Journal of Photochemistry & Photobiology, B: Biology 162 (2016) 72–76

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

# Dermoscopy and methyl aminolevulinate: A study for detection and evaluation of field cancerization☆

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ARTICLE INFO

*Article history:*
Received 28 January 2016
Received in revised form 14 June 2016
Accepted 15 June 2016
Available online 16 June 2016

*Keywords:*
Dermoscopy
Actinic keratosis
Field cancerization
Methyl aminolevulinate
Fluorescence
Score

ABSTRACT

Actinic keratosis (AK) is a keratinocyte intraepidermal neoplasia UV light–induced that frequently appears in sun-exposed areas of the skin. Although historically AK was defined as "precancerous", actually it is considered as the earliest stage of squamous cell carcinoma (SCC) in situ. Since AKs can progress into invasive SCC, their treatment is recommended. AKs rarely develop as a single lesion; usually multiple lesions commonly affect an entire area of chronically actinic damaged skin. This has led to the concept of "field cancerization", an area chronically sun-exposed that surrounds peripherally visible lesions, in which are individualized subclinical alterations. One of the main principles endpoint in the management of AKs is the evaluation and the treatment of field cancerization. In this view, in order to detect and quantify field cancerization, we employed a method based on the topical application of methyl aminolevulinate (MAL) and the detection of the fluorescence emitted by its metabolite Protoporphyrin IX (PpIX); then, considering the extension and the intensity of measured fluorescence, we create a score of field cancerization. The results show that patients underwent to daylight PDT had a reduction of total score, from T0 to T2. Whereas in the group untreated we observed a stability of total score or a slightly worse. So, the method and the score used allows to evaluate with a good approximation the dimension of field cancerization and show the modification of it after treatment.



## 1. Introduction

Actinic keratosis (AK) is a common UV light–induced cutaneous lesion that results from the proliferation of atypical epidermal keratinocytes. The incidence of development of AK in Caucasian population increases with age, proximity to the equator and outdoor occupation. Australia has the highest skin cancer rate in the world. AKs are discovered in up to 40–50% of the Australian population older than 40 years. Although historically AK was defined as "precancerous" or "premalignant", in agreement with recent histopathologic and molecular studies it is considered as the earliest stage of squamous cell carcinoma (SCC) in situ [1–4]. Transformation of AK into invasive SCC happens following three progressive stages of keratinocyte intraepidermal neoplasia (KIN); in particular, KIN I and KIN II are characterized by proliferation of atypical keratinocytes in the epidermis lower third and in the lower two-third respectively, while KIN III is characterized by the presence of atypical keratinocytes in all layers of the epidermis. So, all AK lesions, regardless of intraepidermal neoplasia thickness, are potentially invasive [1] In according with the literature, the risk of progression of a single AK to an invasive SCC ranges from 0.25% to 20% per year and up to 60% of invasive squamous cell carcinoma arose from AKs [5–6]. The main risk factors for the development of AKs include: fair skin (Fitzpatrick type 1 or 2), old age, immunosuppressive therapy, PUVA-therapy and arsenic exposure. The most important cause of AK formation is UV-B radiation (wavelength 315–280 nm) and UV-A radiation (400–315 nm) from sun-light, in fact AKs are typically seen on fair-skinned people in chronic sun-exposed areas and their frequency correlates with cumulative UV exposure. The main mechanisms of AKs formation are genetic mutations which cause proliferation of altered keratinocytes, oxidative stress, immunosuppression, and altered apoptosis due to dysregulation of p53 pathways [5]. Clinically AKs appears at begin like small, rough spots that are easier felt than felt; afterwards the lesions enlarge, usually becoming red and scaly, often covered by yellow or brown adherent scales. Most lesions are only 3–10 mm, but

☆ Conflict of interest disclosure: None declared.

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http://dx.doi.org/10.1016/j.jphotobiol.2016.06.028
1011-1344/

they may enlarge to several centimeters in size; except in their hypertrophic form, the lesions show little or no infiltration. The surrounding areas may show evidence of widespread chronic sun damage with telangiectasia and yellowish discoloration [7]. Histologically AKs is characterized by dysplasia and architectural disorder of the epidermis, such as: abnormal keratinocytes of the basal layer with nuclear atypia, altered cellular polarity, hyperkeratosis and parakeratosis of the epidermis; an irregular acanthosis may be present [5]. Since AKs can progress into invasive SCC, their treatment is recommended. Approaches to AK can be broadly divided into lesion-directed (cryotherapy, laser therapy, surgery and curettage), or field-directed (diclofenac 3%, imiquimod 5%, 5-fluorouracil, ingenol mebutate, chemical peels and ALA/MAL + PDT) [8]. Lesion-directed therapies are reserved for patients who have only a few isolated lesions and no elevated risk for development of invasive SCC. Field-directed therapies target both clinically visible lesions and preclinical alterations in the normal appearing skin surrounding the lesions; because AK is a visible marker of more extensive damage caused by chronic UV radiation exposure, the REAKT Working Group recommends field directed therapy as the optimal treatment approach for most patients [9]. The apparently normal skin which surround lesions is exposed to the same insults and could already reveal carcinoma-associated genetic alterations; this area is known as the 'field cancerization'. The concept of "field cancerization" was introduced by Slaughter in 1953 [10]. In recent molecular findings, it was established the following definition of field cancerization: "the presence of one or more areas consisting of epithelial cells that have genetic alterations. A field lesion (or shortly 'field') has a monoclonal origin, and does not show invasive growth and metastatic behavior, the hallmark criteria of cancer." A field lesion may have histological aberrations characteristic for dysplasia [11,12]. The field cancerization is an area chronically sun-exposed that surrounds peripherally visible lesions, in which are identified subclinical alterations detectable through different methods: histologically, molecular biology, confocal microscopy and through fluorescence [12,13]. One of the main principles endpoint in the management of AKs is the evaluation and the treatment of field cancerization. As the field cancerization is, for its definition, a subclinical lesion, it is not possible to recognize specific dermoscopy alterations, except those produced by chronic sun damage (telangiectasia, epidermis atrophy and yellowish discoloration, honeycomb pattern) [14]. Histologic features related to field cancerization are epidermal atrophy, increased pigmentation in basal keratinocytes, variable grade of atypia of basal keratinocytes, multiple vascular ectasias in the superior dermis, loss of normal keratinocyte polarization and intense degeneration of the dermic collagen with solar elastosis [15]. The biopsy for the evaluation of the field is invasive and not very useful, because it may not identify with precision the field of cancerization. Reflectance confocal microscopy (RCM) is a noninvasive imaging technique that allows the visualization of cellular and subcellular structures of the skin in vivo with near histological resolution. It was postulated that initial changes of epidermal morphology and cellular atypia may be observed by RCM before becoming clinically apparent. Therefore, RCM may be useful for the evaluation of actinic field cancerization and the detection of subclinical AK. The main features observed at RCM are: at the level of the stratum spinosum, discrete cellular and nuclear atypia of the keratinocytes, resulting in focal disruption of the epidermal architecture; at the level of the dermis, bright irregular bundles were observed, suggestive of solar elastosis. Furthermore, small, branched, bright structures morphologically corresponding to dilated blood vessels were seen [16]. Photodynamic therapy (PDT) with topical application of 5-aminolevulinic acid (ALA) or its methyl ester [methyl aminolevulinate (MAL)] as photosensitizers has proven to be clinically effective in AKs, Bowen's disease and superficial basal cell carcinoma (BCC) [17]. ALA or MAL is metabolized in Protoporphyrin IX (PpIX). All the cells own enzymes able to transform ALA to PpIX, although the altered cells with increased rate of replication and metabolism synthesize an excess of PpIX [18]. Preferential accumulation of PpIX in altered skin is used to treat various skin diseases. As PpIX has

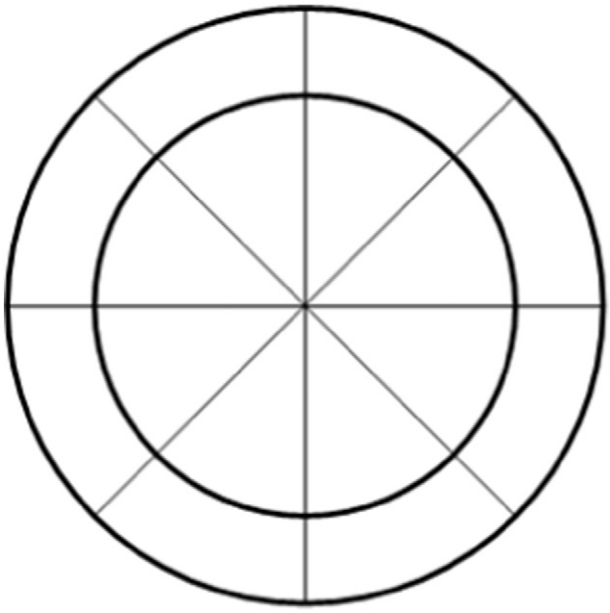

**Fig. 1.** Probe circular area.

fluorescence properties, its preferential accumulation in altered cells can be used as a marker of neoplastic keratinocytes. PpIX when excited by blue light (408 nm) shows red fluorescence. So, the field of cancerization could be identified by the detection of fluorescence emitted by PpIX accumulated in the keratinocytes [19] and the degree of fluorescence depends on the differential uptake of the photosensitizer by neoplastic cells [20].

## 2. Materials and Methods

In this paper we provide a method based on the topical application of MAL on AKs and perilesional areas and the successive detection of the fluorescence emitted by its metabolite (PpIX), in order to evaluate the score of field cancerization, considering the extension and the intensity of the recorded fluorescence. The method was applied to 20 patients (10 male and 10 female), affected by AKs on sun-exposed skin, aged between 58 and 80 years old with photo-type 2 and 3. The exclusion criteria were: perilesional scarring and hypopigmentation, previous treatment on AKs and immunosuppression. Moreover, we considered as control group 10 patients not affected by clinically evident AKs, applying MAL on not sun-exposed skin. We provide an example of application and validity of this method for identifying the field cancerization surrounding the lesions through the fluorescence emitted by PpIX, a metabolite of MAL, which is a topical photosensitizer applied at the level of altered skin with a cream formulation containing 16% MAL (Metvix®). The choice of MAL is due to greater selectivity, reduced time of occlusion and for the issuance of a more intense fluorescence for greater formation of PpIX than ALA. In the first visit (T0), patients signed the informed consent and have been subjected to clinical evaluation in

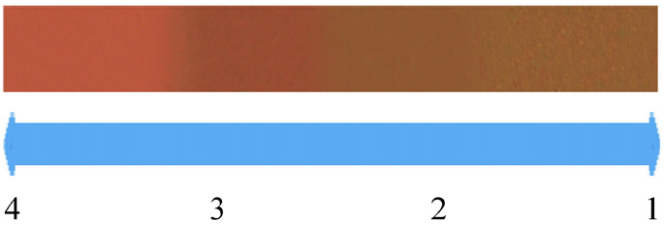


**Fig. 2.** Fluorescence scale.