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Original article

Non-invasive skin biomarkers quantification of psoriasis and atopic dermatitis: Cytokines, antioxidants and psoriatic skin auto-fluorescence

Meital Portugal-Cohen [a,b], Liran Horev [c], Claas Ruffer [d], Gerrit Schlippe [d], Werner Voss [d], Ze'evi Ma'or [b], Miriam Oron [b], Yoram Soroka [e], Marina Frušić-Zlotkin [e], Yoram Milner [e], Ron Kohen [a,*]

[a] School of pharmacy, Institute of Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, P.O.B. 12065, 91120 Jerusalem, Israel
[b] AHAVA – Dead Sea Laboratories, Dead Sea, Israel
[c] Hadassah Medical Center, Department of Dermatology, Jerusalem, Israel
[d] Dermatest® Medical Research Company, Muenster, Germany
[e] Department of Biological Chemistry, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

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ABSTRACT

Background: Psoriasis and atopic dermatitis (AD) are challenging to treat due to the absence of suitable monitoring procedure and their recurrences. Alteration of skin hydrophilic biomarkers (SHB) and structural elements occur in both disorders and may possess a distinct profile for each clinical condition.
Objective: To quantify skin cytokines and antioxidants non-invasively in psoriatic and in AD patients and to evaluate skin auto-fluorescence in psoriatic patients.
Methods: A skin wash sampling technique was utilized to detect the expression of SHB on psoriatic and AD patients and healthy controls. Inflammatory cytokine (TNFα, IL-1α and IL-6) levels, total antioxidant scavenging capacity and uric acid content were estimated. Additionally, measurement of the fluorescent emission spectra of tryptophan moieties, collagen cross-links and elastin cross-links were performed on psoriatic patients and healthy controls.
Results: Our findings demonstrate significant alterations of the SHB levels among psoriasis, AD and healthy skin. Differences were also observed between lesional and non-lesional areas in patients with psoriasis and AD. Ultra-structural changes were found in psoriatic patients both in lesional and non-lesional areas.
Conclusion: Employing non-invasive measurements of skin wash sampling and skin auto-fluorescence might serve as complementary analysis for improved diagnosis and treatment of psoriasis and AD. Furthermore, they may serve as an additional monitoring tool for various diseases, in which skin dysfunction is involved.

1. Introduction

Psoriasis and atopic dermatitis (AD) are the most common diseases of the skin [1,2]. However, their mechanisms for skin inflammation and propensity for skin infection are quite different. The immune response in psoriasis is Th1-mediated and associated with local neutrophil infiltration [3]. In contrast, the immune response in AD is Th2-mediated, contributing to the high IgE levels [4]. In spite of the fact that both skin diseases are characterized by defective skin barriers, 30% of AD patients suffer from frequent serious skin infections whereas only 6.7% of psoriasis patients suffer from skin infections [5]. The excessive activity of the immune system in psoriasis and AD can trigger the production of ROS [6] and thereby, to enhance the biological damage. Overproduction of superoxide radicals ($O_2^{\cdot -}$) play an important role in the oxidation process of psoriasis and AD [6,7], whereas the expression of the enzyme iNOS overproducing nitric oxide radicals ($NO^{\cdot}$), increases mostly in psoriatic patients [8,9].

Although psoriasis and AD are expressed mainly as skin dysfunctions, it is important to note that they are both associated also with intrinsic events. Psoriasis is linked to psychological distress [10,11], psoriatic arthritis [12] diabetes mellitus [13,14], heart disease [14] and stroke [15] and Metabolic stress syndrome. AD is linked to asthma, food allergy and allergic rhinitis [16] and emotional stress [17]. Therefore, they possess high complexity regarding their diagnosis and treatment. There are no specific blood tests or diagnostic procedures for psoriasis and AD. Furthermore, sometimes invasive procedures such as a skin biopsy, or scraping, may be needed to rule out other disorders and to confirm the diagnosis. The difficulty in distinguishing psoriasis and AD from other skin disorders might lead occasionally to misdiagnosis. Thus, the need to define additional non-invasive

* Corresponding author. Tel.: +972 2 6758659; fax: +972 2 6757246.
E-mail address: ronk@ekmd.huji.ac.il (R. Kohen).

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procedures for skin biomarkers and to examine the interplay among them for diagnosis is arisen. Simple and non-invasive procedures can be used in order to diminish patient's discomfort in diagnosis, and to illustrate typical hallmarks of psoriasis and AD.

The purpose of this present study was to evaluate skin biomarkers by using two non-invasive techniques:

- detection of secreted skin hydrophilic biomarkers (SHB);
- measurements of skin auto-fluorescence in psoriatic patients.

SHB secretion was measured on the surface of the patients' skin by a non-invasive technique based on skin wash sampling. This technique was already employed by us for the characterization of SHB in patients suffering from chronic renal failure. We found significant alterations of SHB such as cytokines and uric acid levels between these patients and healthy subjects. Moreover, such alterations were correlated with detected changes in blood biochemistry and dermatology severity score [18].

The auto-fluorescence of human skin has previously been shown to serve as indicator for structural changes and to vary with chronological aging and exposure to UV radiation in a predictable manner [19–24] The major fluorophores are tryptophan moieties, which are mainly part of the epidermis, as well as fluorophores representing the connective tissue in the dermis such as collagen and elastin cross-links.

The results achieved by the assessment of these biomarkers are offered as a step towards the establishment of biological profiles corresponding psoriasis and AD in order to ease their diagnosis.

2. Methods

2.1. Study population

Secreted SHB were evaluated in healthy volunteers (age ranged 23–60 years, men and women), psoriatic patients (age ranged 22–68 years, men and women) and patients with AD (age ranged 18–47 years, men and women). Patients were recruited By Dermatest® Medical Research, Germany.

For auto-fluorescence measurements, serial of fluorescence emission spectra were measured from healthy human volunteers (age ranged 27–69 years, men and women) and psoriatic patients (age ranged 20–69 years, men and women) from Hadassah Medical Center, Israel.

All studies were carried out following informed consent. Controlled use of oral administration was allowed. No creams or ointments were applied prior to measurements of the patients at least 12 hr before the measurements.

The samples taken from AD patients were obtained from Dermatest® Germany. Auto-fluorescence measurements were not conducted for the AD patients.

2.2. Measurement of secreted skin hydrophilic biomarkers

Samples from skin were collected from healthy volunteers, as well as AD and psoriatic patients. As previously described [18,25], this method consisted of non-invasive SHB extraction of the skin from the inner side of the wrist with a PBS solution pH = 7.4 (Sigma-Aldrich, Steinheim, Germany). The extract was collected by placing a well (1 cm in diameter) on the skin of the patient and pressing it down with a parafilm foil. No cream or ointment were applied at least 12 hr prior to putting on the well. The well had a small opening on its top to allow injection of 1 ml of sterile PBS. The opening was then sealed with an additional parafilm foil. After 30 minutes, during which the arm kept static, the PBS solution was collected through the opening on the well, and the well removed. Samples were aliquoted and stored at −80°C for future analysis. Total scavenging capacity of antioxidants (TSC), uric acid (UA) levels and cytokines levels were assessed up to 30 days since sample collection.

2.3. Quantification of the total scavenging capacity of hydrophilic antioxidants

In order to determine the overall amounts of secreted hydrophilic antioxidants present in well extract, the oxygen radical absorbance capacity (ORAC) assay was conducted. A procedure described by Cao et al. [26], later modified to allow the use of fluorescein (FL) (Sigma-Aldrich, Steinheim, Germany) as a fluorescent label was used [27]. The ORAC assay was carried out on a FLUOstar Galaxy plate reader (BMG, Offenburg, Germany) equilibrated at 37 °C. Excitation and emission were set up at 485 nm and 520 nm, respectively. 2,2′-Azobis(2-amidino-propane) dihydrochloride (AAPH) (Sigma-Aldrich, Steinheim, Germany) was used as a peroxyl generator, and Trolox (Sigma-Aldrich, Steinheim, Germany) as a calibration standard. All reagents were prepared in 75 mM phosphate buffer (pH 7.4). Forty microliter aliquotes of sample, blank or Trolox dilutions, were transferred into a 96-well microplate. One hundred microliter FL were added, to reach a final concentration of 96 nM. $ORAC_{FL}$ fluorescence was read every 2 min for 70 min. Peroxyl radical-induced oxidation started immediately after AAPH addition, and results were evaluated by reference to the Trolox calibration curve. The final results were calculated by using the differences of areas under the fluorescent decay curve between the blank and the sample (expressed as mmol Trolox Eq/g protein.).

2.4. Determination of secreted uric acid content

To determine the UA levels secreted from skin surface, we used the HPLC system [28]. The well extract were thawed and then injected to the HPLC system composed of a Kontron system Pump LC 320 Pump model (San-Diego, CA, USA), an injection device containing a loop with a capacity of 20 μl, a reversed phased 4 μm-pore 250 × 4.6 mm c-18 column (Supleco, Bellefonte, PA, USA), and a voltametric detector. Twenty-five microliter of each sample was injected. The mobile phase consisted of 100 mg/ml EDTA, 0.1 M acetic acid buffer, and 1% tetrabutylammonium hydroxide (pH 4.75). This was delivered at a flow rate of 0.8 ml/min. The voltage applied to the samples was (+)600 mV with a sensitivity of 50 nA. The UA levels were calculated from a calibration curve prepared under the same experimental conditions. Stock solutions of UA (Sigma-Aldrich, Steinheim, Germany) were used as standards.

2.5. Evaluation of cytokines secretion

IL-1α, TNFα and IL-6 levels were assayed by ELISA kit (Biolegend, San-Diego, Ca, USA). Briefly, ELISA plates (Nunc-lmmuno Plate Maxisorb, Neptune, NJ) were coated with a cytokine-specific capture antibody (supplied from manufacture kit) and incubated overnight at RT. The plates were washed three times (using PBS containing 0.05% Tween-20), blocking solution (supplied from manufacture kit) was added, and the plates were incubated for 1 to 2 h at RT. Standards and samples from well extract were then introduced into the wells and incubated for 2 h at RT. The plates were then washed, and rabbit anti-human IL-1α, TNFα, or IL-6 antibody was added for a further incubation at RT for 2 h. Avidin-horseradishperoxidase was diluted 1:5000 and added. The plates were again incubated for 30 min at RT. The plates were washed, and substrate solution was added (supplied from manufacture kit)). Color development proceeded for 4 to 5 min at RT before being stopped by the addition of 2N H_2S0_4 (Sigma-Aldrich, Steinheim, Germany). The absorbance was then measured at 450 nm using a Bio-tek PowerWave 340 microplate scanning spectrophotometer (Bio-TEK ELx, Winooski,