Analysis of photodynamic therapy influence on expression of inflammation and proliferation markers in actinic keratoses

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Introduction

Actinic keratosis (AK) is a benign intra-epidermal growth that is a pre-malignant neoplasm. It presents as an erythema with irregular and rough surface, sometimes covered with callous epidermis, gray-brown, pink or normal colored skin. Usually it appears in areas that are exposed to ultraviolet (UV) light, such as the skin. It occurs most frequently in elderly patients (80% of patients are in the age of 60-69), especially with fair skin phototype (Fitzpatrick phototype I and II), who have been chronically exposed to UV radiation. The literature reports that the risk of neoplastic transformation from AK to squamous cell carcinoma (SCC) is approximately 16%. It is also not uncommon to transform AK into Basal cell carcinoma (BCC). The more AK lesions on the skin, the greater risk of malignant lesions. Since AK is a pre-malignant noeplasm - cancer in situ, it is crucial to start treatment early enough to prevent cancer progression.

Photodynamic therapy (PDT) is a topical treatment based on a phototoxic reaction that occurs as a result of the reaction of a photosensitizing substance, light and oxygen.

Objectives

The primary objective:
Analysis of photodynamic therapy influence on expression of inflammation and proliferation markers in adult patients with clinical, dermoscopic and histopathological diagnosis of actinic keratoses.

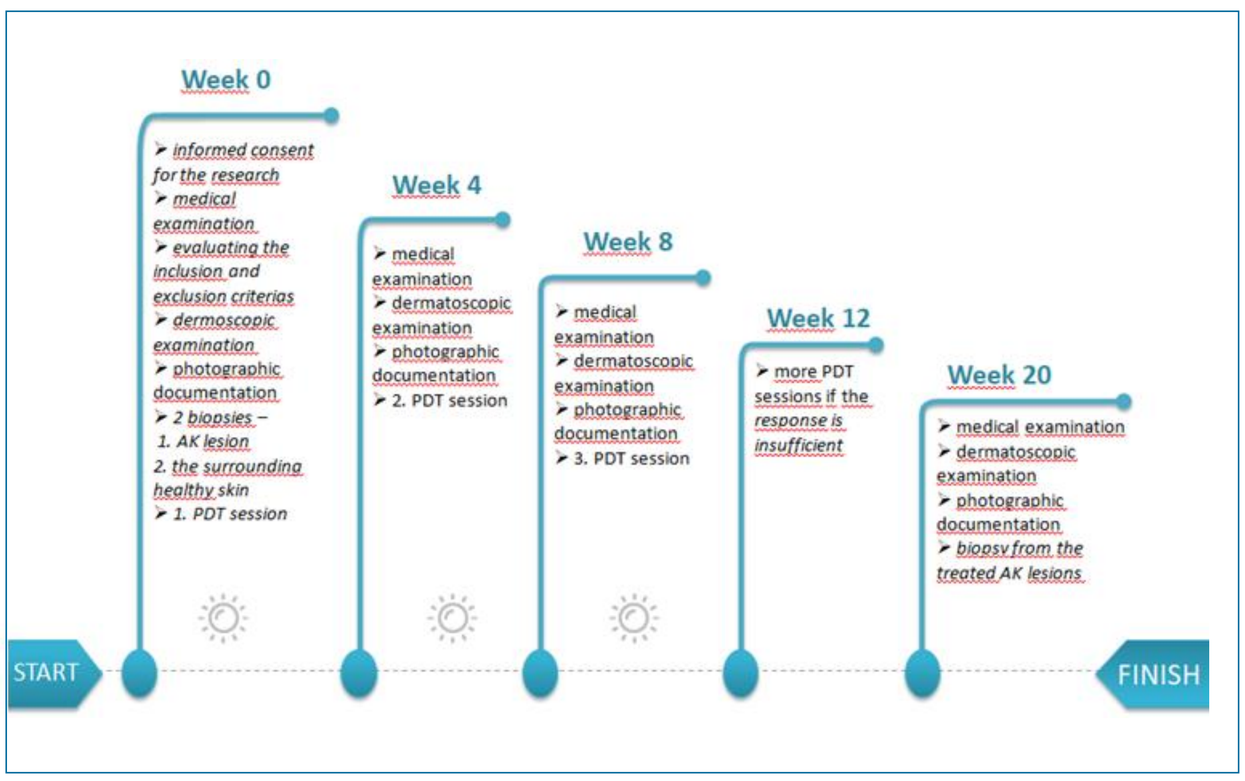
Specific objectives of the study:

- Clinical remission of the actinic keratosis and inhibition of the carcinogenesis to squamous cell carcinoma (SCC).
- The statistically significant ($p<0.05$) reduction in the markers COX-2, TP53 and Ki67 in the field cancerization.
- Analysis of the correlation between clinical regression of AK and expression of the markers COX-2, TP53, Ki67 in the skin lesions treated with photodynamic therapy.

Hypothesis

Hypothesis:
Photodynamic therapy contributes to regression of the AK lesions, what correlates with the reduction of the markers COX-2, TP53 and Ki67 in AK lesions treated with PDT.

Investigation plan



Materials and Method

Material: In all patients included in the study, skin sections were taken from lesions with AK morphology, before and after therapy. Skin sections were also taken from the area of the lesions, from skin that looked healthy/diseased.

Study group
31 patients (24 men, 7 women)
42 - 92 years (mean age 72.4 +/- 8.64).

Location of the AK lesions	
head/scalp	17 patients (54.84%)
Face	9 patients (29.03%)
Trunk	2 patients (6.45%)
forearm	3 patients (9.68%)

Severity of the AK lesions before the treatment (according to Zalaudek et al. dermatoscopic severity scale)	
I°	4 patients (12.90%)
I/II°	3 patients (9.68%)
II°	7 patients (25.81%)
II/III°	11 patients (35.48%)
III°	6 patients (19.35%)

Histopathological types of AK before treatment	
proliferative	8 patients (25.81%)
proliferative with features of dysplasia	11 patients (35.48%)
atrophic	3 patients (9.68%)
atrophic with features of dysplasia	4 patients (12.90%)
with no specific type with features of dysplasia	5 patients (16.13%)

Results

The severity of AK lesions after the treatment ((according to Zalaudek et al. dermatoscopic severity scale)	
complete clinical reduction	8 patients (25.81%)
I°	16 patients (51.61%)
II°	7 patients (22.58%)

The severity of the AK lesions after the treatment was reduced in every patient (100%)

Ki67 expression in epidermis	
1. <5% immunopositive cells	0 patients (0%)
6-15% immunopositive cells	3 patients (9.68%)
16-30% immunopositive cells	13 patients (41.94%)
31 - 0% immunopositive cells	3 patients (9.68%)
>50% immunopositive cells	12 patients (38.71%)

Ki67 expression in the epidermis was reduced in 20 patients (64.52%); Ki67 expression increased in 2 patients (6.45%)

TP53 expression in the epidermis	
<5% immunopositive cells	8 patients (25.81%)
6-15% immunopositive cells	1 patients (3.23%)
16-30% immunopositive cells	7 patients (22.58%)
31-50% immunopositive cells	1 patients (3.23%)
>50% immunopositive cells	14 patients (45.16%)

TP53 expression in the epidermis was reduced in 19 patients (61.29%), TP53 expression increased in 1 patient (3.23%)

COX-2 expression in the epidermis	
weak immunoexpression	1 patient (3.23%)
medium immunoexpression	12 patients (38.71%)
high immunoexpression	18 patients (58.06%)

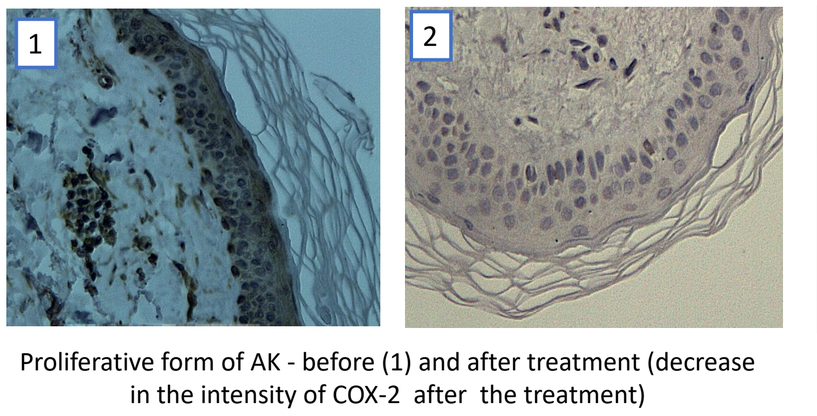
The intensity of COX2 immunoexpression in the epidermis among treated AK lesions was reduced in 17 patients (54.84%)
It increased in 9 patients (29.03%)

The range of Ki67 immunopositive in the epidermis	
<1/3 of the lower epidermal /lesion level	2 patients (6.45%)
1/3 - 2/3 of the epidermall/lesion level	25 patients (80.65%)
2/3 - 1, or the entire epidermis	4 patients (12.90%)

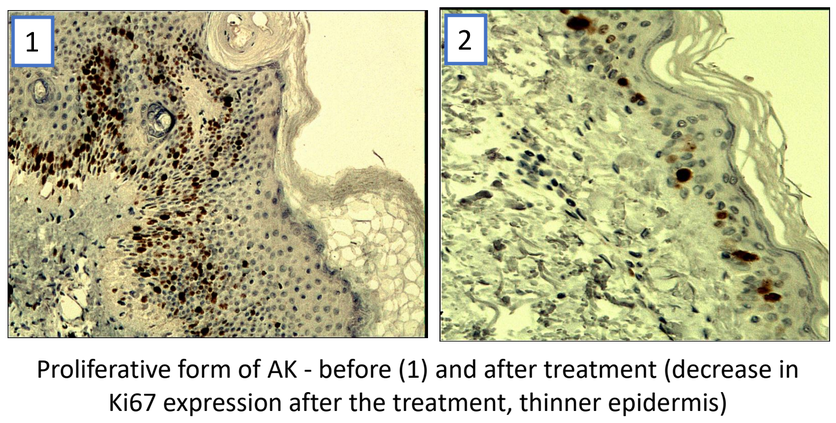
The extent of Ki67 immunocompetent cells in AK lesions was reduced in 20 patients (64.53%), what correlated with a reduction in Ki67 expression in AK treated lesions but the extent of these cells increased in 3 patients (9.68%).

The range of TP53 immunopositive cells in the epidermis	
no immunocompetent cells	7 patients (22.58%)
<1/3 of the lower epidermal/lesion level	6 patients (19.35%)
1/3 - 2/3 of the epidermis/lesion level	15 patients (48.39%)
2/3 - 1, or the entire epidermis	3 patients (9.68%)

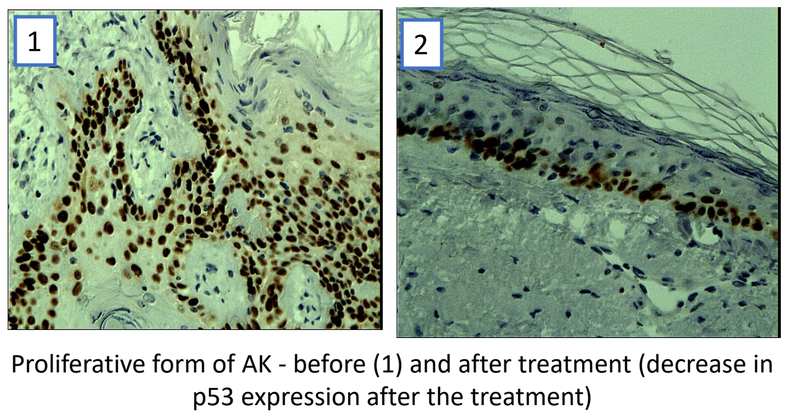
The extent of TP53 immunocompetent cells expressing TP5 in AK lesions was reduced in 11 patients (35.48%), but the extent of these cells increased in 4 patients (12.90%)



Proliferative form of AK - before (1) and after treatment (decrease in the intensity of COX-2 after the treatment)



Proliferative form of AK - before (1) and after treatment (decrease in Ki67 expression after the treatment, thinner epidermis)



Proliferative form of AK - before (1) and after treatment (decrease in p53 expression after the treatment)

Conclusions

- The results of this study indicate that photodynamic therapy (PDT) is an effective treatment for solar keratosis - it results in clinical resolution of skin lesions on physical examination and reduction of lesions on dermoscopic examination.
- Depending on the severity of the skin lesions, a repeated number of PDT irradiation is required.
- The results of immunohistochemical studies also confirm the effectiveness of the PDT therapeutic method. Analysis requires a deviation from the assumed conclusions - an increase in the expression of Ki67 and TP53 in individual patients. This could be a consequence of the destruction of lesion cells by reactive oxygen species during PDT therapy, which leads to the healing of lesions and their replacement by healthy epidermal cells (proliferation processes, of which Ki67 and TP53 are markers, are involved in this). During PDT therapy, and within a few days after phototherapy, there is also an increase in the inflammatory process of the skin (erythema, increased heat) - this process also leads to the replacement of lesional cells with new keratinocytes - which we observed in the results as an increase in COX2 immunoexpression (a marker of inflammation) in AK foci after PDT therapy in almost 30% of patients.