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Research Article

Complementary Non-Invasive Methods to Assess Disease State in Psoriasis Vulgaris Patients

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Abstract

A complementary tool for management and monitoring of psoriasis, consisting of hydrophilic biomarkers collected from human skin surface and a biomarker measured by autofluorescent techniques, is proposed. A two-centre bilateral clinical study was performed on 48 psoriatic patients undergoing phototherapy and using two emollients as adjuvant treatments. Emollient A is a cosmetic preparation enriched with minerals and mud from the Dead Sea and emollient B is the carrier for A and served as a control. SHB (Skin Hydrophilic Biomarkers) were collected from patients and their autofluorescence measured using non-invasive methods. Bilateral post-treatment improvement was shown in clinical scores after the 8-week study period. Differences between lesional and unaffected psoriatic skin were seen in IL-8 and IL-1β levels and in autofluorescent signals of tryptophan moieties ($p<0.05$). IL-8 levels and TSC (Total Scavenging Capacity) were significantly different in psoriatic lesional skin vs. healthy skin and IL-8 and IL-1β levels differ in psoriatic unaffected vs. healthy skin ($p<0.05$). Post treatment scores differed when compared to baseline scores in IL-1β levels in both emollient treatments, A and B, and in tryptophan moieties autofluorescent signal only in group A. No significant differences were detected between emollients A and B. This study demonstrates that skin biomarkers measured in a non-invasive manner may be linked to the visual clinic classification i.e., affected and unaffected psoriatic skin and potentially be applied as indicators to predict therapeutic response and early relapse.

Keywords: Biomarkers; Non-Invasive Methods; Psoriasis; Skin Autofluorescence

Abbreviations

TRY	:	Tryptophan Moieties
SHB	:	Skin Hydrophilic Biomarkers

Introduction

The study of biomarkers has been constituently growing in the fields of human health and disease during the past three decades. Rapidly advancing technologies, such as' omics', have opened novel opportunities to explore aspects of biological and chemical diversity which were previously inaccessible. Detection

of pools of biomarkers provides a dynamic and powerful approach to understanding various pathologies. Their analysis can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of certain disease [1-3]. Validated correlations between biomarkers expression and disease severity and activity are the key to a successful contribution to the current clinical practice. The approach of using biomarkers to predict therapeutic response is now in routine practice in some cases of cancer. One successful example is the BRAF V600E mutation: a biomarker used to predict response to treatment of metastatic melanoma with the drug Vemurafenib. There has been growing interest for biomarkers applications in autoimmune diseases, partially as a result of the poor long-term success of existing treatments and challenging management of these ailments [4,5].

Psoriasis is a chronic autoimmune disease with genetic predisposition, involving inflammation and abnormal keratinocyte differentiation. The disease is associated with psychological stress [6] and metabolic stress syndrome and its constituent pathologies (insulin resistance, obesity, hypertension and atherogenic dyslipidemia) [7,8]. This may explain the variety in clinical presentation and severity of symptoms, as well as the unpredictable response to the various therapies. Its extensive dermal manifestations are extremely detrimental to the quality of life of those affected by the disease. According to a recent report of the World Health Organization [WHO] [9], in 2014 Psoriasis was recognized as a serious non-communicable disease. Improved care of patients around the world on all levels, social, psychological and healthiness was declared as a major target by the WHO. Among the actions of this resolution is priority to studies involving a better categorization of the disease, as well as development of tools to evaluate and predict clinical therapeutic efficacy. The study of biomarkers involved in Psoriasis has contributed to elucidating the pathophysiology of the disease. Current advancements in emerging omics technologies have opened opportunities for improved mechanistic studies as well as clinical applications [10-14]. Biomarkers analysis has become essential for effective translation [14] from preclinical models to human disease, predict response to therapy and early relapse [1,14-17].

A wide range of biomarkers, mainly genetic and immunologic markers, have been identified in blood and tissue of psoriatic patients [1,8,17]. These biomarkers are better categorized according to their bio-mechanistic pathophysiology rather than physiological location (blood, dermis, epidermis). These include oxidative stress, hyperproliferation, abnormal differentiation and inflammation. The (IL)-23/Th17 cell axis has been reported to play a pivotal role in the pathogenesis of psoriasis [10,18]. This involves upregulation of IL-17, IL-22, IFN-γ, and tumour necrosis factor (TNF) cytokines and oxidative imbalance [19] which lead to the inflammation typically observed in psoriatic lesions [10,20]. The abnormal keratinocyte differentiation and proliferation is associated with upregulation of K6 and K16 [21], p35, antigen Ki67, HSP60, Cx26 and Cx30 [22] in lesional epidermis. Increased levels of TNF-α, IFN-α, IL-2, IL-6, IL-8, IL-12, IL-23, IL-31, IL-23R and LIF-1 [19] have been found in psoriatic lesions. Most of these interleukins are general markers of inflammation and found in other inflammatory diseases. IL-36γ has been reported as specific for psoriasis [23]. Nevertheless, most of the psoriasis biomarkers are based on invasive procedures such as skin biopsies and blood sampling. Some non-invasive and minimally invasive methods for monitoring biomarkers on human skin have been recently developed, though, not yet validated [15, 24-26]. Therefore, there is a need for quick non-invasive sampling procedures combined with high throughput methods that will enable routine sampling of all skin areas of the patient (unaffected, mildly affected and severely affected). Large data collection is expected to allow method validation and diligent choice of biomarkers as indicators of disease in psoriasis. Biomarkers can be surrogate endpoints in clinical practice once these methods are validated. In a future perspective, the application of skin biomarkers analysis with pharmacogenomics will open new avenues for personalized medicine [14].

In this study, we propose a complementary tool for management and monitoring psoriasis, based on hydrophilic biomarkers collected from human skin and a biomarker measured by autofluorescent techniques. The samples were collected using non-invasive techniques and results were gathered with the relevant clinical scoring indices of the disease:

- PASI -Psoriasis Area Severity Index.
- IGA -Investigator Global Assessment.
- PSA - Patient's Subjective Assessment.

Our purpose was to assess the potential of four biomarkers; IL-8, IL-1β, Total Scavenging Capacity (TSC) and Tryptophan Moieties (TRY), as disease indicators and complement the classical disease indices used in clinical practice. For studying their potential as predictors of therapeutic response and of early relapse, we sampled the lesions of psoriatic patients before and after phototherapy, as well as their unaffected skin areas. Since patients received emollient treatment as adjuvant to phototherapy sessions we measured the hydration level of their skin as well. We propose a "Biomarkers Print" that includes a combination of relevant disease biomarkers which play different roles in the pathogenesis: hyper-proliferation, abnormal differentiation, inflammation and oxidative stress. Their selection for this study was also based on technical aspects such as assay detection limits, accuracies and reproducibility of results. IL-8 and IL-1β were chosen as inflammation markers, though they are common to many inflammatory diseases. IL-8 is a chemoattractant for neutrophils and IL-1β is an important mediator ("master switch") of inflammatory response. Furthermore, they both stimulate proliferation of keratinocytes and IL-1β is involved in cell differentiation [27]. The autofluorescence of Tryptophan moieties is an indicator of the structural changes occurring in the skin as a result of the disease. Higher

tryptophan moieties in psoriatic skin are probably a result of enhanced epidermal proliferation or higher epidermal thickness [24]. For monitoring the change in redox balance of the skin inflicted by the disease TSC (Total Scavenging Capacity) was measured on the hydrophilic skin wash samples collected.

This two-cantered study was performed on patients undergoing narrow band phototherapy (nbUVB) and using emollient preparations as adjuvant therapy. Bilateral use of the two emollients, A and B, allowed comparing their efficacy as adjuvants. A is a commercial cream enriched with Dead Sea Minerals, Dead Sea Mud and natural plant extracts. B is a simple emollient that served as the carrier for preparation A. The beneficial effects of Dead Sea in inflammatory skin disorders have been repeatedly demonstrated [28-30]. Clinical studies show soaking in Dead Sea Minerals relieves symptoms of Psoriasis Vulgaris [28, 29]. The Dead Sea mud and Dead Sea water are rich in magnesium, calcium, sodium, potassium, zinc, strontium, sulphides and bromides [31,32]. Magnesium and Calcium ions were shown to act as immunomodulators in cutaneous immune cells [33] and to modulate keratinocyte differentiation and proliferation [34-36]. Product A was specially developed as a non-drug preparation to deliver the Dead Sea therapeutic qualities to alleviate symptoms of psoriasis. This preparation was shown to attenuate symptoms in psoriatic patients using it a single treatment (data not shown).

Materials and Methods

Study Design

This two-centered bilateral double-blind study was carried out on Psoriatic patients undergoing narrow band phototherapy (nbUVB). The efficacy of two topically applied skincare preparations, A and B, was evaluated as adjuvant treatment to phototherapy, using two methods:

- "Classic" clinical Psoriasis indices e.g. PASI, investigator and subject evaluations.
- Non-invasive methods employing biochemical and fluorescent markers.

Patients were instructed to apply both products twice a day (morning and evening) on two halves of their body area, except for the mornings before phototherapy and study visits. Tubes were labeled "Left" and "Right" and randomization to avoid preference of one side. Two symmetrical psoriatic lesions were selected by the investigator for monitoring and measurements, usually in the arms. The study lasted for 8 weeks and included four visits T(0), beginning of the study, T(2) second visit after 2 weeks, T(4) third visit after 4 weeks, and T(8) fourth and last visit after 8 weeks, PASI, investigator and subject assessments were recorded for each side. Skin hydration was measured on each of the lesions. Skin biochemistry was collected from patients and autofluorescence measured at T(0) and again at either T(2) or T(4), depending on the PASI score. Clinical scores were correlated with autofluorescence and skin biochemistry results, at baseline and vs the two treatments. Autofluorescence and skin biochemistry baseline results were also correlated vs a group of healthy volunteers.

Study Population

Eligible patients, aged 18-70 diagnosed with moderate to severe chronic stable plaque type Psoriasis, for at least 6 months and candidates for phototherapy treatment, were enrolled in the study. Patients were excluded if they were in the process of diagnosis or treatment for cancer / kidney disease / liver disease or if they were pregnant or lactating. Subjects undergoing treatment with medication such as anti-inflammatories, anti-histamines, corticosteroids, systemically or topically applied, unless stopped for 4 weeks prior to the trial in the case of systemic treatment and 2 weeks in the case of topical treatment were excluded from the trial. No patient was enrolled without his or her signed and witnessed informed consent.

A group of 14 healthy volunteers (not suffering from Psoriasis or any other known skin condition) were asked to participate in the study. The volunteers were aged 19-68 and of equal gender distribution. After signing informed consent forms skin wash samples from their wrists were collected and skin autofluorescence was measured on 8 of them.

Adverse Events

Patients were asked to report any side effects, such as dryness, burning sensation, peeling of the skin, redness etc., immediately to the principal investigator or study monitor, in addition to keeping a record of all side effects during the study.

Study Treatment Preparations

The skincare preparations used in this study were developed and manufactured by AHAVA-Dead Sea Laboratories, Ltd., Israel. A is a commercial product, Clineral PSO body cream, enriched with minerals and mud from the Dead Sea. It was specially designed as adjuvant and maintenance treatment to alleviate symptoms of Psoriasis. B is a regular emollient, without ingredients from the Dead Sea, specially formulated for this study. It was used as a control for A in this study and therefore was formulated to be similar to A in colour and odour. Their lists of ingredients in INCI (International Nomenclature of Cosmetic Ingredients) are given in decreasing orders.

A: Aqua (Mineral Spring Water), Ethylhexyl Palmitate, Glycerin, Cetyl Alcohol, Glyceryl Stearate, Silt (Dead Sea Mud), Ceteareth-30, Cetearyl Alcohol, Propanediol, Allantoin, Stearalkonium Hectorite, Maris Aqua (Dead Sea Water), Peg-40 Stearate, Propylene Carbonate, Caprylic/Capric Triglyceride, Caprylyl Glycol, 1,2 Hexanediol, Dimethicone, Aloe Barbadensis Leaf Extract, Propylene Glycol, Butylene Glycol, Calendula Officinalis Flower Extract, Potassium Sorbate, Zinc Oxide, Triethoxycaprylylsilane, Dipotassium Glycyrrhizate, SorbitanTristearate, Bisabolol, Hip-

pophaeRhamnoides (Oblipicha) Fruit Oil, Lactic Acid, Tocopheryl (Vitamin E) Acetate, CymbopogonSchoenanthus Oil, Citral, Geraniol, Limonene.

B: Aqua (Mineral Spring Water), Ethylhexyl Palmitate, Cetyl Alcohol, Glyceryl Stearate, Ceteareth-30, Cetearyl Alcohol, Propanediol, Stearalkonium hectorite, PEG-40 Stearate, Propylene carbonate, Caprylic/capric triglyceride, Caprylyl glycol, 1,2-Hexanediol, Dimethicone, Glycerin, Potassium sorbate, SorbitanTristearate, Illite, Lactic Acid, CymbopogonSchoenanthus Oil, Citral, Geraniol, Limonene.

Blinding and Study Product Dispensing

This was a bilateral, double-blind study. A and B were packed in two kits, one labeling A as "Left" and B and "Right" and the opposite labeling in the second kit. The kits were sequentially dispensed so that both sides (left and right) are sampled with both study creams. This was done to avoid influence of uneven factors between the two sides of the body, such as exposure to the sun (trucker's tan). A and B were identical in color, texture and scent. The two creams were packaged in containers void of labeling except for the treatment code number and "Left" and "Right" indication and were identical in terms of shape, size and color so that identification of treatment assignment was unknowable to the participant, study investigators and medical personnel. The code for treatment identification was held by a company representative and revealed only after results were analyzed and study terminated.

Ethics

The study was approved by the Israeli Ministry of Health (registration code 20120306) and by the two Institutional Ethics Committees. Research Ethics Committee Reference Number are; 6851 for the Rabin Medical Center (RMC) and 0086-12-HMO for the Hadassah Medical Center (HMC). The study was registered at the NIH clinical studies portal (#NCT1651559). Two separate consent forms were signed by patients, one for participating in the two-emollient trial (all patients) and the other for agreeing to participate in the autofluorescence measurements and submit skin wash samples.

Study Endpoints

Primary Endpoints

The two primary endpoints of the study were modified PASI and Investigator's Global (IGA) assessment, evaluated for each of the two lesions of the patient at all 4 visits. Modified PASI was calculated for each half of the patient's body, left and right, on a scale from 0-12. IGA was also rated for two lesions separately for each side, on a scale from 0-5. For IGA scoring the following was used: 0=clear, 1= almost clear, 2= mild, 3=moderate, 4=severe, 5=very severe.

Secondary Endpoints

Secondary endpoints were patient's subjective assessment (PSA) and skin hydration level. For the PSA subjects were asked to rate 4 symptoms (Erythema, Induration, Desquamation and Pruritus) on a scale from 0-3 (0=Absent, 1 = Slight, 2 = Moderate, 3 =Severe). The PSA will be calculated by multiplying the score as follows: ∑Erythema(0-3) + Induration (0-3) + Desquamation (0-3) + Pruritus(0-3), scoring 0 in the absence of all three symptoms and 12 when all three are severe. Hydration level was measured using a Corneometer, CM 825 from Courage &Khazaka, CK. Skin hydration was measured on each visit on both sides of the body, on the lesions chosen for monitoring the of skin surface wash and fluorescence.

Measurement of Secreted Skin Hydrophilic Biomarkers (SHB)

Skin surface wash samples were collected from patients at T(0) and when their lesions had reached more than or equal to 50% of its initial modified PASI score, either T(2) or T(4). At T(0) a sample from their non-lesional skin was extracted from their arms, as well as samples from two symmetrical lesions. Skin surface wash samples from wrists of 14 healthy volunteers were collected. The methodology of skin extraction and their analysis was previously described [26]. Briefly, the skin is extracted using 1ml of a sterile 1% PBS solution at pH=7.4 (Sigma-Aldrich, Steinheim, Germany). The cap of a 10 milliliter Teflon test tube was used to hold the PBS solution on the skin for 30 minutes and then poured into the test tube and sealed with parafilm foil around the cap. Aliquots of 200 microliters were stored at -80◦C for future analysis. Total Scavenging Capacity of Antioxidants (TSC) and 2 interleukins levels were assessed up to 30 days from sample collection.

Quantification of the Total Scavenging Capacity (TSC)

The overall amounts of hydrophilic antioxidants, secreted by the skin in the samples collected, were determined using the Oxygen Radical Absorbance Capacity (ORAC) assay. The procedure was described [37] and consists of exposing the sample to a peroxyl generator 2,2′-Azobis(2-amidino-propane) dihydrochloride and comparison of its radical quenching capacity to Trolox, which is used as the standard. The $ORAC_{FL}$ 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. All reagents were prepared in 75 mM phosphate buffer (pH 7.4). 40 µl aliquots of sample, blank or Trolox dilutions were transferred into a 96-well microplate. 100 µl Fluorescein were added, to reach a final concentration of 96 nM. ORACFL fluorescence was read every 2 min for 70 min.

Evaluation of Interleukins Secretion

IL-1β and IL-8 levels were assayed by ELISA kit (Peprotech, Rehovot, Israel). Briefly, ELISA plates (Nunc-lmmuno Plate Maxisorb.Neptune, NJ) were coated with a cytokine-specific capture antibody and incubated overnight at RT. The plates were washed three times (using PBS containing 0.05% Tween-20), blocking solution (PBS containing 1% BSA) was added, and the plates were incubated for 1 to 2 hours at RT. Standards and samples from well

extract were then introduced into the wells and incubated for 2 hours at RT. The plates were then washed, and the appropriate 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 (TMB/E solution (Chemicon international, Temecula, Canada). Color development proceeded for 4 to 5 min at RT before being stopped by the addition of 2 N H_2S0_4. The absorbance was then measured at 450 nm using a Bio-tekPowerWave 340 microplate scanning spectrophotometer (Bio-TEK ELx, Winooski, VT, USA).The concentrations of both interleukins were calculated based on their corresponding standard calibration curve.

Skin Autofluorescence Measurements

Auto fluorescence spectroscopy of human skin is performed using a SPEX SkinSkanspectrofluorimeter (JY Horiba, Edison, NJ, U.S.A.). The excitation source is a Xenon arc lamp. The emission is recorded using a pen shaped probe with a flat tip that touches the skin. The evaluation of autofluorescent emission of skin was done by calculating the area beneath the spectral curve at each of the following wavelengths. Detection of tryptophan moieties was conducted upon excitation at 295 nm and emission was scanned from 310 nm to 450 nm (the peak is maximal at 350 nm).

9 patients were sampled at T(0) and then, when their lesions had reached more than or equal to 50% of its initial modified PASI score, either T(2) or T(4) and again at T(8). At T(0) a sample from their non-lesional skin was recorded, as well as samples from the two symmetrical lesions. Autofluorescence was measured on the arms of 8 healthy volunteers.

Data Quality Assurance

The study was conducted according to GCP guidelines and all documentation relating to the clinical study was monitored to verify adherence to the study protocol and the completeness and exactness of data entered on the CRF.

Data Analysis and Statistic Methods

The data was analyzed using the SAS® version 9.1 (SAS Institute, Cary North Carolina) by MediStat Ltd., Israel. All measured variables and derived parameters were listed individually and tabulated by descriptive statistics. For categorical variables summary tables were provided giving sample size, absolute and relative frequency. For continuous variables summary tables were provided giving sample size, arithmetic mean, standard deviation, minimum, maximum and 95% CI (Confidence Interval) for means, (by study group if available).

Primary Endpoint

Paired t-tests were applied for testing the statistical significance of the absolute and relative changes in modified PASI (total score) and in Physician's Global Assessment at weeks 2, 4, 8 from baseline. Wilcoxon tests and Signed Rank tests were applied for testing the statistical significance of the differences in the relative changes in modified PASI (total score) and in Physician's Global Assessment on last measurement (using LOCF method), from baseline. Chi-square tests were applied for testing the statistical significance of the difference in percent of lesions reducing by 30% and 50% in PASI on last measurement between the study groups (Responders analysis). Chi-square tests were applied for testing the statistical significance of the difference in percent of lesions reducing by 30% and 50% in Physician's Global Assessment on last measurement between the study groups (Responders analysis).

Secondary Endpoint and Other Assessments

Paired t-tests were applied for testing the statistical significance of the absolute and relative changes in Patient's Global Assessment at weeks 2, 4, 8 from baseline. Signed Rank tests were applied for testing the statistical significance of the relative changes in hydration level, at weeks 2, 4, 8 from baseline. Signed Rank tests were applied for testing the statistical significance of the relative changes in biomarkers results after treatment. Signed Rank tests were applied for testing the statistical significance of the relative changes in auto fluorescent results at last visits from first visits. Wilcoxon tests were applied for testing the statistical significance of the difference in biomarkers results between affected areas before treatment, unaffected areas after treatment and healthy patients. Wilcoxon tests were applied for testing the statistical significance of the relative changes in biomarkers results after treatment between the study groups.

Results

Subject Disposition

48 patients aged 18-70 were enrolled in the study, 26 males (54%) and 22 females (46%). Out of the 33 patients that completed the study, 18 agreed to sample skin SHB and 8 of them agreed to autofluorescence skin measurements. Table 1 shows patient distribution per centre and per study groups. The creams were dispensed in two versions of left/right labelled kits to assure bilateral randomization Table 2.

Number of patients enrolled	n	%
Hadassah Medical Center	17	35
Rabin Medical Center	31	65
Total enrolled from both centers	48	100
Completed the study	33	69
Withdrew	15	31
Skin wash samples collected	18	40
Autofluorescence measurements	8	17

Table 1: Subject Disposition

Treatment by side	n	%
Left labeled A/Right labeled B	25	52
Right labeled A/ Left labeled B	23	48
Total	48	100

Table 2: Patient Distribution by Treatment Kits Dispensing, To Assure Bilateral Randomization.

Adverse Events and Premature Termination of the Study

The reasons for premature termination of the study and adverse events are listed in Table 3. Three adverse events were reported during the study, one a burning sensation upon application of the creams, one patient's skin erythema and another patient's pruritus worsen after beginning the study. All three patients were excluded from the study. Since each subject had used both tested preparations, it was not possible to attribute the appearance of these adverse events to either emollient A or B.

Number of drop out patients	Number of patients	% of drop outs
Total	15	100
Unrelated health issues	7	47
Lost to follow up	5	33
Adverse event (erythema and pruritus) - possibly treatment related	2	13
Adverse event (burning sensation)- probable treatment related	1	7

Table 3: Adverse Events and Drop Out Patients.

The unrelated health issues for patients' termination of the study were: headache, urinary tract infection, alopecia, pyrexia, upper respiratory tract infection, rash and cessation of the phototherapic treatment.

Pre-treatmentBaseline Scores

Pre-treatment baseline scores and hydration measurements at 1st visit (T (0)) are given in Table 4. Biomarkers baseline levels of patients and healthy volunteers are given in Table 5. Table 6 shows the p values analysis of comparison between the baseline biomarkers means the groups studied, after combining A and B baseline scores.

Clinical Parameter/group	Affected skin - A (n = 48)	Affected skin - B (n = 48)
Modified PASI (0-12)	6.79±1.77	6.83±1.80
IGA (0-5)	2.88±0.70	2.88±0.70
PSA (0-12)	8.33±2.54	8.35±2.55
Corneometry (arbitrary units)	248±729	276±959

Table 4: Pre-Treatment Baseline Mean Scores and standard deviations of The Two Study Groups A and B.

Biomarker/ group	Affected skin A	Affected skin B	Unaffected skin	Healthy
IL-8 (in pg/ ml)	106.3±196.6 (n=17)	67.6±144.8 (n = 17)	8.7±14.6 (n = 17)	0.4±0.1 (n = 14)
IL-1β (in pg/ml)	21.4±32.5 (n=16)	12.1±15.8 (n=16)	2.2±5.2 (n=16)	3.2±1.0 (n=14)
TSC (uM of Trolox equivalents)	72±46 (n=17)	67±39 (n=17)	52±36 (n=17)	39±22 (n=26)
TRY - peak area $X10^5$ (arbitrary units)	783±613 (n=8)	457±291 (n=8)	247±223 (n=9)	301±168 (n=8)

Table 5: Pre-treatment Biomarkers Baseline Mean Levels of Patient's Affected and Unaffected Skin and of Healthy Volunteers' Skin.

No differences were found between baseline scores of the two treatment groups A and B. The reason being that, equally affected bilateral lesions, were selected for monitoring during the study. When combined, some baseline scores of affected areas, A and B, showed significant different values for biomarker levels and in tryptophan moieties signal (TRY) compared to unaffected and healthy skin (Table 6).

Parameter/ group	Affected vs Unaffected skin	Affected vs Healthy skin	Unaffected vs Healthy skin
IL-8 (in pg/ml)	0.0033	<.0001	<.0001
IL-1β (in pg/ ml)	0.0004	0.1624	0.0002
TSC (uM of Trolox equivalents)	0.0821	0.0019	0.2583
TRY - peak area in arbitrary units	0.0188	0.1046	0.3123

Table 6: Comparison of the Mean Baseline of SHB and TRY Levels Between the Groups, P Value Obtained by Wilcoxon Test Affected Scores are Results of Combined Pre-treatment Scores with A and B.

As can be seen from Table 5 and Table 6, IL-8 levels are a useful biomarker to distinguish between psoriatic, affected and unaffected, to healthy skin. IL-1β expression is significantly higher in psoriatic affected vs. unaffected skin and differs in psoriatic unaffected vs healthy skin. The TSC results are in line with a previous study [24] showing significant differences between affected and healthy skin. But, we did not find significant changes in TSC of

affected vs unaffected psoriatic skin in contrast with the previous study [24]. The higher TRY signals in psoriatic patients has been previously describes [24,38] and attributed to enhanced epidermal proliferation or higher epidermal thickness.

Change in Post Treatment Clinical Scores Within and Between Study Groups

All patients participating in the study experienced bilateral improvement in their psoriatic symptoms, as shown in Table 7. Improved scores were similar in both treatment sides A and B, and most patients experienced more than 50% improvement in all four clinical parameters after only eight weeks of treatment. Skin hydration improved significantly on every visit, though amelioration peaked after four weeks. This is in contrast to the three clinical indices which kept improving on every visit.

Parameter	Skin Preparation A			Skin Preparation B		
week	2	4	8	2	4	8
Modified PASI	20 (p<0.0001)	32 (p<0.0001)	54 (p<0.0001)	21 (p<0.0001)	31 (p<0.0001)	54 (p<0.0001)
IGA	10 (p=0.0258)	23 (p=0.0007)	49 (p<0.0001)	8 (p=0.0596)	23 (p=0.0014)	50 (p<0.0001)
PSA	25 (p<0.0001)	43 (p<0.0001)	60 (p<0.0001)	26 (p<0.0001)	42 (p<0.0001)	64 (p<0.0001)
Corneometry	23 (p=0.0107)	244 (p=0.0052)	56 (p<0.0001)	56 (p=0.0498)	213 (p=0.0156)	68 (p=0.0021)

Table 7: Percentage of Improvement from Baseline Mean Scores, on Each Visit Within Treatment Groups. Percentage was Calculated As: (Difference from Baseline Mean Score/ Baseline Mean Score) X 100. P Value from Paired T-Test, And for Corneometry Signed Rank Test Are Given for Each Difference Calculated.

p-value from Wilcoxon and Signed Rank tests were used to compare relatives change from baseline scores between the two treatment groups at weeks 2, 4 and 8. No significant differences were found between the two treatment groups (emollients A and B) on none of the time points for neither, PASI, IGA, PSA nor in the Corneometric measurements.

Pre-and post-treatment scores of biomarkers from skin wash samples (SHB) and TRY peak area were compared in all groups and between them. Fig 1 shows IL-8 and IL-1ßlevels in all groups. As shown in the graphs, following treatment, both interleukins in the affected areas, decrease to healthy and unaffected levels, in both groups. IL-1β showed significant post treatment levels compared to baseline, in both groups A and B. Figure 2 shows the differences in pre-to post treatment of each parameter for each of the groups. TRY showed significant change in signal from baseline only in the group A. Although the biomarkers were sampled according to the modified PASI score for each patient, no correlation was found between biomarkers change form baseline to modified PASI change from baseline.

Unfortunately, Inclusion of interleukins 17A, 12, 23, 20 and 31 in this study was not possible due to poor detection limits on samples collected by the SHB method (data not shown). These interleukins were considered due to their strong role in the pathogenesis of the disease.

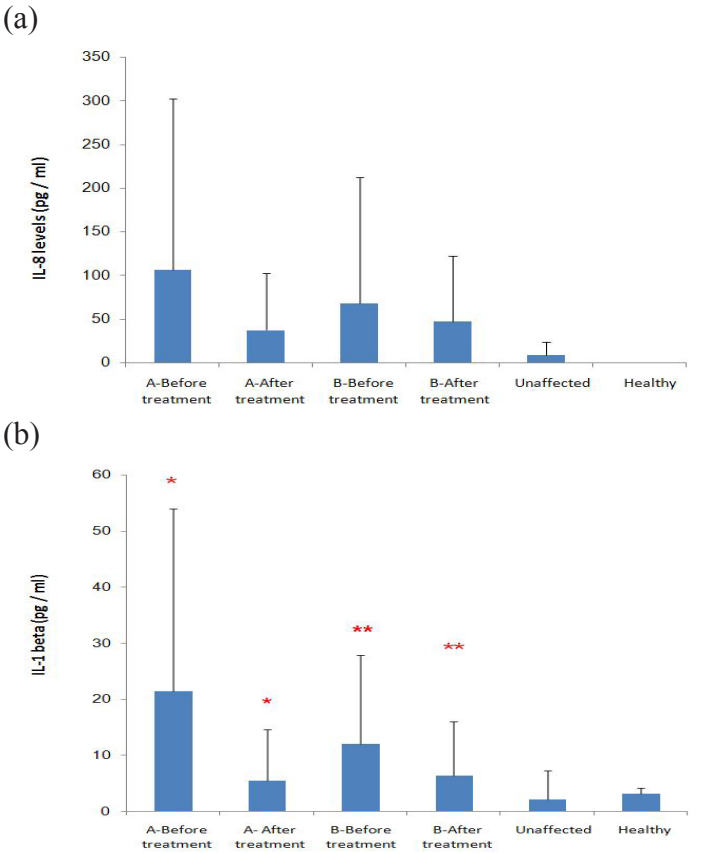

Fig 1: Mean levels of interleukins on healthy, unaffected and affected skin before and after phototherapy and treatment with emollients A and B. (a) IL-8 pg/ml measured in secreted hydrophilic skin wash samples. The number of samples is 17 in each group except for the healthy volunteers which is 14. (b) IL-1ß pg/ml measured in secreted hydrophilic skin wash samples. Error bars are given in the graphs. The number of samples is 16 for each group except for the healthy volunteers which is 14. Changes in pre post treatments in IL-1ß levels were significant ($p < 0.05$) for both groups A (*) and B (**).

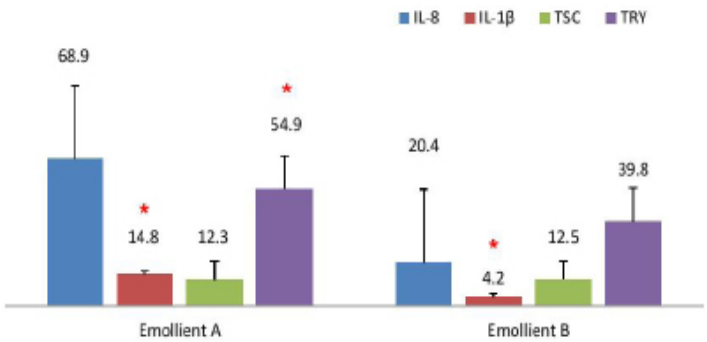

Fig 2: Differences of mean scores measured at reduction of 50% modified PASI for IL-8, IL-1β (pg/mL) and TSC (uM of Trolox equivalents) to mean baseline levels. TRY (in AU X105) peak area were compared at T(0) and T(8). Significant differences ($p < 0.05$), marked with *, were found between pre and post treatment with emollient A in TRY values and in IL-1β levels for both emollients tested. Standard error bars are given for each

Discussion

Psoriasis is a severe chronic disease, affecting more than 100 million people around the globe [9]. In spite of the numerous clinical studies carried out worldwide, management of this complex disease remains a major health and economical challenge [9]. The diagnosis of Psoriasis and its severity level are primarily based on clinical morphological evaluation of a skin lesion (mainly PASI), due to the absence of validated methods for monitoring the expression of disease at the biochemical level. This study proposes a new strategy for the evaluation of psoriasis severity by combining clinical scores and non-invasive biochemical analysis in psoriatic patients- a "Biomarker Print". The new strategy is demonstrated on psoriatic patients prescribed with phototherapy and topical skincare preparations as a complementary treatment.

Clinical Outcome of The Study

Phototherapy is the first line of treatment for psoriatic out breaks on account of its proven efficacy and relatively low cost [39,40]. Both primary and secondary endpoints were met as patients experienced bilateral improvements of more than 50% in all four clinical scores. Emollient therapy has been recognized as important adjuvant treatment in the management of Psoriasis during flare ups as well as for daily maintenance between flare-ups [41]. Daily application of emollients helps to protect skin barrier functioning via conserving the hydration level of the epidermis and in some cases calming irritation. This is the first study evaluating the efficacy of Dead Sea based emollients as adjuvants to phototherapy in psoriatic patients. The results demonstrated post treatment bilateral improvements in all patients' clinical scores. This suggests that both skin preparations A and B are effective for psoriatic patients as adjuvant treatments in addition to phototherapy. The failure to detect treatment wise differences between emollient A and B may be due to limitations in study design. The high efficacy of phototherapy in alleviating the symptoms of psoriasis, compromises our ability to assess the contribution of the emollient to the success in treatment. A larger number of study participants and longer periods of monitoring and follow up may have demonstrated otherwise.

Skin Biomarkers Analysis Using Non-Invasive Methods

Most biochemical data of psoriatic patients have been collected from blood and biopsies but data on skin surface biomarkers is scarce. As valuable as this information is, using invasive methods in regular clinical monitoring is strictly limited. Furthermore, repeated tests on a specific lesion, to access efficacy of treatment, multiple samples to access location related lesion characteristics and unaffected skin of patients cannot be regularly studied by sole biopsies. For these reasons, there is a quest for rapid non-invasive methods for topical monitoring of dermal biomarkers in psoriasis affected individuals. Along with previous studies on patient's skin performed by our groups, this study demonstrates the potential and feasibility of non-invasive methods.

Some of the biomarkers analyses were in line with the clinical scores, showing post treatment amelioration of the lesional skin. Post treatment decrease in IL-1ß levels was observed in both treatment groups, while both treatments did not affect the levels of TSC and IL-8. TRY levels decreased only by treatment with skin preparation A. This may indicate a higher sensitivity of these biomarkers compared with the clinical visual scoring (PASI and IGA). The higher sensitivity of biomarkers to the pathogenesis may account for the lack of correlation between patients' modified PASI scores and their biomarkers levels.

In this study, we found higher levels of TRY in affected vs unaffected skin and no significant difference between them to the healthy results. A previous study [24] found the differences in TRY are between the affected and unaffected to the healthy group. In both studies, the highest TRY levels were observed in psoriatic lesional skin area. Since in both studies the number of patients was small and the method is highly sensitive, more studies are needed to elucidate the potential non-invasive fluorescent methods to distinguish between various stages of psoriatic conditions.

All four biomarkers discriminate between psoriatic lesional and either or both, unaffected and healthy skin. IL-8 and IL-1β proved to be sensitive and efficient for this task. Additional studies with lager groups are necessary to further evaluate these four markers as a tool to support a clinical evaluation and disease monitoring of psoriatic patients.

Conclusion

Taken together, this study demonstrates that some skin biomarkers may be measured in a non-invasive manner and be linked to the visual clinic classification i.e. affected and unaffected psoriatic skin. The fact that IL-8 and IL-1β levels show significant distinction between psoriatic unaffected and healthy skin proposes they may be potential subclinical predictors of a flare up. This study suggests the potential of SHB and autofluorescence technologies to support the clinic with a quick, non-invasive measurement. In view of the complexity of the pathogenesis of psoriasis, we believe a "biomarkers print", rather than expression of a single biomarker, should be validated for a complementary platform for clinical diagnosis and prediction of treatment response.

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