Relation of Aquaporin 3 Expression to Skin Aging in Egyptian Population; Immunohistochemical Study

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Abstract

**Background:** AQP3 is a water channel protein concerned with glycerol and water transport in the skin. It is also involved in several physiological processes in the skin including skin hydration, proliferation and wound healing.

**Aim of Work:** The current study aimed at examination of AQP3 pattern and expression in healthy non sun exposed skin in selected sample of Egyptian population. This was to assess if AQP3 shows changes with age and can be involved in skin aging in Egyptian population.

**Patients and Methods:** This study included twenty healthy controls. Each patient was subjected to short medical history and cutaneous examination. Skin biopsies were examined and immunohistochemical study was done using anti aquaporin 3 antibodies.

**Results:** AQP3 stained the epidermis in both the basal and spinous layers. No staining reaction was detected within the stratum corneum. There was no statistical significant correlation of AQP3 expression among healthy subjects as regards the age and gender.

**Conclusion:** AQP3 expression didn’t show significant difference with age in the studied sample of Egyptian population in contrast to previous studies conducted in other countries denoting that genetic factors and/or skin phenotype may affect AQP3 expression.

**Key Words:** Aquaporin 3 – Histopathology – Immunohistochemistry.

Introduction

THE aquaporins (AQPs) are a family of integral membrane water transporting proteins. To date 13 aquaporins are discovered (0-12). Aquaporin 3 (AQP3) is the main and most important aquaporin found in the skin. In the epidermis, it is localized mainly in the plasma membranes of the basal and the suprabasal layers [1].

AQP3 is involved in the regulation of keratinocyte proliferation, differentiation, and apoptosis, as well as regulation of wound healing and maintaining skin elasticity [2].

The ultraviolet radiation induces downregulation of AQP3 which occurs as well with aging and may explain the dryness of the skin and delayed wound healing in the elderly and in cases of photoaging as AQP3 is also involved in fibroblast migration in human skin, which occurs during normal wound healing [3].

**Aim of work:** Our objective is to examine the pattern and expression of AQP3 in normal skin in different age groups in in order to evaluate its possible role in the process of aging in skin phenotype III and IV.

**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. It was approved by the Dermatology research ethical committee (Derma REC).

Skin samples were obtained from twenty healthy individuals undergoing abdominoplasty or mammoplasty.

**Inclusion criteria:**
1- Age above 18 years old with skin phenotype III and IV according to Fitzpatrick skin types (Table 1).
2- Non sun exposed areas.
Table (1): Fitzpatrick’s skin phototype.

<table>
<thead>
<tr>
<th>Skin Phototype</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Very fair, always burns and never tans</td>
</tr>
<tr>
<td>II</td>
<td>Fair, burns frequently and tans with difficulty</td>
</tr>
<tr>
<td>III</td>
<td>Fair, occasionally burns and tans gradually</td>
</tr>
<tr>
<td>IV</td>
<td>Medium, never burns and always tans, common in Mediterraneans.</td>
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<tr>
<td>V</td>
<td>Light brown, never burns and tans profusely, common in Indians.</td>
</tr>
<tr>
<td>VI</td>
<td>Dark brown, never burns and is deeply pigmented, Dark Africans.</td>
</tr>
</tbody>
</table>

Exclusion criteria:
1- Pregnant and lactating women.
2- Cases using Anti-aging treatment.

A 5mm punch biopsy was taken from the specimens of abdominoplasty and mammoplasty 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, washed twice for 5min in PBS. 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 (USBiological, USA; 1:100 dilution) in a humidified chamber at 4ºC. Following primary antibody incubation, antigen visualization was performed using Super Sensitive™ 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). Mayer’s hematoxylin was used for counterstaining.

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).

Statistical method:
Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22. Data was summarized using mean, standard deviation, median minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. For comparing categorical data, Chi square ($\chi^2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. $p$-values less than 0.05 were considered as statistically significant.

Results

There were 13 females (65%) and 7 males (35%), ages ranged from 20-60 years with a mean of $44.80\pm 12.474$.

AQP3 stained the basal and suprabasal layers and no staining reaction was detected within the stratum corneum in all cases and controls (Fig. 1)

There was no statistical significant correlation of AQP3 expression as regards the age and gender.

Discussion

AQP3 is a glycerol water channel that is expressed in epithelial tissues. It is involved in water transport and hydration in the human epidermis, in the regulation of keratinocyte proliferation, cell migration, and tumorigenesis [4].

Downregulation of AQP3 occurs with aging and may explain the dryness of the skin and delayed wound healing in the elderly [3].
The current study aimed at examination of AQP3 pattern and expression in normal skin obtained from specimens of abdominoplasty and mammoplasty of twenty healthy individuals.

To our knowledge, this is the first study to examine AQP3 expression in relation to aging in Egyptian population.

In the current study AQP3 stained the basal and spinous layers. No staining was detected in the stratum corneum in all cases.

In accordance with our findings, Olsson et al., [5] reported the expression of AQP3 in the basal and suprabasal layers and its absence from the stratum corneum in normal skin. They stated that there is a gradual reduction in AQP3 from basal to suprabasal layers thus creating a gradient of AQP3 that may lead to a corresponding water gradient in the epidermis, with a sharp decrease of water content in the SC. In this way, water loss is prevented at the same time as the living layers of the epidermis are optimally hydrated.

In our work, AQP3 intensity and distribution were not affected by neither age nor gender.

Opposite to our finding, Seleit et al., [6] reported that AQP3 expression intensity showed a gradual decrease from the 20 to 35-year-old group to the 35 to 50-year-old group, with the least immunoreactivity in the above 50-year-old group.

Moreover, Bonté [7], also reported that the expression of AQP3 in human skin is strongly affected by aging.

However, neither of these studies was conducted on ethnic population or skin phenotype similar to that of ours. The variable results may be related to the different genetic background or skin phenotype of our patients.

Conclusion and recommendations: Aquaporin 3 expression in the studied sample of Egyptian population didn’t change with aging, in contrast to previous studies conducted on other populations. Therefore, it may not be related to the process of aging in our population due to genetic or skin phenotype related factors. Further studies are needed on a larger sample of patients to verify its role in the skin aging in different ethnic groups.

References
Relation of Aquaporin 3 Expression to Skin Aging in Egyptian Population

الملخص العربي

أوكاپورين 3 هو قناة للماء والجسيمات في الخلايا ويعد هو الأكثر وفرة من كل الأوكاپورينات في الجلد، كما أنه يشارك في تنظيم انتشار الخلايا الجلدية وحركة الخلية والانتشار الجروجي وتكون الأورام.

تهدف هذه الدراسة إلى فحص نسبه من الجلد السليم من مشاركين أصحاء باستخدام علم الباحثي والصيغة الكيميائية المناعية لتقسيم درجة ونطاق انتشار الأوكاپورين 3 في الأعمار المختلفة لبحث وجود علاقة بين أوكاپورين 3 والتقدم في العمر في العينة المختارة.

تمت هذه الدراسة على 20 من المشاركين الأصحاء من مختلف الأعمار بناءً على بيانات الراقبة، ثم تم أخذ وفحص عينات الجلد مصمية. جميع الحالات بالصيغة الكيميائية المناعية باستخدام الأوكاپورين 3

وقد كشفت الدراسة عن وجود أوكاپورين 3 في جدار الخلايا الجلد في الأصحاء وعدم اختلاف مستواها باختلاف الأعمار المدربة أو النوع.

تعمقاً على هذه الدراسة، وجد أن نبض الأوكاپورين 3 وشديها لم يختلف باختلاف الأعمار التي تم دراستها وفقاً للمستوى، إذ أن الأوكاپورين 3 ليس له دور في تغيرات الحالة في الجلد مع تقدم العمر في العينة المدربة ويفضي بأعادة الدراسة على عدد أكبر من المشاركين.