of focal areas of parakeratosis without acanthosis and spongiosis in three acquired ichthyosis skin fragments, as well as the focal and intense positivity of K6, indicate alteration in epidermal differentiation in these cases besides keratinocyte activation, at least in these areas.

The lack of concurrent K6 and K16 positivity in most of our cases is intriguing, as the pairs of cytokeratins are usually jointly expressed. The literature reports K6 and K16 coexpression in inflammatory processes, and just K6 expression in some neoplastic proliferations. This fact has led to the interpretation that the mechanisms causing K6 and K16 gene activation are different. As three cases in this study displayed areas of intense K6 expression under parakeratosis, besides K16 expression throughout the extent of the epidermis, we suggest that an additional factor stimulating keratinocytes might be focally modifying the process of epidermal differentiation.

In conclusion, the evidence of epidermal flattening, the lack of acanthosis, and the absence of increased cell kinetics, as demonstrated by the low rate of Ki-67 antigen expression in basal cells, allow us to infer that
the pattern of acquired ichthyosis related to HTLV-I-associated myelopathy may be retentive. The foci of parakeratosis encountered in three histological specimens showed that, at least in certain areas of some cases, alteration in keratinocyte differentiation might occur. There is clearly an activation of keratinocytes, probably dependent on the production of cytokines liberated by the HTLV-I-infected lymphocytes at the cutaneous inflammatory infiltrate, as demonstrated by the diffuse p16 expression and the focal K6 expression. This must lead to an interference in keratinocyte differentiation and migration, with the formation of an anomalous cytoskeleton resulting in a defect in the process of normal desquamation, accumulation and retention of cornocytes, culminating in the formation of ichthyotic squames.

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