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Original article

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

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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

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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,

VT, USA), the concentrations of IL-1α and TNFα was calculated based on the standard curve.

2.6. Skin auto-fluorescence measurements

In vivo fluorescence spectroscopy was performed using a spectrofluorimeter.

The excitation source was a Xenon arc lamp. Acquisition of emission spectra was the preferred method of measuring *in vivo* skin auto-fluorescence. Measurements were performed by placing the fibreoptic probe in contact with the skin site of interest of the patients.

The measurements on psoriatic patients were performed on lesional areas and non-lesional areas located on the volar inner side of the wrist. The distance between lesional and non-lesional areas was at a range of 1–2 cm. Acquisition of emission spectra was the preferred method of measuring skin fluorescence intensity, which was calculated by the area beneath the spectral curve. Serial fluorescence emission spectra were used:

- for detection of tryptophan moieties, excitation was set at 295 nm, and emission was scanned from 310 nm to 450 nm (maximum at 350 nm);
- for pepsin-digestible collagen cross-links (PDCCL), excitation was set at 335 nm, and emission was scanned from 360 nm to 450 nm (maximum at 380 nm);
- for collagenase-digestible collagen cross-links (CDCCL), excitation was set at 370 nm, and emission was scanned from 420 nm to 550 nm (maximum at 460 nm);
- for elastin cross-links (ECL), excitation was set at 420 nm, and emission was scanned from 480 nm to 550 nm (maximum at 500 nm) [23].

2.7. Data analysis

For pairwise comparisons, Two-tail Student *t*-test was applied: unpaired *t*-test for comparison between groups and paired *t*-test for comparison between lesional and non-lesional areas. Data are expressed as mean ± SEM. Findings were considered significant at $P < 0.05$.

3. Results

3.1. Patients with psoriasis and AD demonstrate a different profile of cutaneous cytokines secretion

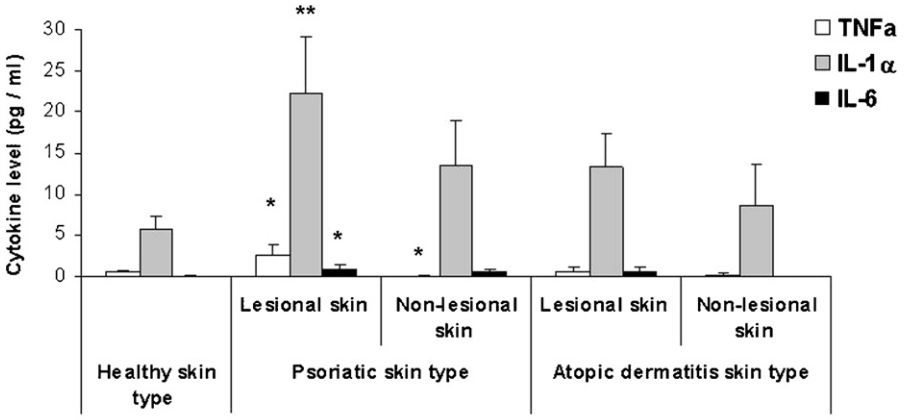
In order to examine inflammatory biomarkers, the secretions of the cytokines TNFα, IL-1α and IL-6 were detected in patients with psoriasis and AD (Fig. 1). The highest levels of TNFα, IL-1α and IL-6 were observed in lesional skin areas of psoriatic patients. TNFα levels were significantly higher in lesional psoriatic areas (2.694 pg/ml ± 1.264) than non-lesional psoriatic areas (0.072 pg/ml ± 0.072) and significantly higher than the healthy subjects (0.503 pg/ml ± 0.219) ($P = 0.0370$). IL-1α levels were significantly higher in psoriatic lesional areas (22.261 pg/ml ± 6.711) than in healthy subjects (5.870 pg/ml ± 1.390) ($P = 0.0014$). IL-6 levels were significantly higher in psoriatic lesional areas (0.630 pg/ml ± 0.423) than in healthy subjects (0.061 pg/ml ± 0.061) ($P = 0.049$).

No significant differences between AD affected areas and healthy controls were found in TNFα, IL-α and IL-6 levels.

3.2. Patients with psoriasis and AD demonstrate a different profile of cutaneous antioxidants secretion

In an attempt to evaluate the oxidation process in psoriasis and AD, the secretion of skin antioxidants was evaluated (Fig. 2).

As shown in Fig. 2a, the total antioxidant scavenging capacity (TSC) in psoriatic lesional areas was the highest (70 μM Trolox eq./ml ± 17) and markedly higher compared to healthy subjects (42 μM Trolox eq./ml ± 4, $P = 0.0329$), to psoriatic non-lesional areas (34 μM Trolox eq./ml ± 6, $P = 0.0038$) and to lesional areas of AD patients (35 μM Trolox eq./ml ± 7, $P = 0.0117$). When UA levels were assessed (Fig. 2b), they were lower in both lesional (77 nM/ml ± 55, $P = 0.0246$) and non-lesional (45 nM/ml ± 19, $P = 0.0058$) areas of



Cytokine	Number of subjects		
	Healthy skin type	Psoriatic skin type	Atopic dermatitis skin type
TNFα	N = 17	N = 13	N = 13
IL-1α	N = 17	N = 13	N = 13
IL-6	N = 12	N = 12	N = 6

Fig. 1. Cytokine secretion from skin surface shows different levels in psoriatic and atopic dermatitis (AD) patients.
Skin wash samples were collected from healthy volunteers, patients with psoriasis and patients with AD as described in the methods section. The cytokines TNFα, IL-1α and IL-6 were quantified by ELISA. The values presented are the mean ± SEM. Two-tail Student *t*-test was applied. Differences between average values were considered as significant for $P < 0.05$. $^{*}P < 0.05$: TNFα and IL-6: healthy skin vs. psoriatic lesional skin; $^{*}P < 0.05$: IL-1α: psoriasis-lesional skin vs. non-lesional skin. $^{**}P < 0.01$: IL-1α: healthy skin vs. psoriatic lesional skin.

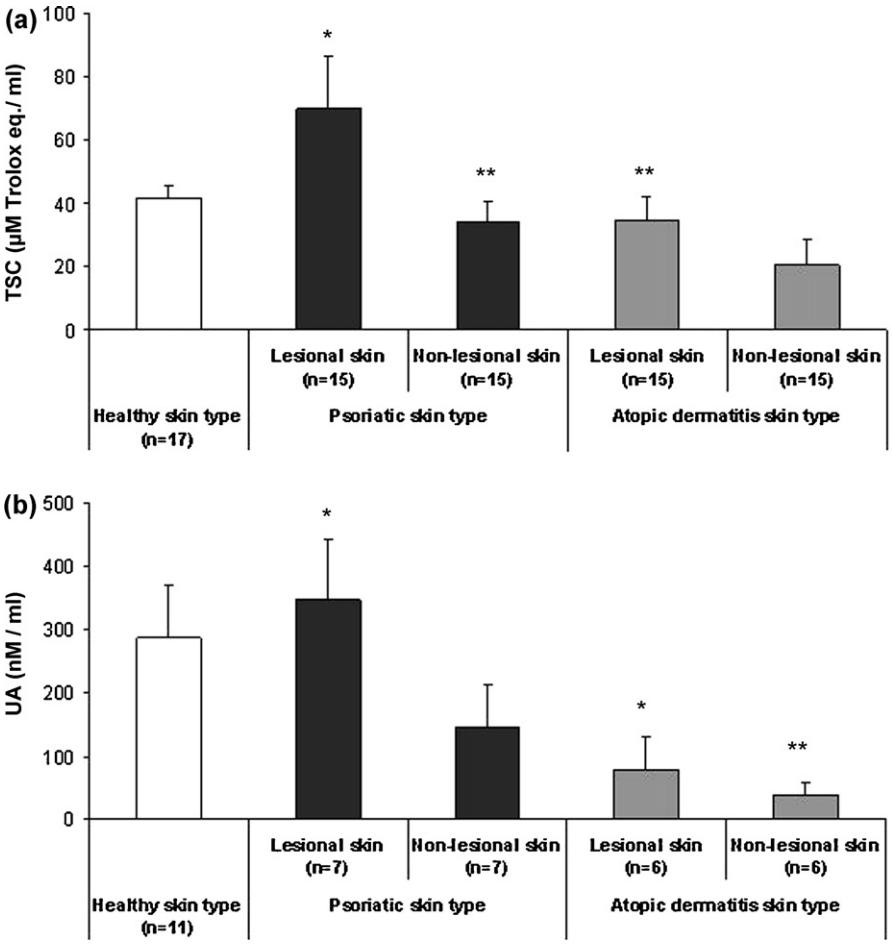


Fig. 2. Antioxidants secretion from skin surface is altered in psoriatic and atopic dermatitis (AD) patients.
Skin wash samples were collected from healthy volunteers, patients with psoriasis and patients with AD as described in the methods section. TSC was evaluated by ORAC assay (a) and uric acid (UA) levels were assessed by HPLC-ECD (b). The values presented are the mean ± SEM. Two-tail Student *t*-test was applied. Differences between average values were considered as significant for $P < 0.05$. TSC: $^{*}P < 0.05$: healthy skin vs. psoriatic lesional skin; $^{**}P < 0.01$: psoriasis-lesional vs. non-lesional skin, lesional skin – psoriasis vs. AD. UA: $^{*}P < 0.05$ normal skin vs. AD lesional skin, psoriatic lesional skin vs. psoriatic non-lesional skin and AD lesional skin; $^{**}P < 0.01$ healthy skin vs. AD non-lesional skin.

AD patients than in healthy subjects (286 nM/ml ± 84). The levels of UA were higher in lesional areas of psoriatic patients (347 nM/ml ± 95) compared to lesional areas of AD ($P = 0.0191$) patients and non-lesional areas of psoriatic patients (40 nM/ml ± 19, $P = 0.0318$).

3.3. Skin auto-fluorescence in psoriatic patients show different spectra compared to healthy volunteers

Fluorescence emission spectra representing the epidermis and dermis layers were different in psoriatic patients compared to healthy subjects (See typical spectra in Fig. 3). When the fluorescence of tryptophan moieties was detected (Fig. 3a), the spectra obtained from lesional and non-lesional areas of psoriasis patients clearly revealed a large signal with red-shift of 50 nm additionally to the obtained peak at 350 nm. The red-shift signal was significantly larger in lesional areas (relative fluorescence – $7.95 \times 10^7 \pm 2.82 \times 10^7$) ($P = 0.0024$) and non-lesional areas (relative fluorescence – $7.10 \times 10^7 \pm 3.57 \times 10^7$) ($P = 0.0281$) of psoriatic patients compared to healthy subjects (relative fluorescence – $1.67 \times 10^7 \pm 3.76 \times 10^6$), in which no such signal was observed.

Collagen cross-links (PDCCL and CDCCL) fluorescence intensities were the highest in lesional and non-lesional areas of psoriatic patients and the lowest in healthy subjects (Fig. 3b, c). PDCCL levels were significantly higher in lesional (relative fluorescence – $1.56 \times 10^7 \pm 3.60 \times 10^6$) ($P = 0.0129$) and non-lesional areas (relative fluorescence – $1.75 \times 10^7 \pm 5.20 \times 10^6$) ($P = 0.0205$) compared to healthy subjects (relative fluorescence – $7.32 \times 10^6 \pm 1.66 \times 10^6$). Moreover, in psoriatic patients, PDCCL spectra split into two peaks at 382 nm and at 404 nm. The first peak was higher in lesional areas than non-lesional areas. Among healthy subject, these peaks were not observed (Fig. 3b). CDCCL levels were also significantly higher in lesional (relative fluorescence – $5.56 \times 10^7 \pm 9.57 \times 10^6$) and non-lesional areas (relative fluorescence – $4.76 \times 10^7 \pm 8.78 \times 10^6$) compared to healthy subjects (relative fluorescence – $2.86 \times 10^7 \pm 4.10 \times 10^6$) ($P = 0.00196$ and $P = 0.0307$ respectively).

ECL fluorescence spectra did not exhibit any statistical differences among lesional (relative fluorescence – $2.50 \times 10^6 \pm 4.89 \times 10^5$) and non-lesional areas (relative fluorescence – $2.81 \times 10^6 \pm 6.86 \times 10^5$) in psoriatic patients and healthy subjects (relative fluorescence – $2.90 \times 10^6 \pm 4.96 \times 10^5$) (Fig. 3d).

4. Discussion

Psoriasis and AD are both chronic inflammatory skin diseases of unknown etiology [29]. Although psoriasis and AD are characterized by alteration of cutaneous cytokines and antioxidants level and activity [8,30], they differ in their damage mechanism. Moreover, even though both are common diseases, their diagnosis is often uncertain and thus, additional tests can help in better understanding of their pathogenesis. The purpose of this present study was to detect skin biomarkers in psoriatic and AD patients by non-invasive techniques in order to provide

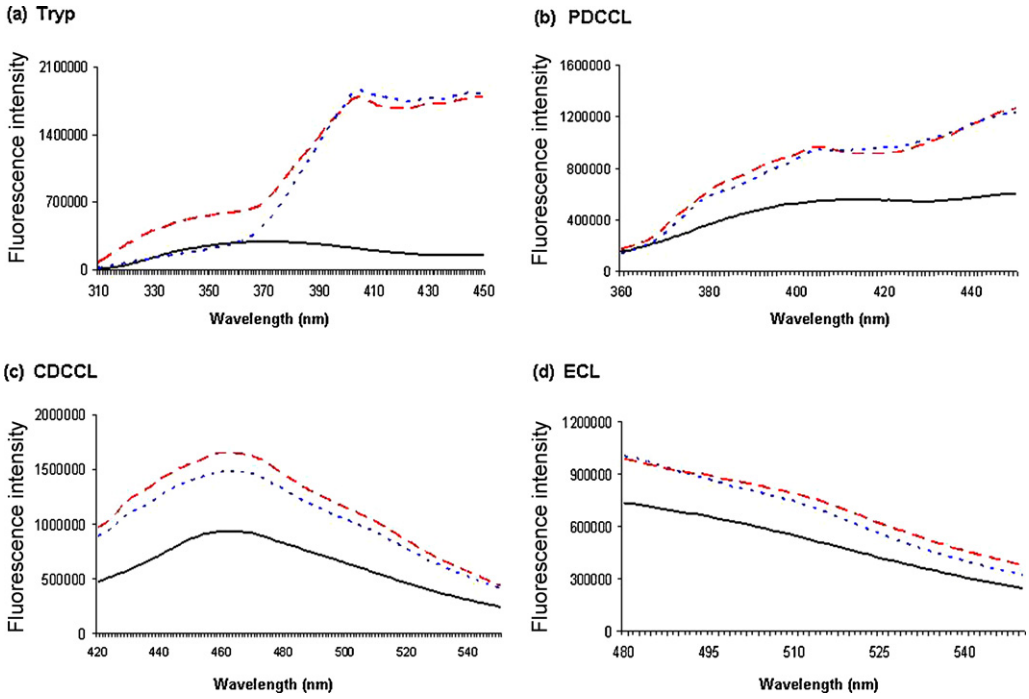


Fig. 3. Psoriasis affects skin auto-fluorescence.
Skin auto-fluorescence was assessed in healthy volunteers (▬▬▬) and patients with psoriasis in lesional (▬ ▬ ▬ ▬) and non-lesional areas (• • • • • • •). Emission spectra of the following fluorophores were measured as described in the methods section and were calculated by area under curve following normalized to baseline: tryptophan moieties (excitation wavelength: 295 nm) (a), pepsin-digestible collagen cross-links (PDCCL) (excitation wavelength: 335 nm) (b), collagenase-digestible collagen cross-links (CDCCL) (excitation wavelength: 370 nm) (c), elastin cross-links (excitation wavelength: 420 nm) (d). Spectra shown represent average spectra ($n = 9$ for psoriatic patients, $n = 16$ for healthy patients). Differences between average values were considered as significant for $P < 0.05$.Tryptophan moieties: $P < 0.05$ normal vs. non-lesional skin, $P < 0.01$ normal vs. lesional skin; PDCCL: $P < 0.05$ normal vs. lesional and non-lesional skin, CDCCL: $P < 0.05$ normal vs. non-lesional skin, $P < 0.01$ normal vs. lesional skin.

novel tools for analysis of cutaneous modifications associated with these diseases.

Using skin wash sampling, we demonstrated a distinct pattern of cytokine and antioxidant secretions from skin surface among patients with psoriasis compared to patients with AD (Figs. 1 and 2). The levels of the inflammatory cytokines TNFα, IL-1α and IL-6 were the highest in lesional areas of psoriatic patients (Fig. 1). These findings by skin wash procedure are in line with previous studies and hence, this procedure confirms an over-expression of TNFα, IL-α and IL-6 in psoriatic lesions; TNFα secretion was higher in psoriatic lesional area compared to psoriatic or AD non-lesional area and healthy skin. This, in agreement with previous reports, indicate that TNFα production is increased in psoriatic lesional skin as compared with non-lesional psoriatic skin and healthy skin [31–33] by various types of skin samples [34], such as skin sections [35] blister fluids [31] and skin extracts [32].

Lesions of psoriatic skin are characterized by epidermal hyperproliferation, leukocyte adhesion molecule expression and leukocyte infiltration. The local release of pro-inflammatory cytokines, such as TNFα, may play an important role in the induction of these events [32]. While in the skin many cell types additionally to keratinocytes can produce TNFα (dermal dendritic cells, macrophages, mast cells, and activated T lymphocytes), keratinocytes are the main source of IL-1. Its activity in normal epidermis is sustained by IL-1α [36,37]. Many conflicting results have been reported by different authors on the relative amounts of IL-1α and IL-1β in psoriatic skin. Several studies have indicated that IL-1α concentrations were reduced in psoriatic lesional skin as compared to non-lesional and normal skin, [38–40] while others have reported that IL-1α concentrations were increased in psoriasis [41–44]. There is also evidence that transgenic mice expressing high levels of IL-1α in the basal epidermis develop inflammatory skin lesions with psoriasis-like histologic features [45]. These researches support our results, in which psoriatic lesions showed an augmentation in IL-1α secretion (Fig. 1).

IL-6 is suggested to play an important role in psoriasis by stimulating keratinocytes proliferation and T lymphocytes activation [46]. Our results demonstrated higher secretion of IL-6 in lesional areas of psoriatic patients compared to healthy subjects. This is with agreement with other data showing that IL-6 mRNA and protein increase in psoriatic plaques [47].

Cytokines secretion is also affected by cellular redox status [48]. Thus, we examined whether psoriasis or AD patients have altered levels of antioxidants in skin by the assessment of the TSC and UA levels. Our results show, for the first time, that psoriatic lesional areas had the highest TSC and UA levels (Fig. 2). There is no previous description of the overall non-enzymatic antioxidants activity in psoriatic or AD skin by *in vivo* measurements. Few studies showed an elevation of the superoxide dismutase (SOD) in psoriatic skin [49,50]. In another study, higher α-tocopherol levels in sera of psoriatic patients were detected while no change in TSC between psoriatic sera and normal sera was observed [51]. Given that ROS, such as, $O_2^{\cdot -}$, $NO^{\cdot}$, and H_2O_2, are excessively formed in psoriasis [52,53], it can be speculated that an excessive antioxidant scavenging occurs in order to overcome the overproduction of ROS in psoriatic lesions.

When UA levels were detected, the pattern was similar to that of TSC (Fig. 2b). It is known that the epidermis in psoriatic skin is characterized by increased levels and activity of the enzyme xanthine oxidase [54]. Thus, this might explain the high levels of UA in psoriatic lesional skin. Pruritus, which is common in psoriatic lesions, can be due to high UA levels. In both lesional and non-lesional areas of AD patients, UA levels were lower than healthy skin type (Fig. 2). Recently, we have also reported that low UA

levels were observed in skin of hemodialysis patients compared to healthy subjects [18].

The profound epidermal keratinocytes hyperproliferation in psoriasis is related to an accelerated and incomplete differentiation and alternations in skin structure. Given that skin autofluorescence might indicate its structural properties, both in epidermis and dermis, its fluorescence spectroscopy was performed using serial emission spectra. The results clearly show that spectra obtained from both lesional and non-lesional areas of psoriatic patients have similar spectra between themselves but significantly different pattern compared to healthy subjects.

The results showed that tryptophan moieties fluorescence was higher in both lesional and non-lesional areas of psoriatic patients compared to healthy subjects. The increased tryptophan signal in psoriatic patients was previously described [19,55] and can be due to epidermal proliferation or increased thickness of the epidermis. The similar intensity of tryptophan fluorescence in lesional and non-lesional skin, points to abnormal keratinocytes proliferation in the entire skin of psoriatic patients.

Our results also revealed dermal changes related to collagen cross-links of both PDCCL and CDCCL in psoriatic patients. Lesional and non-lesional sites of psoriatic patients had similar emission spectra, which differ significantly from healthy subjects. No statistical differences were observed in the signal of ECL, although it tended to be higher in psoriatic lesional areas. Previous studies demonstrated an increased collagen synthesis in psoriasis by using *in vitro* and *in vivo* models [56–58]. Furthermore, the reported rate of collagen synthesis higher in biopsies obtained from lesional and non-lesional areas of psoriatic patients compare with healthy volunteers [56]. Our results indeed indicated higher collagen fluorescence. However, they also give information about collagen cross-links and therefore, suggest higher cross-linking of collagen in lesional and non-lesional areas of psoriatic patients. The literature lacks the information about the degree of collagen cross-links in psoriatic patients' dermis. Nevertheless, our data is supported by previous work, showing that the fibroblasts of psoriatic patients exhibit an enhancement of oxidative damage as shown by the high levels of carbonylation *in vivo* and *in vitro*. The level of carbonyl groups, induced mainly by H_2O_2, was found to be higher in both fibroblasts and tissue sections prepared from skin biopsy samples of lesional and non-lesional tissue from psoriatic patients [59]. Since carbonylation of macromolecules, such as collagen, catalyzed by H_2O_2 might lead to cross-links [60], it can be assumed that the augmentation in carbonyl groups encourages cross-linking of collagen. Additionally, the PDCCL signal is split into two peaks at 382 nm and at 404 nm only in psoriatic patients. This phenomenon is enigmatic and could be explained by the interaction of PDCCL with other fluorophores to form component that modify the emission spectra. The ultra-structural modification in the dermal region detected by fluorescence measurements possesses an important part in the progress of psoriasis. Dimon-Gadal et al. already showed that fibroblasts in the skin of psoriatic patients undergo changes even before psoriatic lesions are formed, and thus may be involved in the abnormal immune reactions leading to the onset of the disease [59]. Therefore, examination of dermal alteration might help in predicting the progress of disease. Moreover, fluorescent measurements are rapid, accurate and involve little discomfort to patient.

In conclusion, the effects of psoriasis and AD on human skin *in vivo* were assessed by the utilization of non-invasive measurements. Skin wash samples of SHB revealed a distinct pattern of pro-inflammatory cytokines and antioxidants in psoriatic, AD and healthy skin types. Moreover, lesional and non-lesional areas of psoriatic and AD skin demonstrated a different pattern. Fluorescent measurements of psoriatic patients also showed ultra-structural cutaneous modification compared with the control group. Our data are offered as a step towards the establishment of biological profiles corresponding to psoriasis and AD.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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This study has been carried out in the spirit of Professor Yoram Milner's tutelage who has passed away before the paper was sent for publication. This work is dedicated as a memorial to his great contribution to skin research.

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