Acta Dermatovenerol Croat 2005;13(4):206-211 ORIGINAL SCIENTIFIC ARTICLE

# Expression of Keratins 14, 10 and 16 in Marginal Keratoderma of the Palms

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**SUMMARY** Marginal keratoderma of the palms (MKP), also known as degenerative collagenous plaques of the hands, is an infrequent disease affecting individuals with photoaged skin and submitted to mechanical trauma of the hands. The aim of the study was to study the expression of keratin 14 (K14) in the basal layer and keratins 10 (K10) and 16 (K16) in the suprabasal layer, and to establish the effect of growth factors and receptors of the epidermal growth factor in the genesis of hyperkeratosis observed in MKP. The study included 14 patients with MKP. Expression of keratins 10, 14 and 16 was visualized by immunohistochemical staining using monoclonal antibodies by standard immunoperoxidase method. As control three normal skin samples were used. Absence or weak expression of keratins 14 and 10, and strong expression of keratin 16 was recorded in MKP patients. It was concluded that MKP patients present weak expression of keratins 14 (basal membrane) and 10 (suprabasal layer) but strong expression of keratin 16, emphasizing the importance of stimulation of epidermal growth factor receptors by ultraviolet radiation and traumatism.

**KEY WORDS** keratoderma; cytokeratins; photoaging; trauma

## INTRODUCTION

Marginal keratoderma of the palms (MKP) was first identified in 1949 by Cozzolino (1) and individualized as a disease by Ramos-e-Silva (2) in 1957. Burks *et al.* (3) described a disease entirely similar to MKP, using the denomination "degenerative collagenous plaques of the hands". Kocsard (4), in Australia, studied 15 farm workers, with keratoderma of the same kind as the foregoing, calling it keratoelastoidosis marginalis of the hands. Recently, Jordaan and Rossouw (5) described a digital papulous calcified dermatosis (DPCD), keratoderma that overlaps to MKP, and called attention to the importance of the "basophilic elastotic masses" as being characteristic of DPCD. By comparing all of the above diseases described, identical clinical and histopathological pictures are observed, solely with emphasis on a certain peculiarity on behalf of each investigator that would make them appear distinct amongst themselves. The subject to be discussed is the pathogenesis of MKP, accepted by most of the authors as a probable result of ultraviolet radiation and local

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traumatism. Several works show that MKP installs in an area with solar elastosis, being, in fact, considered a component of the photo-aging picture. Its intriguing location, at the sides of the hand, may be due to both genetic pattern and mutation. Reviewing the literature, there is no data about immunohistochemical study of the expression of keratins in MKP. The authors believe that the analysis of the expression of keratins 14, 10 and 16 might create conditions for speculation on the pathogenesis of MKP.

In normal skin, keratins 5 and 14 are expressed by cells of the basal layer disappearing gradually with the cells of the Malpighi layer, near the granular layer, expressing keratins 1 and 10. Near the horny layer, keratin 2e prevails. In hyperproliferative states, the pair of keratins 6 and 16 is expressed, with a decrease or absence of keratins 5 and 14, and 1 and 10 (6).

## PATIENTS AND METHODS

Fourteen patients with MKP were studied (Table 1). The material for immunohistochemical examination was collected from the radial border of the left indicator with a punch # 6. As control, skin biopsies from three individuals without skin changes were used.

The skin fragments collected were immediately immersed in a fixing solution containing formaldehyde in a PBS tampon, pH 7.2-7.4, in a volume at least 20 times greater than the fixed material. Afterwards, duly tagged, they were referred to the laboratory for histologic processing, where they remained in the fixing solution at 4° C for a period ranging from three to five days, whereafter they were washed in running water for 10 minutes, processed, paraffin embedded and cut.

To improve adherence of paraffin sections to the slides in immunohistochemical studies (7), the slides were previously rinsed in a salt solution and, after drying, the paraffin sections were placed in an oven at 40° C for 24 hours.

The sections of the skin fragments of the patients affected by MKP were deparaffinizated and hydrated. Heat induced epitope retrieval was done (8), using a citrate buffer (pH 6.0) (9) Slides were incubated in 0.3% of $H_20_2$ in methanol for 30 minutes, in order to block endogenous peroxidase, washed in PBS and incubated at room temperature for 60 minutes with primary antibodies. Antibodies used were: primary monoclonal antibody against keratin 10 (LHP1 clone, Novocastra Laboratories Ltd., UK), keratin 14 (LL002 clone, Novocastra) and keratin 16 (LL025 clone, Novocastra). After this slides were treated as follows with: secondary universal biotinylated antibody (Novocastra) for 10 minutes, streptavidin-peroxidase complex (Novocastra) for five minutes, peroxidase (Novocastra), containing DAB (diaminobenzidine tetra-hydrochloride) for seven minutes. As negative controls were used same slides by omitting primary antibodies. The slides were then slightly stained with Mayer's hematoxylin and mounted using Dabco mixture (diazabicyclooctane). Slides were analized using a Nikon Optiphot microscope, thus obtaining images that were recorded and reproduced by a PhotoScan image manager, by Spectrolab with a Hyper HAD model Sony camera and IBM Lexmark Z-42 printer.

**Table 1.** Patients with marginal keratoderma of the palms

| Patient No. | Sex (M/F) | Age (yrs) | Phototype | Occupation |
|---|---|---|---|---|
| 1 | M | 50 | III | Waiter |
| 2 | M | 63 | III | Agricultural worker |
| 3 | M | 78 | III | Stevedore |
| 4 | F | 70 | IV | Seamstress |
| 5 | M | 72 | III | Agricultural worker |
| 6 | F | 63 | III | Agricultural worker |
| 7 | F | 68 | IV | Maid |
| 8 | M | 72 | III | Mason |
| 9 | M | 61 | IV | Agricultural worker |
| 10 | F | 59 | III | Maid |
| 11 | F | 53 | III | Seamstress |
| 12 | M | 65 | IV | Driver |
| 13 | M | 65 | III | Trader |
| 14 | M | 58 | IV | Agricultural worker |

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## RESULTS

Histologicaly strong expression of K 14 and K 10 was observed (Figures 1a, b) in the basal and prickle-cell layers, while K 16 was negative (Figure 1c, Table 2). On the other hand, in MKP patients (Figure 2a) there was no expression of keratin 14 in the basal layer (Figure 2b), or of keratin 10 in the prickle-cell layer (Figure 2c).

In MKP patients, strong keratin 16 expression was recorded throughout the basal and prickle-cell layers (Figure 2d), and keratin 14 expression in sweat glands and intraepidermal ducts (Table 3).

## DISCUSSION

Literature review revealed no studies on the expression of keratins in MKP patients. It is believed that solar elastosis and trauma to the hands are the main causes of the disease; however, there is no consensus on the physiopathologic explanation for the occurrence of hyperkeratosis in MKP. Possibly, genetic factors or even mutations of the keratins, especially keratin 9, peculiar of the thick skin of the palms and soles, favor the onset of the disease exclusively at the sides of the hands. Ultraviolet radiation and mechanical trauma produce microscopic effects similar to those of burns and compression, leading consequently to the liberation of factors that stimulate epidermal regeneration. In cutaneous repair, families of epidermal growth factors (EGF) and epidermal growth factor receptors (EGFr) are activated by both epidermal and mesenchymal stimuli (10).

In fetus and neonatal human epidermis, EGFr are located along all layers of the nucleated cells of the epidermis, which also occurs in cutaneous diseases with fast epidermal proliferation (like psoriasis and traumatized skin) and in hyperproliferative interfollicular epidermis of the normal adult skin, although their main location is in the basal layer of the adult skin. The role of these EGFr is difficult to determine because several variables interact on its expression, such as age, anatomic location, stage of proliferation, degree of differentiation, pre-existent cutaneous and systemic abnormalities, temporary post-trauma intervention and type of trauma, besides other undefined genetic and environmental factors (10). Furthermore, EGFr are found in the cells of the germinative layer of the hair follicles, sebaceous glands and sweat glands, these supposedly being a strategic structure because the keratinocytes located in depth have an important function in tissue repair after serious traumas such as burns or loss of tissue (10). *In vitro* studies have suggested that substances related to EGF may regulate the expression of keratin 8, influencing in this way the phenotype of keratinocyte (11).

One cannot, however, simplify the EGFr performance model, considering its operation purely by epidermal stimulation, but should suppose the presence of a mesenchymal-epidermal interaction. The levels of EGFr in the epithelial cells are low, however, there is evidence for the involvement of the cascade of cytokines in the

**Table 2.** Control subjects

| Patient No. | Sex (M/F) | Age (yrs) | Phototype | Occupation | Location | K14 | K10 | K16 |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 42 | III | Maid | Palm | ++++ | ++++ | + |
| | | | | | Buttocks | ++++ | ++++ | + |
| 2 | F | 28 | III | Maid | Thenar region | ++++ | ++++ | + |
| 3 | F | 50 | III | Maid | Indicator (outer border) | ++++ | ++++ | + |

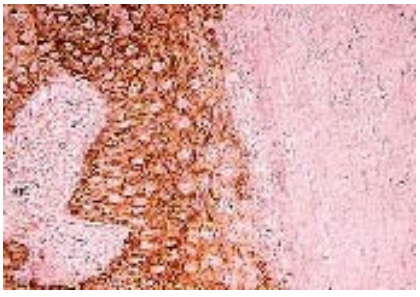

**Figure 1a**

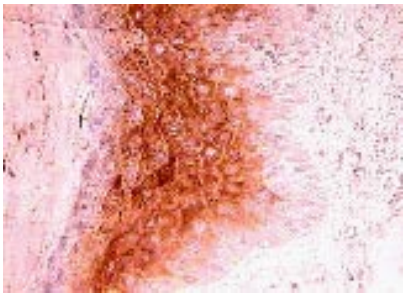

**Figure 1b**

**Figure 1c**

**Figure 1.** (a) Normal individual #2, normal thick skin, antibody LL002 (K14) (40X); (b) normal individual #2, normal thick skin, antibody LHP1 (K10) (40X); (c) normal individual #2, normal thick skin,

**Table 3.** Keratin 14, 10 and 16 expression in patients with marginal keratoderma of the palms

| Patient No. | Sex (M/F) | Age (yrs) | Phototype | Occupation | K14 | K10 | K16 |
|---|---|---|---|---|---|---|---|
| 1 | M | 50 | III | Waiter | ++ | + | ++++ |
| 2 | M | 63 | III | Agricultural worker | ++ | + | ++++ |
| 3 | M | 78 | III | Stevedore | ++ | + | ++++ |
| 4 | F | 70 | IV | Seamstress | ++ | + | ++++ |
| 5 | M | 72 | III | Agricultural worker | ++ | + | ++++ |
| 6 | F | 63 | III | Agricultural worker | + | ++ | ++++ |
| 7 | F | 68 | IV | Maid | ++ | + | ++++ |
| 8 | M | 72 | III | Mason | ++ | + | ++++ |
| 9 | M | 61 | IV | Agricultural worker | ++ | + | ++++ |
| 10 | F | 59 | III | Maid | ++ | + | ++++ |
| 11 | F | 53 | III | Seamstress | ++ | + | ++++ |
| 12 | M | 65 | IV | Driver | ++ | + | ++++ |
| 13 | M | 65 | III | Trader | ++ | + | ++++ |
| 14 | M | 58 | IV | Agricultural worker | ++ | + | ++++ |

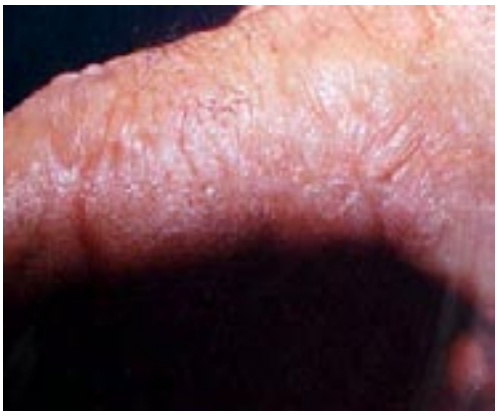

**Figure 2a**

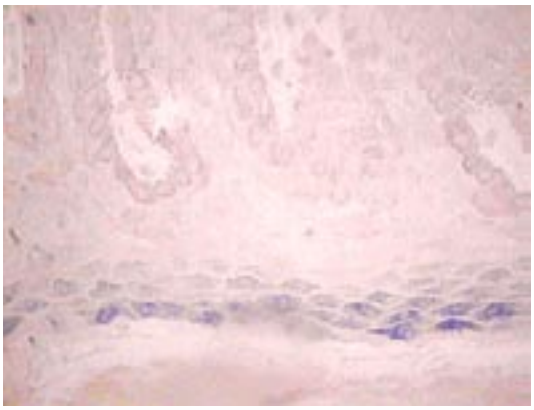

**Figure 2b**

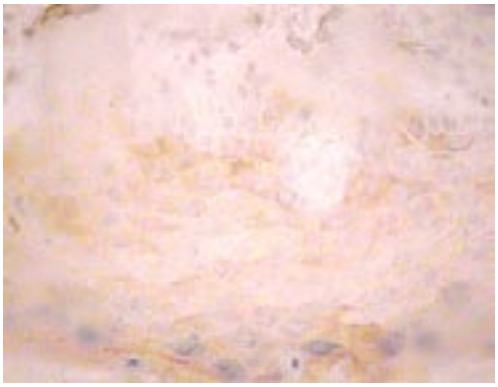

**Figure 2c**

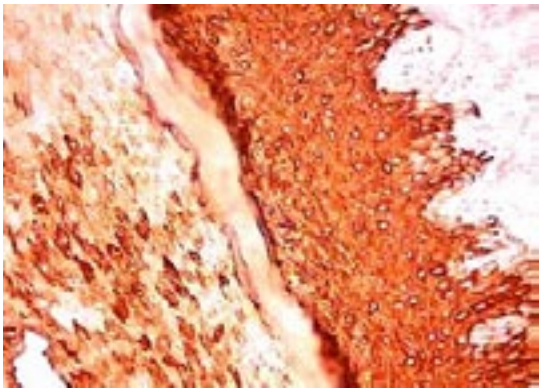

**Figure 2d**

**Figure 2.** (a) Patient #1 with marginal keratoderma of the palms; (b) patient #1, weakly expressing antibody LL002 (K14) (40X); (c) patient #1, weakly expressing antibody LHP1 (K10) (40X); (d) patient #1, strongly expressing antibody LL025 (K16) (40X).

interactions that occur between the epithelial and mesenchymal cells. EGF acts in the fibroblasts that synthesize a great variety of matrix proteins, ranging from glycosaminoglycans to collagenases and stromelysin (10). Furthermore, keratinocytes also respond to EGF, increasing their expression as degrading proteins of the matrix, such as gelatinases with a molecular weight of 92 kDa (12). The initial amounts of EGF and alpha transforming growth factor (TGF-$\alpha$), liberated in damaged

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areas, as well as of other cytokines (beta transforming growth factor, TGF-β) and of plaque derived growth factor (PDGF) are, without doubt, originating from platelet degranulation (10). Additionally, the repair tissue cells are temporarily induced by three other molecules similar to EGF, which are EGF molecules linked to heparin, as well as the sequences similar to EGF present in tenascin-C and laminin, located in the dermoepidermal junction. Recently, a new member of the family of the basic fibroblast growth factor (bFGF), known as keratinocyte growth factor (KGF), showed great relevance in tissue repair, in that it acts in a paracrine fashion, stimulating *in vitro* the keratinocytes neighboring the damaged area, both present in the epidermis as in the hair follicles, through the connection to the specific KGF receptor located in the keratinocyte (13). KGF also stimulates keratinocyte proliferation through the activation of TGF-α (11).

Macrophage, in turn, has capital importance in the repair of cutaneous lesion, being attracted by several chemotactic factors, among them TGF-β. Additionally, it operates as a true cytokine factory, among them molecules similar to EGF linked to heparin, EGF and TGF-α. It also takes part in the remodeling phase of the damaged area, increasing the expression of structural matrix proteins, such as stromelysin and collagenases (10). EGF and TGF-α, in turn, stimulate angiogenesis.

In the human keratinocyte culture *in vitro* and in the human skin *in vivo*, ultraviolet radiation of small wavelength, acting on EGFr promotes its expression, together with cytokine receptors, by the activation of c-Jun-kinase (14). Additionally, it causes a change to occur in its post-translation conformation or modification, directly or through other signaling proteins (15). On the other hand, ultraviolet radiation can trigger the mechanisms of survival of epidermal cells by the activation of PI 3-kinase/AKT *via* EGFr, although its performance in the induction of cell apoptosis is well known (14). The same may also happen through the activation of JNK (protein kinase with c-Jun aminoterminal) and ERK by EGF that operates as an apoptosis inhibitor, during the activation of cell death by ultraviolet B radiation, through p38 (16).

Ultraviolet B radiation in doses under 10 mJ/cm$^2$ stimulates protein kinase C (PKC or EGFr) increasing mRNA of keratin 5 and 14 expression, and of human SV40 transformed keratinocytes, without affecting keratins 1 and 10. However, when the ultraviolet B dose exceeds 10 mJ/cm$^2$, the effect becomes cytotoxic (17), although Smith and Rees (18) observed an increase in the expression of keratins 1 and 10 after ultraviolet B irradiation.

Analyzing the results obtained, low keratin 14 and 10 expression was verified in the marginal keratoderma of the palms, while keratin 16 expression was intense in all study cases. These results apparently contradict the work of Kinouchi *et al.* (17), already commented, according to which it was expected that, with small doses of ultraviolet B, an increase in the expression of keratin 14 and maybe of keratin 10 would occur, since marginal keratoderma of the palms develops on photoaged skin. However, this was not confirmed in the present work, possibly suggesting that the hyperkeratosis trigger mechanism in marginal keratoderma of the palms is actually linked to the accumulation of ultraviolet radiation because, as pointed out by Lavker *et al.* (19), this would lead to reactive hyperkeratosis. The explanation of that hyperkeratosis can be found in the work of Kinouchi *et al.* (17), suggesting that high doses of ultraviolet B radiation cause an inflammatory reaction through cytotoxic effect. Such a post-radiation inflammatory process, amplified by the inflammation caused by the trauma itself (10), can activate a cascade of reactions that lead to EGF overproduction together with AP-1, in order to promote an increase in keratin 16 expression, with the consequential hyperproliferative response of the epidermis (20), however, without observing keratin 14 and 10 expression.

It is possible that the mechanism of hyperkeratosis formation in marginal keratoderma of the palms is due to mechanical factors (trauma and extension and contraction of the fingers, and movement of the fists), in an area modified by solar elastosis. One might also raise a hypothesis that fluctuations or mutations of the gene responsible for the expression of keratin 9 propitiate hyperkeratosis, as a consequence of poor adaptation of the elastotic skin of the marginal area of the hands. When considered from the histologic point of view, due to the hyperkeratotic band observed on microscopic examination, MKP reminds of actinic porokeratosis, which could be an issue for future research.

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