Expression of Aquaporin 3 in the Skin of Psoriatic Patients in Egyptian Population; Immunohistochemical Study

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Abstract

Background: Aquaporin 3 (AQP3) is a glycerol water channel that is involved in water transport and hydration of the epidermis, in the regulation of keratinocytes proliferation, cell migration, and tumorigenesis.

Objectives: Examination of AQP3 expression in psoriatic epidermis compared to healthy controls to assess if it can be related to the complex pathogenesis and severity of the disease.

Patients and Methods: This study included forty patients with psoriasis and twenty healthy, age and sex matched, controls. Skin biopsies from cases and controls were examined and immunohistochemical study was done using anti aquaporin 3 antibodies.

Results: Among psoriasis patients, the cytoplasmic expression of AQP3 was significantly higher than control (p-value = <0.001). On the other hand, there was no significant difference between patients and controls as regards the membranous expression of AQP3 in all layers of the epidermis.

Conclusion: In psoriasis, the abnormal cytoplasmic location of AQP3 may affect its function and lead to dryness of the lesion. The high expression of AQP3 can be related to hyperproliferation of the epidermis and increased T cell trafficking seen in psoriasis.

Key Words: Psoriasis – Clinical – Immunohistochemistry – Aquaporin 3.

Introduction

Psoriasis is a chronic inflammatory hyperproliferative skin disorder. It varies in severity with important implications in terms of medical costs and treatment strategies. Its exact pathophysiology is unclear [1].

Agre et al., in 1993 suggested the name aquaporins (AQP) from the Latin words: Aqu means water and porus means passage) for water channel proteins. The aquaporins (AQP) are a family of integral membrane water transporting proteins expressed in many mammalian epithelial, endothelial and other cell types [2,3].

Aquaporin 3 (AQP3) is the main and most important aquaporin found in the skin. AQP3 is involved in skin hydration, the regulation of keratinocyte proliferation, differentiation, and apoptosis [4,5].

Aim of the study: Our objective is to examine the expression of AQP3 in psoriatic skin and compare it to healthy controls, in order to evaluate its possible role in the pathogenesis of psoriasis.

Patients and Methods

This study was conducted at the Dermatology Outpatient Clinic, Dermatology Department, Faculty of Medicine, Cairo University in the period between November 2013 and August 2015 after approval of Dermatology Research Ethical Committee (Derma REC).

Forty patients with psoriasis vulgaris and twenty healthy controls were recruited and informed consents were signed.

Inclusion criteria included:

1- Psoriasis vulgaris affecting more than 30% of the body surface area.
2- Age above 18 years old.

Exclusion criteria included:

1- Other types of psoriasis.
2- Pregnant and lactating women.

Each patient was subjected to: Medical history including personal history (age, sex, occupation,
residence and special habits) and present history (onset, course, duration, history of previous treatment and history of associated medical conditions).

Clinical examination of the lesions was done to confirm the diagnosis, extent of involvement and to exclude possible differential diagnosis.

A 5mm punch biopsy was taken from the lesion and also from the specimens of abdominoplasty of healthy controls. Then it was fixed in 10% natural buffered formalin dehydrated in ascending grades of alcohol, cleared in xylol & processed into paraffin blocks.

Slides for AQP3 immunostaining were deparaffinized, washed twice for 5min in Phosphate-buffered Saline (PBS), incubated 30min in 3% hydrogen peroxide. Sections were then blocked with 10% Bovine Serum Albumin (BSA) for 20 minutes at room temperature followed by overnight incubation with rabbit polyclonal anti-AQP3 (US-Biological, USA: 1:100 dilution) in a humidified chamber at 4ºC. Antigen visualization was performed using Super SensitiveTM Link-Label Detection Systems Concentrated Format (BioGenex, Fremont, CA, USA), avidin-biotin complex technique and 3,30-diaminobenzidine tetrahydrochloride (DAB chromogen) (BioGenex, Fremont, CA, USA).

The expression of aquaporin 3 was assessed in the form of intensity and distribution whether membranous and/or cytoplasmic in the epidermis. The AQP3 staining intensity was scored using a scale of 0-3 (0=No staining; 1=Weak staining; 2=Moderate staining; 3=Strong staining). The distribution of AQP3 staining was scored using a scale of 0-2 (0=No staining; 1=Focal/patchy staining; 2=Diffuse staining).

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**Results**

The current study included forty patients, fourteen females (35%) and twenty six males (65%) with psoriasis vulgaris. Their ages ranged from 18-75 years (45.82 years ± 13.94). It included also 20 healthy controls, 11 females (55%) and 9 males (45%). Their ages ranged from 24-54 years (38.35 years ±9.73). There was no statistically significant difference between patients and control as regards age and sex.

AQP3 stained the psoriatic and normal epidermis in both the basal and spinous layers. No staining reaction was detected within the stratum corneum in all cases and control (Fig. 1).

The AQP3 reaction was mainly membranous, with thirty six cases showing cytoplasmic positivity as well. There was a statistically significant higher number of patients showing cytoplasmic reaction compared to controls (p-value=<0.001). The cytoplasmic expression in the spinous layer (2.70 ± 1.20) was significantly higher than control group (0.75 ± 1.33) (p-value=<0.001) (Table 1) (Fig. 1).

<table>
<thead>
<tr>
<th>Lesional area</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal layer (Mean±SD)</td>
<td>4.10±1.01</td>
<td>3.85±0.49</td>
</tr>
<tr>
<td>Spinous layer (Mean±SD):</td>
<td></td>
<td></td>
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<tr>
<td>Cytoplasmic</td>
<td>2.70±1.20</td>
<td>0.75±1.33</td>
</tr>
<tr>
<td>Membranous</td>
<td>4.12±1.18</td>
<td>4.05±0.51</td>
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</tbody>
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*p-values less than 0.05 were considered as statistically significant.

In the control group, there was no statistical significance as regards the number of patients with membranous pattern compared to control.

Fig. (1): Lesional skin revealing a diffuse strong immunohistochemical reaction for AQP3, with negative parakeratotic zone (DAB, x 400 original magnification).
There was no statistical significant correlation between AQP3 expression in patients and control as regards the age and gender. There was no statistical significant correlation between AQP3 expression among patients as regards disease duration and the PASI score.

Discussion

Psoriasis is a very troublesome disease with complex pathophysiology and the precise pathogenesis is not clear yet. Therefore, its pathogenesis is an area of continuous research [4].

AQP3 is a glycerol water channel, the functional role of which in human epidermis is still an area of research interest, as it is abundantly expressed in the epidermis and there are contradictory theories about its expression and role [6].

In the current study, AQP3 expression in the epidermis in forty psoriatic patients and twenty healthy controls was examined. AQP3 stained the psoriatic and normal epidermis in both the basal and spinous layers. No staining was detected in the stratum corneum in all cases and controls.

Consistent to our findings, AQP3 in the normal epidermis was localized in the plasma membranes of the basal and the suprabasal layers in normal skin. The membranous pattern of AQP3 is consistent with the fact that AQP3 is an integral membrane protein [4,7,8].

In our study, among psoriasis patients, the cytoplasmic expression of AQP3 was significantly higher than control. However, there was no statistical significance as regards the membranous expression.

Voss et al., [4] and Lee et al., [9], reported the same pattern of AQP3 in psoriasis where it is diffusely expressed in the cytoplasm rather than in the plasma membrane. They stated that since AQP3 has an important role in regulating the hydration status of the epidermis, thus its abnormal localization in the cytoplasm may lead to impairment of its function and can explain the dryness of the psoriatic lesions.

Hara-Chikuma and Verkman [10] and Olsson et al. [11], found a positive correlation between AQP3 mediated glycerol transport, ATP content in keratinocytes and increased proliferation of cells.

Hara-Chikuma et al., [6], suggested that AQP3-mediated intracellular $H_2O_2$ uptake in keratinocytes and lymphocytes is required for TNF-$\alpha$-induced signal activation and T-cell trafficking, and hence development of psoriasis.

Moreover, Kusayama et al., [12] suggested a potentially important role of AQP3 in maintaining cell to cell adhesion and reported that its suppression results in anoikis which is a form apoptosis. Thus, AQP3 helps to maintain a dynamic balance between cell turnover and survival which is the core of regulation of cellular proliferation.

Conclusion and recommendations: Our study revealed that in psoriasis, the abnormal cytoplasmic location of AQP3 may affect its function leading to skin dryness. The high expression of AQP3 can be related to hyperproliferation of the epidermis and increased T cell trafficking.

Our recommendations include: The measurement of AQP3 on a genetic level and mRNA expression levels in a larger group for better understanding of its role in the skin.

References

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