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Research Article

Investigation of potential biomarkers for psoriasis in skin with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and ambient ionization mass spectrometry

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ABSTRACT

Background: Psoriasis is a chronic inflammatory disease with an unclear etiology that affects skin, nails, and joints and often accompanies comorbidities. Recent studies indicate that alterations in metabolites within psoriatic lesions might be linked to inflammation. Studying bioactive lipid mediators or metabolites in skin inflammation and immunity might provide new potential biomarkers and therapeutic prediction factors.
Methods: Lipids and peptides in the scale extracts from psoriasis patients and healthy controls were characterized by thermal desorption-electrospray ionizationmass spectrometry and matrix-assisted laser desorption/ionization time-of-flightmass spectrometry, respectively. Principal component analysis (PCA) was then applied to these data to identify potential differences between psoriasis patients and healthy controls.
Results: Psoriatic plaques show reduced wax esters and triglycerides and a predominant increase in human neutrophil defensins (HNPs), compared to non-lesional sites of psoriatic patients and healthy control. Additionally, when medical treatments were administered to psoriasis patients, levels of HNPs were significantly reduced, suggesting that they may serve as potential biomarkers to evaluate therapeutic efficacy for psoriasis.
Conclusion: Two mass spectrometric techniques were used to rapidly and non-invasively identify and monitor potential biomarkers between psoriasis patients and healthy controls. However, PCA results only showed slight differences, and we intend to broaden the sample base in the future to increase the statistical power of the investigation.

Abbreviations: AIMS, ambient ionization mass spectrometry; BSA, body surface area; DART, direct analysis in real time; DESI, desorption electrospray ionization; ELISA, enzyme-linked immunosorbent assay; GC-MS, gas chromatography-mass spectrometry; HCCA, alpha-cyano-4-hydroxycinnamic acid; HNP, human neutrophil peptide; LC-MS, liquid chromatography-mass spectrometry; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; PASI, psoriasis area and severity index; PCA, principal component analysis; PEG, polyethylene glycerol; PPG, polypropylene glycol; qRT-PCR, quantitative reverse transcription polymerase chain reaction; TD-ESI-MS, thermal desorption-electrospray ionization mass spectrometry; TFA, trifluoroacetic acid.

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1. Introduction

Psoriasis, a multifactorial skin disease, presenting with clear-demarcated erythematous plaques overlying silver scales, has an estimated worldwide prevalence ranging from 0.51 to 11.43 % [1]. While the histological features of psoriasis are well-defined, the precise etiological factors triggering psoriasis remain unclear. Several lipidomic and proteomic studies have been conducted to search for biological markers involved in inflammation and immune responses related to psoriasis etiology, severity grading, and prognosis [2–5]. Epidermal lipidomics is crucial in understanding the lipid structure, function of the epidermal barrier, and immune status [6]. A psoriatic skin lesion, one of the most common chronic inflammatory cutaneous disorders, gives rise to disturbances in epidermal lipidomics [7]. The disturbance triggers the release of arachidonic acid from phospholipids in the cell membrane, leading to the synthesis of potent mediators, such as prostaglandins and thromboxanes via the cyclooxygenase pathway, and leukotrienes via the lipoxygenase pathway. The arachidonic acid derivatives, potent mediators on the skin, promote inflammation. On the contrary, eicosapentaenoic acid and docosahexaenoic acid are released from blood endothelial cells to synthesize resolvins, protectins, and maresins, which aid in inflammation resolution and help return the body to homeostasis [3,4].

For skin barrier dysregulation in psoriasis, inflammation provokes cytokines that affect the skin's lipid biosynthesis and T-cell immunity [8,9]. Alterations in wax esters and triglycerides lead to an impaired skin barrier and a loss of protection against radiation and oxidation [10]. In addition, amino acids, acylcarnitines, amines, cholines, and histamine have been studied in relation to psoriasis to elucidate the causes and effects of the inflammatory process and cell hyperproliferation on the skin [2].

Traditionally, psoriasis diagnoses are made through physical examination, with the distinct observation of well-demarcated plaques featuring silver and coarse scales on various sites, such as the scalp, elbows, knees, shins, and gluteal areas. The psoriatic nail is also an important clue that supports the diagnosis of psoriasis in complicated cases [11]. It develops in psoriatic patients due to inflammation in the nail units, causing nail deformities, such as pitting, crumbling, leukonychia, or subungual hyperkeratosis. Skin biopsies are rarely used as a diagnostic tool for psoriasis, except in certain challenging cases, such as cutaneous T-cell lymphoma or pityriasis rubra pilaris. There are no specific laboratory or genetic tests for evaluating psoriasis [12]. Blood and urine are the tissue surrogates in psoriasis studies [13].

Techniques, such as ELISA (Enzyme-linked immunosorbent assay), immunohistochemistry, qRT-PCR (Quantitative Reverse Transcription Polymerase Chain Reaction), and nuclear magnetic resonance (NMR) have been used to search for psoriatic biomarkers in blood and urine [14–17]. However, without concrete molecular-level evidence for the pathophysiology, such histopathological examinations and immunoassays cannot effectively provide valuable insights into diagnosing and predicting the prognosis of psoriasis.

Besides immunoassays and other time-intensive techniques, modern mass spectrometry has emerged as a powerful tool for bioanalytical studies. Chromatography combined with mass spectrometry is commonly used to detect small biomolecules in psoriatic specimens compared to healthy ones. The serum samples are analyzed by liquid chromatography-mass spectrometry (LC-MS) to identify differences in the concentrations of acylcarnitines, phosphatidylcholines, amino acids, urea, phytol, and 1,11-undecanedicarboxylic acid [18]. In addition, multivariate analyses coupled with gas chromatography-mass spectrometry (GC-MS) have been used to detect α-ketoglutaric acid and other amino acids, such as asparagine and glutamine, in blood samples [19]. However, high-throughput analysis of the samples remains challenging due to the time- and labor-intensive sample pretreatment processes required for GC-MS and LC-MS.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a high-throughput technique, was developed to rapidly identify large molecular compounds, such as peptides and proteins, in biospecimens. The sample preparation processes for MALDI-TOF MS are usually straightforward: mixing the sample solution with an equal volume of saturated matrix solution. After drying, the sample spot is irradiated with a pulsed laser beam in a vacuum to desorb/ionize the analytes, which are then detected by a TOF mass analyzer attached to the MALDI source. For compounds with smaller molecular weights, ambient ionization mass spectrometry (AIMS) enables rapid in situ detection by ionizing targeted analytes under atmospheric conditions [13]. Sample pretreatment is usually unnecessary or minimal for AIMS techniques, such as direct analysis in real time (DART) [20] and desorption electrospray ionization (DESI) [21], which have been applied extensively in bioanalytical studies.

Thermal desorption-electrospray ionization mass spectrometry (TD-ESI-MS), an AIMS technique, functions on the interactions between thermally desorbed molecules and the charged solvent species generated by electrospray ionization of acidic solvent in open air [22]. The technique uses an inoculation loop to sweep through a solid sample surface to collect trace analytes or dip-and-remove from a liquid sample. The TD-ESI source contains a thermal desorption unit for analyte desorption and an ESI device for ionization before the analyte ions are drawn into a mass analyzer for characterization. Previous publications have demonstrated excellent performance of TD-ESI-MS and TD-ESI-MS/MS in biomedical studies, including pesticide and over-the-counter drug detection from saliva, gastric lavage, and residuals for emergency care and medication detection on the skin, oral fluids, and gastric lavage [23–31].

As mentioned above, this study employed TD-ESI-MS for the rapid and non-invasive detection of compounds in psoriasis scale extracts. Given the polarity range of the target compounds, a dual analytical approach was utilized: TD-ESI-MS for volatile and thermally stable molecules and MALDI-TOF MS for biomolecules such as proteins and peptides to compare lipid or metabolite differences among psoriatic plaque, non-lesional skin from psoriatic outpatients, and the normal skin of healthy controls. This approach allowed us to evaluate possible biomarkers for the pathogenesis and prognosis of psoriasis in a rapid and non-invasive manner.

2. Experimental section

2.1. Study cohort

A total of 45 participants were recruited for this cohort study, including 40 diagnosed outpatients and five healthy controls from the dermatology department of Hualien Tzu Chi Hospital from November 2022 to March 2023 (IRB110-284-A). Ten participants who had visited twice were included to compare the differences in lipid and protein profiles in psoriatic plaque. The participants, who received biologic therapy #1 or traditional treatment #2 (oral medications, topical ointments, and phototherapy), were classified into two groups: mild and moderate-to-severe, based on the severity grading of their psoriasis. Severity was determined by assessing body surface area (BSA) and psoriasis area and severity index (PASI). BSA is a simple self-assessment method commonly used following the "rule of nines" to estimate the distribution of psoriatic plaques. Mild psoriasis was defined as psoriasis plaques covering less than 10 %, moderate psoriasis covering 10 to 20 %, and severe psoriasis covering over 20 % of the body area. PASI accounts for the psoriatic body area and the presenting plaques in the signs of erythema, induration, and desquamation to assess disease severity as a percentage. Those scoring under 10 % are classified as mild psoriasis, scores of 10 to 20 % are classified as moderate psoriasis, and scores exceeding 20 % are classified as severe psoriasis [32]. While BSA is a simple self-assessment method for patients, PASI is a more formal evaluation used in clinical trial research. The two methods are meant to complement, not contradict, one another, with most clinical trials using

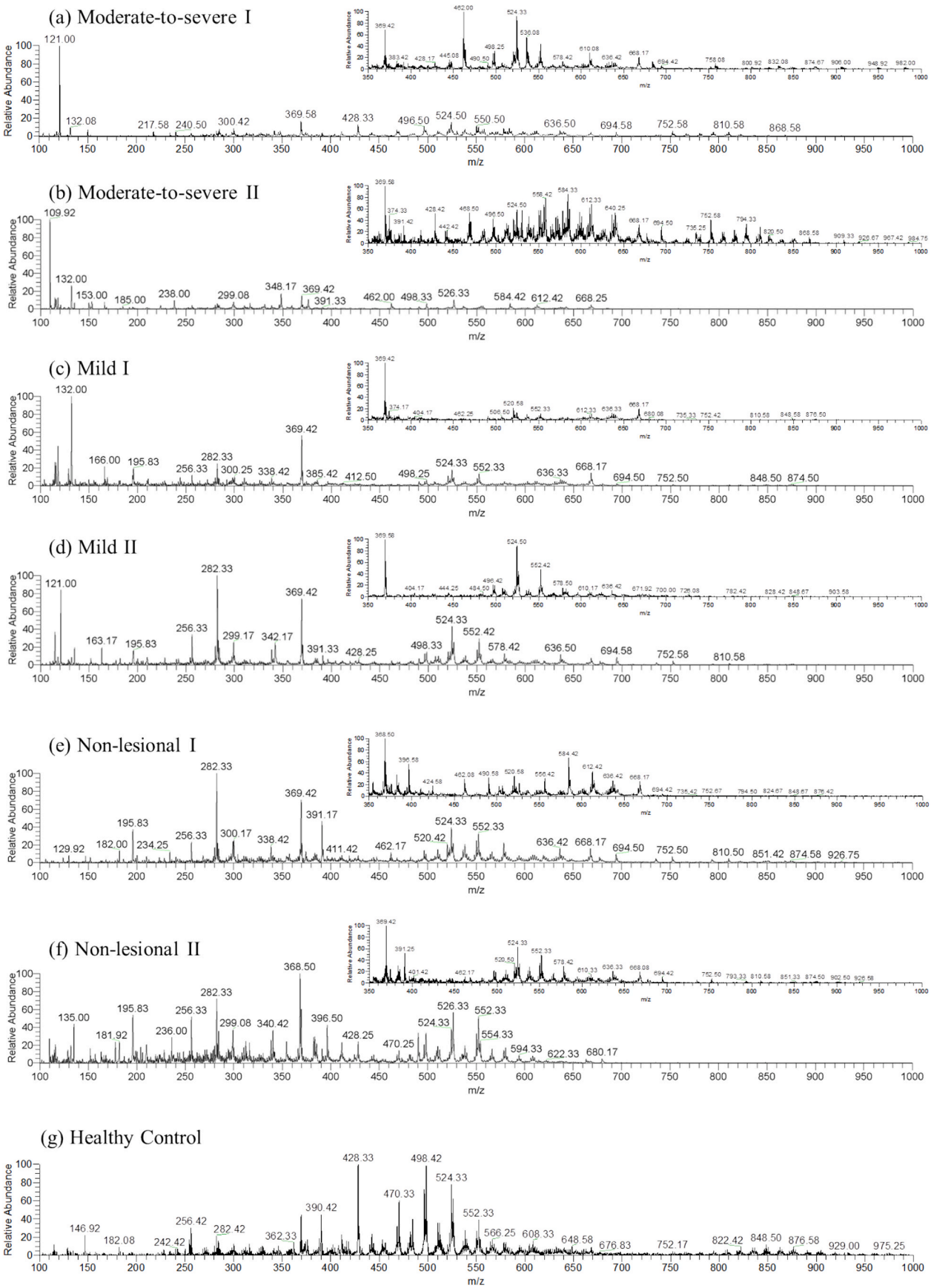

Fig. 1. The full scan of TD-ESI mass spectra (*m/z* 100–1000) of epidermal extracts obtained from psoriatic patients with varying severity: (a,b) moderate-to-severe, (c,d) mild, (e,f) non-lesional, and (g) healthy control. Each mass spectrum of psoriasis patients has a specific range (*m/z* 350–1000) indicated in its inset.

both assessment methods to define disease severity.

2.2. Sampling and sample pretreatment

Epidermal specimens were obtained using a blunt-edge scalpel by shedding the scales from the psoriatic plaque and their peripheral non-lesional normal skin. Each patient was sampled from both a psoriatic plaque and non-lesional normal skin at two distant body regions. The scales of five healthy controls were also collected from similar sites as those of psoriatic patients. Each collected specimen was placed individually in a glass container and stored at −20 ℃. In addition, all samples were collected within one month to ensure that the analytes in the samples did not decay. The specimens were allowed to stand at 25 °C for 10 min before adding 500 μL of methanol for further analysis. The specimens with methanol were subjected to 20 s of vortex mixing for thorough extraction. The resulting extracted solution (400 μL) was transferred to a vial for storage and mass spectrometric analyses. For the TD-ESI-MS analysis, the electrospray solution consisted of methanol, distilled-deionized water, and formic acid (50/50/0.1, v/v/v) and was prepared and employed to facilitate the ionization of the lipids in the epidermal extractions. For the MALDI-TOF MS analysis, the matrix solution was prepared by dissolving 10 mg of alpha-cyano-4-hydroxycinnamic acid (HCCA) in a solvent of 70 % acetonitrile with 1 % trifluoroacetic acid (TFA) to assist in the desorption and ionization of peptides in the epidermal extractions. All chemical reagents used in the study were obtained from Merck (Darmstadt, Germany). HCCA was purchased from Sigma Aldrich (St. Louis, MO, USA). Distilled-deionized water was purified using a water distiller (PURELAB Classic UV from ELGA, Marlow, UK).

2.3. ESI-MS analysis

ESI-MS analysis was performed in positive ion mode using a Shimadzu LC-MS system (Kyoto, Japan). The system included a binary pump (Nexera X2, LC-3AD), degasser (DGU-20A5R), column oven (CTO-20AC), and a triple quadrupole mass analyzer (LCMS-8045). Mobile phase A consisted of 5 mM ammonium acetate in double distilled water with 0.1 % formic acid, while mobile phase B comprised 5 mM ammonium acetate in methanol with 0.1 % formic acid. During analysis, the injection volume and the flow rate of the mobile phase were set at 3 μL and 0.4 mL/min, respectively. The elution gradient was set at 40 % of mobile phase B throughout the analysis. The operational parameters for the mass spectrometer included an interface voltage of 4.0 kV, a nebulizer gas flow rate of 3 L/min, a heating gas flow rate of 10 L/min, an interface temperature of 300 °C, a desolvation line temperature of 250 °C, a drying gas flow rate of 10 L/min, and a heat block temperature of 400 °C.

2.4. TD-ESI-MS analysis

The details of the instrumental setup have been described previously [22,33]. A sterilized sampling probe consisting of a metallic inoculating loop (length: 60 mm, radius: 2 mm, inner diameter: 1.5 mm; Yu Shuan Technology, Kaohsiung, Taiwan), attached to an acrylic holder, was loaded with a liquid sample (2 μL) transferred by a micropipette. The loaded probe was inserted into a quartz tube in the TD-ESI source to desorb and ionize the analyte. The temperature of the TD unit was kept at 280 ℃ by a temperature controller (AT-502; ANLY, Taipei, Taiwan) to evaporate the analytes on the probe. The desorbed analytes were subsequently carried by nitrogen gas (5 L/min), streaming down from the top of the TD unit to an ESI plume. Before entering a linear quadrupole ion trap mass analyzer (LTQ XL Thermo Scientific, Waltham, MA, USA), the desorbed analytes underwent ion-molecular reactions with the charged solvent species in the ESI plume. The skin extracts were analyzed in positive ion mode with unit mass resolution and a mass range of *m/z* 100 to 1000. A complete TD-ESI-MS analysis was completed in approximately sixty seconds, involving ten seconds for sampling, thirty seconds for analysis, and twenty seconds for burning away the residual sample on the inoculating loop with a torch after it was ready to be reused for sampling.

2.5. MALDI-TOF MS analysis

One microliter of each epidermal extract sample solution was deposited on a spot on a MALDI target plate and mixed with an equal volume of alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix solution. After air drying, the sample plate was transferred into a MALDI-TOF (AutoFlex III, Bruker Daltonics, Leipzig, Germany) ionization source operated using the FlexControl 2.2 software (Ver. 3). While in the MALDI source, each sample spot was irradiated with a pulsed Nd-YAG laser (355 nm) for desorption and ionization. One thousand laser shots were averaged per sample to obtain a representative mass spectrum. Triplicate analyses were performed for each sample. To avoid the sweet spot effect that occurred in MALDI-TOF analysis and to import high-quality mass spectra into subsequent statistical software, MALDI mass spectra were recorded under manual operation at the beginning of this study. Random walk mode was then applied to collect MALDI data based on the optimized V_{sample}/V_{matrix} ratios (1:1) for further analysis. The positive ion MALDI mass spectrum was recorded in linear mode at an acceleration voltage of 20 kV in delayed extraction mode. External calibrations were performed using horse apomyoglobin (*m/z* 16,952), bovine cytochrome *c* (*m/z* 12,361), insulin (*m/z* 5734), and ACTH 18–39 (*m/z* 2466) standards. The tolerance of mass error was set at ± 100 ppm in this study.

2.6. Statistical analysis

Principal component analysis (PCA) was applied to the mass spectrometric data using GraphPad Prism 10.0 (GraphPad Prism, Prism 10 for Mac, version 10). PCA reduces the dimensionality of the data while preserving the most variation (i.e., different mass-to-charge ratios and signal intensities) in the original data set [34]. The similarities and differences in acquired mass spectra among psoriatic plaques, the non-lesional parts of the patient's epidermis, and healthy controls were compared based on the PCA analysis.

3. Results and Discussion

3.1. Composition of skin surface lipids detected by TD-ESI-MS

Since the skin samples were extracted in methanol, the solution was directly injected into the ESI-MS to study the chemical composition in the solution. Fig. S1 shows the representative ESI mass spectra selected from the moderate-to-severe psoriasis plaques **(**Fig. S1**a)** and non-lesional epidermis **(**Fig. S1**b)** extracts obtained from one of the patients. The predominant ions detected in the ESI mass spectra are mostly from synthetic polymers with mass differences of 44 (polyethylene glycol, PEG) and 58 (polypropylene glycol, PPG). The origin of the polymers may be the ointment used for psoriasis treatment, and these polymer signals seriously suppressed the signals of biological compounds in the sample solution. TD-ESI-MS was used to reanalyze the samples to avoid interference from polymer signals. Polyethylene glycol and polypropylene glycol are non-volatile and will not be desorbed and ionized in the TD-ESI process, allowing the signals of biological compounds in the scale extracts to be observed.

Previous studies revealed that the predominant ions detected on skin with TD-ESI-MS originated from lipids, including free fatty acids, cholesterol (*m/z* 369), squalene (*m/z* 411), wax esters (*m/z* 450–700), and triglycerides (*m/z* 750–1000). These ions are either decomposition products of epidermal cells or primary components of sebum secreted by the sebaceous and sweat glands [33]. Fig. 1 displays the TD-ESI mass spectra of the extracts from psoriasis plaques **(**Fig. 1**a-f)** and the healthy

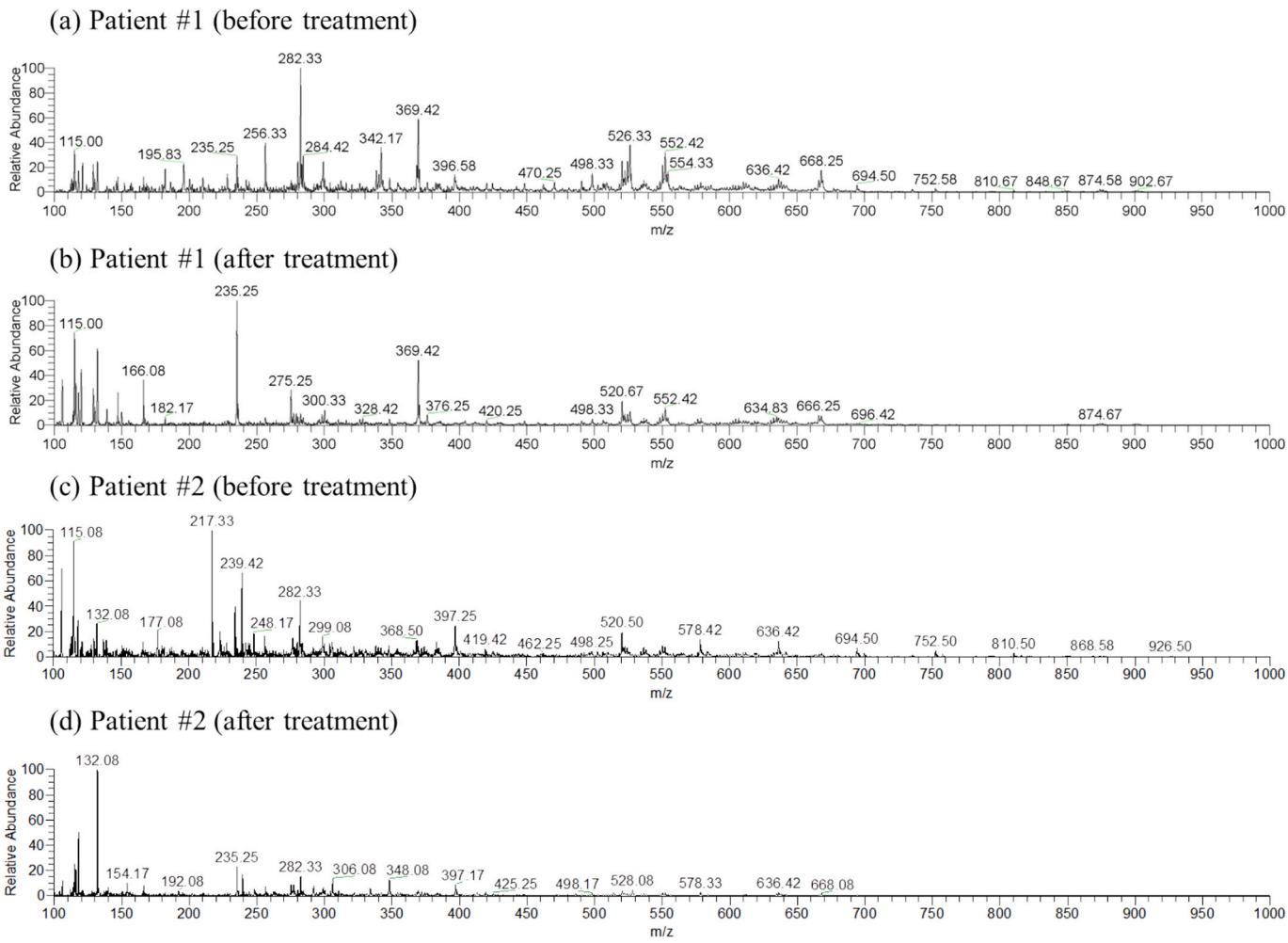

Fig. 2. The full scan of TD-ESI mass spectra was obtained from two representative patients: (a,c) before and (b,d) after treatment with psoriatic plaque extracts. Note that #1 received biologic therapy, while #2 received traditional treatment.

control's epidermis (Fig. 1**g**). Two representative mass spectra (I & II) were selected from distinct individuals. A representative mass spectrum from triplicate analyses of each sample is presented. The ion patterns of the healthy controls in the TD-ESI mass spectra show similar results to those of previous studies [33,35]. Keratinocytes and sebocytes produce lipids such as triglycerides and cholesterol esters to cover the skin's surface [36]. The extracellular lipid matrix also contains cholesterol, supporting the epidermal keratinocytes [37]. As for free fatty acids, most are produced by the epidermis and sebaceous glands [38]. The absence of ion signals from fatty acids could be due to the sampling of lipid-lacking skin areas, such as the gluteal region and limbs. By comparing the ion patterns of the seven mass spectra in Fig. 1**a-g**, there seems to be no significant difference between the non-lesional epidermis of psoriatic patients (Fig. 1**e and f**) and the normal skin of healthy controls (Fig. 1**g**). However, the intensities of wax esters (*m/z* 450–700) and cholesterol (*m/z* 369) were less abundant in the psoriatic plaques of severe psoriasis patients (Fig. 1**a and b**) compared to the non-lesional epidermis of psoriatic patients (Fig. 1**e and f**) and healthy controls (Fig. 1**g**).

The non-lesional epidermis of psoriasis patients appears to have similar mass spectra to those of the control group. This is because the epidermis of both skin samples is primarily covered with triglycerides, wax esters from sebum, and cholesterol from keratinocytes [39]. Although research has shown that the metabolites can serve as psoriasis biomarkers in skin extracts, they are rarely detected due to the matrix effects of the predominant epidermal lipid ions in this study. Since TD-ESI-MS counts as AIMS, it is susceptible to matrix effects that can impact results in a short amount of time. Therefore, more specimens must be collected for an optimized sample pretreatment process to minimize the matrix effects as much as possible.

3.2. *PCA of lipid ion signals from psoriatic plaques, non-lesional skin, and healthy controls*

PCA was applied to the mass spectrometric results for classification based on the significant features of the following sample types: psoriatic plaques, non-lesional epidermis from psoriatic patients, and normal epidermis from healthy controls. **Figs. S2-S4** illustrate the results of PCA based on the ions detected in different mass ranges. **Fig. S2** shows the results of the PCA score and loadings plots based on the ions detected in the mass range from *m/z* 100 to 1000. Even though there is a distinction between normal controls and others, the group of psoriatic plaque samples was not distinguished from those of non-lesional epidermis. **Figs. S3 and S4** display the results of the PCA score and loadings plots based on the ions detected in the mass ranges of *m/z* 100 to 500 and *m/z* 501 to 1000, respectively. Since the absence of sample pretreatment limits the detected analytes, the results indicated that regardless of the selected mass range, the differences in ion types and signal intensities of the prominent lipid ions did not effectively distinguish the psoriatic plaques from the non-lesional epidermis. Nevertheless, some unique lipid ions (*m/z* 400–1000) may serve as potential biomarkers to differentiate patient and control samples according to the PCA loadings plots.

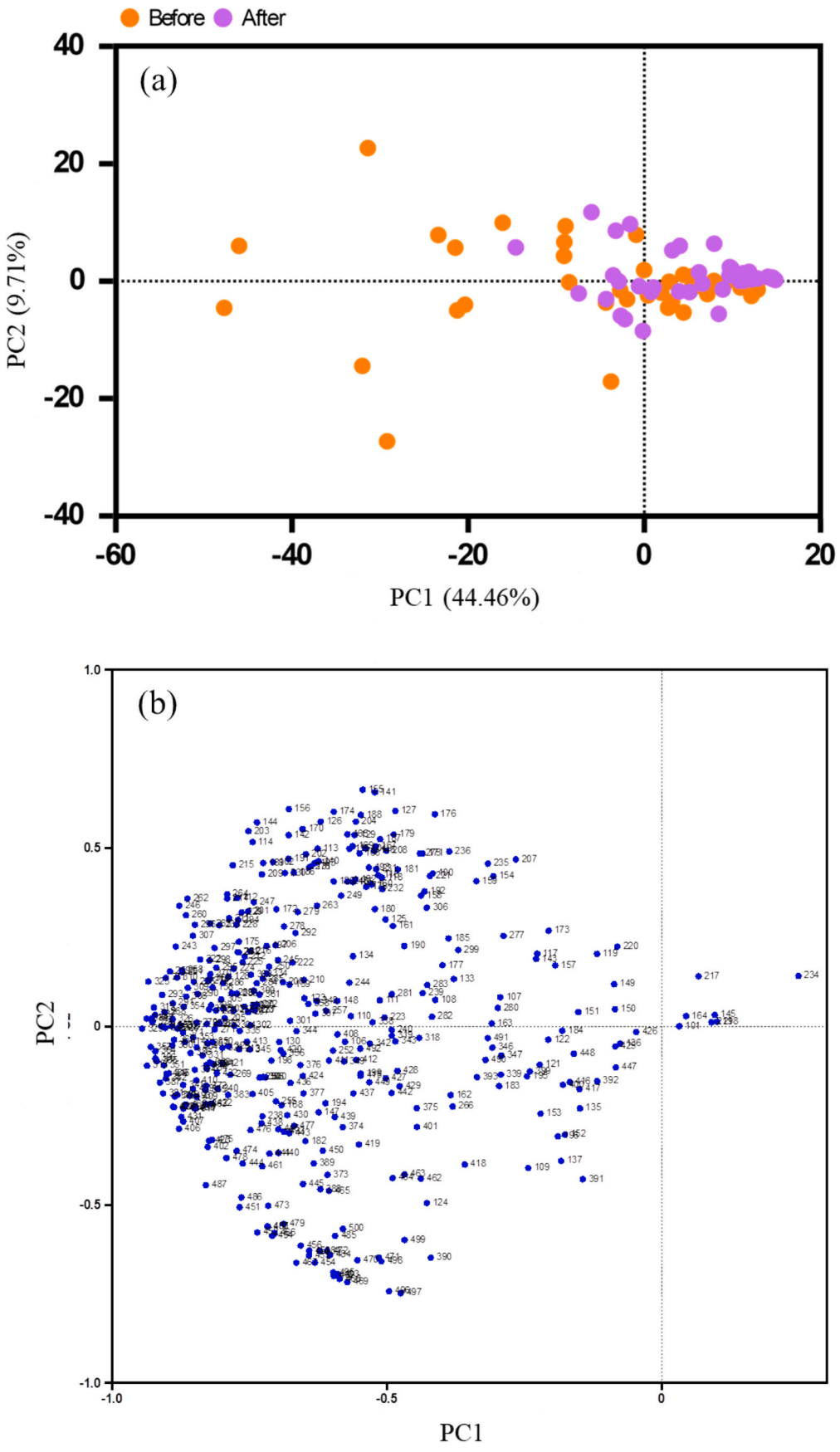

Fig. 3. The results of (a) PCA score plots and (b) loadings plots were obtained from TD-ESI mass spectra ranging from *m/z* 100–500 to compare differences before and after treatment.

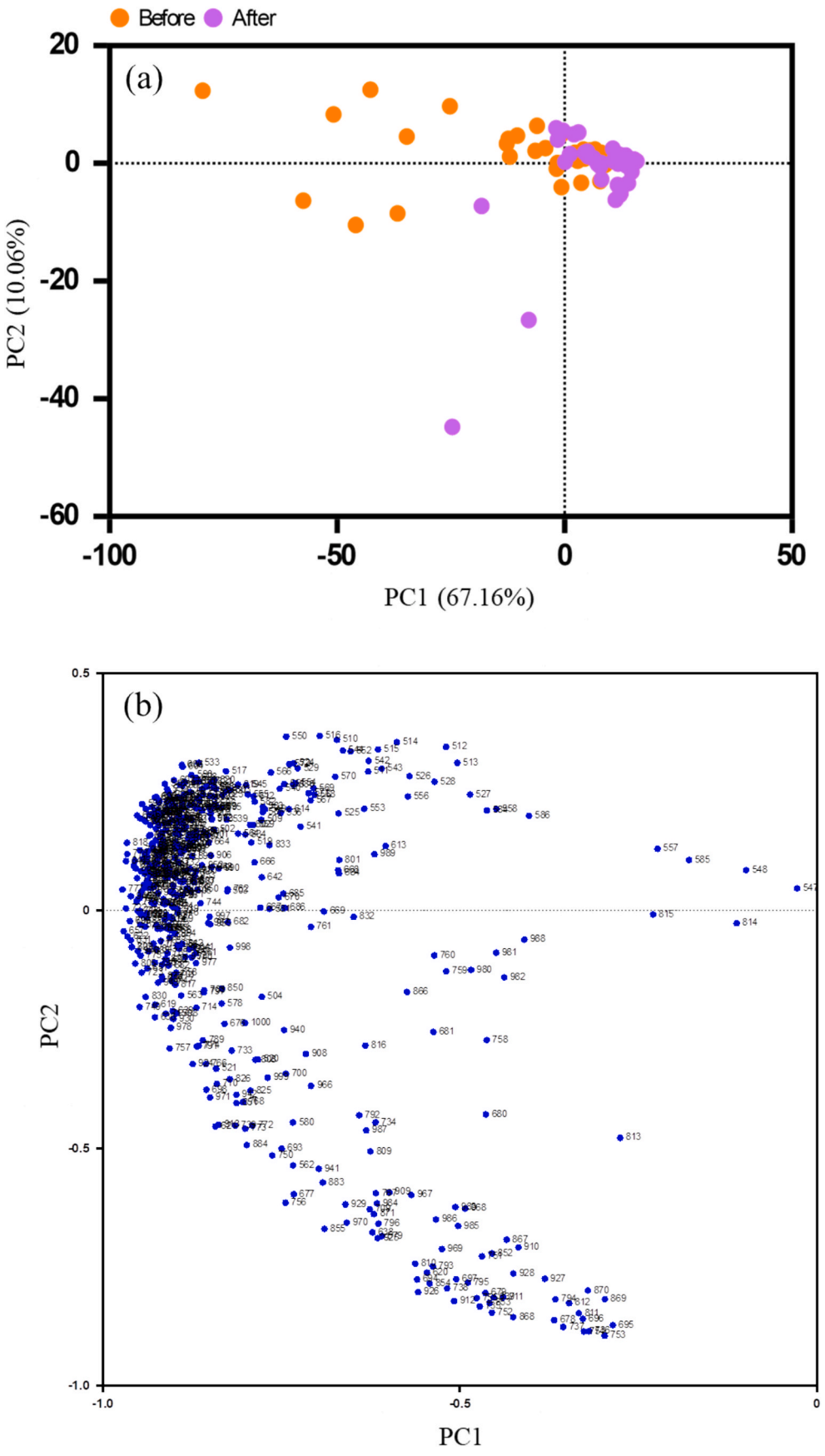

Fig. 4. The results of (a) PCA score plots and (b) loadings plots were obtained from TD-ESI mass spectra ranging from *m/z* 501–1000 to compare differences in before and after treatment.

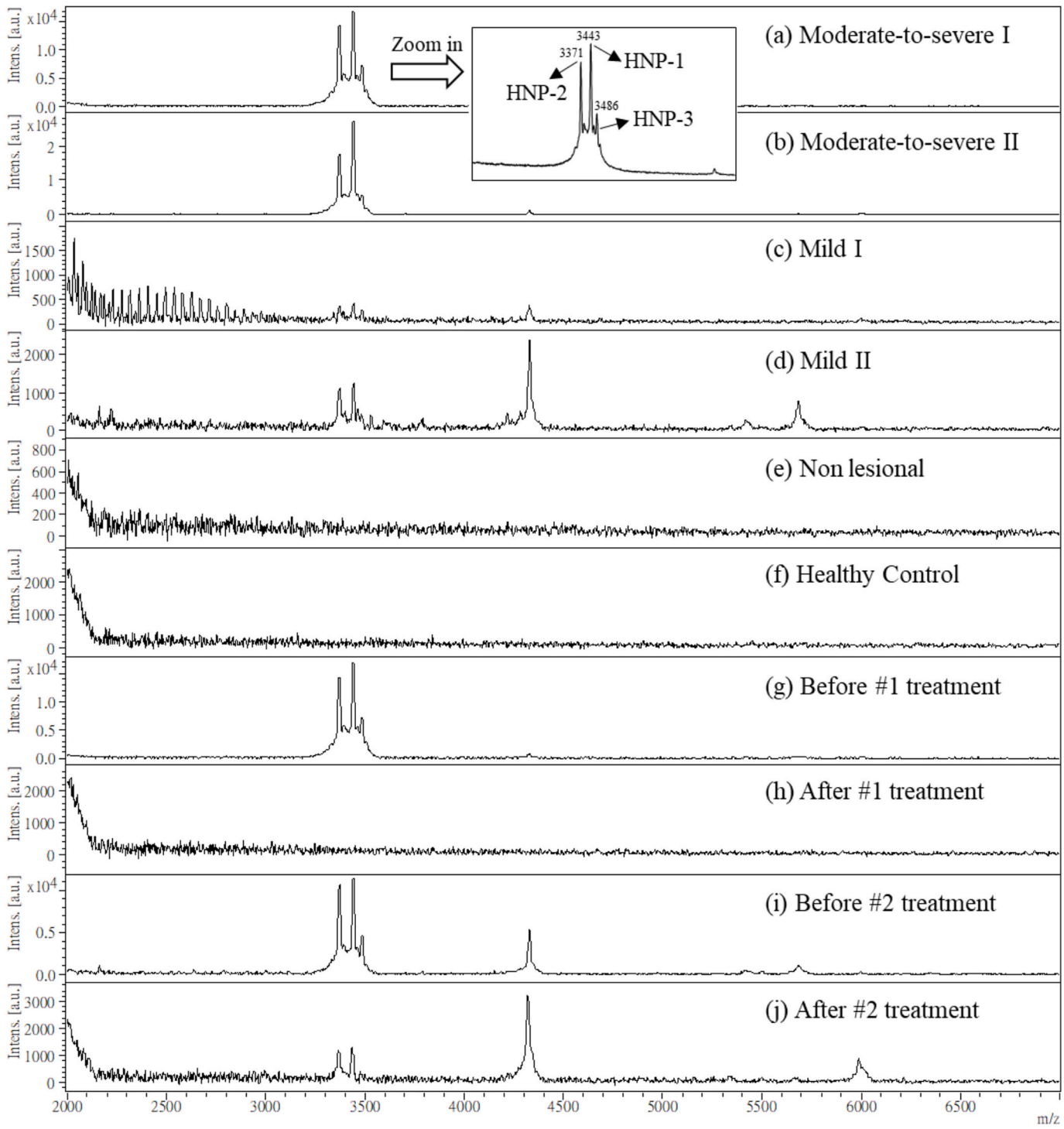

Fig. 5. The full scan of MALDI-TOF mass spectra (*m/z* 2000–7000) of epidermal extracts obtained from psoriatic patients of varying severity: (a,b) moderate-to-severe, (c,d) mild, (e) non-lesional, and (f) healthy control. The inset in (a) shows HNPs exhibited at *m/z* 3371, 3443, and 3486, respectively. Additionally, there are changes in psoriatic plaque extracts (g, i) before and (h, j) after treatment.

The mild and moderate psoriasis samples are also not well separated on the PCA score plots due to the similar lipid patterns in the individual mass spectra (Fig. 1**a-d**). As previously described, the main difference between the psoriasis groups is that the lipid ion intensities for the moderate-to-severe group (Fig. 1**a and b**) are lower than those of the mild group (Fig. 1**c and d**). The decrease in epidermal lipid ions may contribute to the inflammatory processes in psoriasis patients, which can alter the composition of epidermal lipids, leading to a damaged skin barrier and impaired skin function [10,40]. The PCA results based on the epidermal lipids in this study are similar to those of the previous study based on amino acids and other metabolites in psoriasis skin biopsies [2]. PCA's limitations in distinguishing among groups are partly due to noise in the dataset caused by matrix effects and restricted analyte detection. To overcome this, it is advisable to include qualitative and quantitative analyses of identified lipids in this study to reduce data noise and enhance the clarity of patterns. This approach would improve PCA's ability to differentiate among psoriasis severity groups and support more accurate biomarker identification.

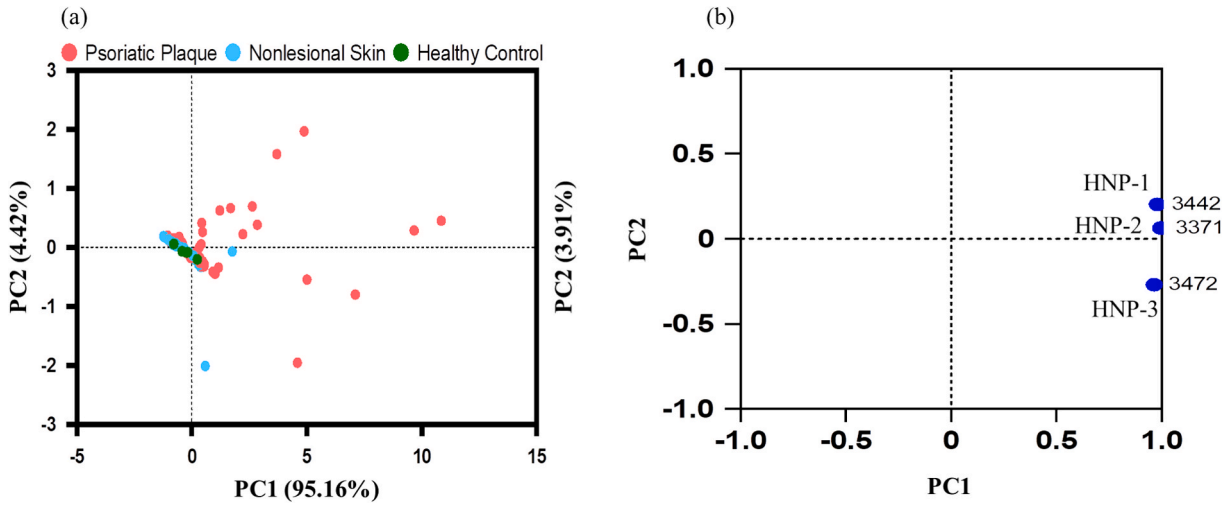

Fig. 6. The PCA results obtained from MALDI-TOF mass spectra of psoriatic plaque, non-lesional epidermis, and healthy control samples: (a) score plots and (b) loadings plots. The mass range of ions was selected *m/z* 2000 to 20,000.

3.3. PCA of lipid ion signals from psoriatic plaques before and after treatment

To evaluate the effectiveness of medical treatment for psoriasis, the analytes of the psoriatic plaque extracts from two representative patients were analyzed and compared before (Fig. 2**a and c)** and after (Fig. 2**b and d)** treatment. The results showed decreased lipid ion intensities after treatment, especially for the ions of wax esters (*m/z* 450–700) and triglycerides (*m/z* 750–1000). As can be seen, different medical treatments (#1 and #2) for psoriasis might change the level of skin barrier repair [41]. Fig. 3 shows the results ranging from *m/z* 100–500 of PCA score plots (Fig. 3**a)** and loadings plots (Fig. 3b) for the samples collected from the psoriatic plaques before and after medical treatment. The other mass range (*m/z* 501–1000) is also presented to observe the difference between before and after medical treatment (Fig. 4). These plots illustrate that specific ions do contribute to the observed—albeit subtle—separation, and these contributions are largely consistent with patterns observable in the raw data. However, there are still various ion signals (left side of loadings plots) that can serve as potential markers according to the results of the PCA loadings plots (Fig. 3**b and 4b)**. The current cohort is limited by an insufficient sample size, which is likely to contribute to the marginal separation of ion signals and constrain the identification of consistent markers for assessing skin barrier repair. To address this limitation, prioritizing the sample size in future studies is a prudent course of action to better capture the mass spectral variations influenced by treatments, enhancing the detection of potential markers associated with treatments.

3.4. Human neutrophil defensins (HNPs) detection with MALDI-TOF MS

Fig. 5**a-f** show the typical MALDI mass spectra of the samples collected from psoriatic patients and healthy controls. Compared with TD-ESI mass spectra, the MALDI mass spectra are relatively simple (**Fig. S5**). The signals of *m/z* 3371, 3443, and 3486 are detected as prominent ions in psoriatic plaques (Fig. 5**a-d)**. They are absent in the non-lesional epidermis (Fig. 5**e)** and healthy controls (Fig. 5**f**). These signals either disappeared or dramatically decreased after treatment (compare Fig. 5**g and i** with Fig. 5**h and j**). Based on the identification approaches, such as SDS-PAGE from previous reports [42–44], ion signals of *m/z* 3371, 3443, and 3486 were found to represent HNP-2, HNP-1, and HNP-3, respectively. In innate immunity, HNPs belong to antimicrobial peptides that exhibit microbial activity against bacterial and fungal infection [45]. They can be found in saliva, oral tissues, tears, and

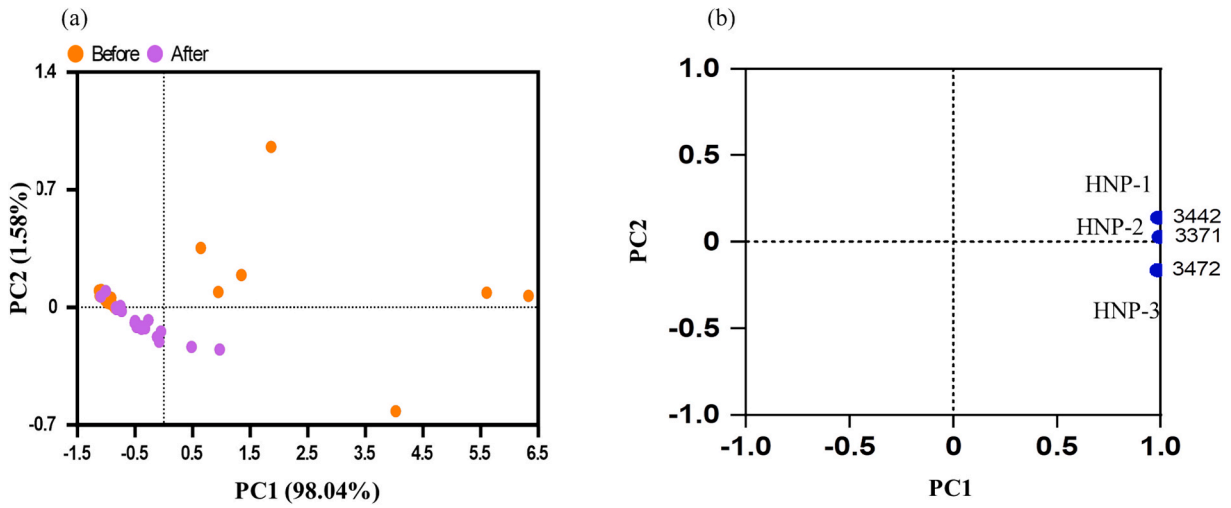

Fig. 7. The results of PCA (a) score plots and (b) loadings plots were obtained from MALDI-TOF mass spectra to compare differences in before and after treatment. The mass range of ions was selected *m/z* 200 to 20,000.

mucosa [46]. Inflammation often occurs in psoriasis, as it is a chronic inflammatory skin disease. Previous research has indicated that HNPs released in psoriatic plaques initiate inflammatory responses and T-cell immunity. This explains why psoriasis is considered a T-cell-mediated disease [47,48]. The presence of HNP in specimens of psoriatic patients indicates that the biomarkers of inflammation and antimicrobial peptides may play a vital role in the skin barrier. Based on our previous research, it can be inferred that HNP is detectable in various body fluids of patients who exhibit signs of inflammation regardless of the underlying disease condition, like the blood samples from patients with schizophrenia [42] and the blister fluids from patients with bullous pemphigoid [49].

3.5. PCA of HNPs from psoriatic plaques, non-lesional skin, and healthy controls

Previously, some proteins have been reported as potential biomarkers for psoriasis, such as C-reactive protein, S100A protein, and substance P [17,50–52]. C-reactive protein concentration in the bloodstream will increase when inflammation occurs; however, the protein is not found on the skin surface. Therefore, analyzing such compounds requires invasive sampling and time-consuming sample preparation. Although S100A and substance P may be present on the skin, they are more abundant in the dermis than the epidermis [17,51,52], and, hence, a tissue biopsy is needed. In addition, the proteins present in the dermis require multi-step isolation to be analyzed. Fig. 6 illustrates the results of PCA score plots (Fig. 6**a)** and loadings plots (Fig. 6**b)** based on the MALDI-TOF analysis for psoriatic plaques, non-lesional epidermis, and healthy controls. In addition, **Fig. S6** shows the results of PCA for the psoriatic plaque samples, indicating a lack of differentiation between mild and moderate-to-severe psoriasis. One possible reason for this is that individual variability in HNP levels influences the observed patterns, making it challenging to directly correlate these results with clinical severity. Moreover, according to the clinical definition, it is likely that the alleviation of psoriasis is associated with compositional changes in cells, which may not be fully captured by HNPs alone.

3.6. PCA of HNPs from psoriatic plaques, before and after treatment

Fig. 7 shows the results of PCA based on the MALDI-TOF analysis of the psoriatic plaques collected from patients before and after medical treatment. After treatment, the ion signals of HNPs detected previously in psoriatic patients either disappeared (Fig. 5**g and i)** or decreased dramatically (Fig. 5**h and j)** due to reduced inflammation and skin barrier repair. The MALDI mass spectra show no significant differences between the non-lesional epidermis of psoriatic patients (Fig. 5**e)** and the normal skin of healthy controls (Fig. 5**f)**. These results indicate that the presence of HNPs is related to whether the medical treatment was successful.

4. Conclusion

TD-ESI-MS and MALDI-TOF MS of skin extracts collected with non-invasive sampling and minimal pretreatment enables rapid analysis in the detection of potential biomarkers for psoriasis. The results of mass spectra of psoriatic plaques showed decreased ion signals of wax esters and triglycerides and increased signals of HNPs, and the PCA of those spectra indicated that the variance showed a minor, potential distinction between psoriasis plaques and non-lesional epidermis. In future studies, we intend to include more samples to strengthen the statistical results and support the meaningful identification of markers in patients with plaque psoriasis.

CRediT authorship contribution statement

Yi-Wen Hsu: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Hung Su:** Writing – review & editing, Methodology. **Deng-Chyang Wu:** Investigation, Conceptualization. **Chi-Wei Lee:** . **Sung-Jen Hung:** Resources, Methodology, Data curation. **Jentaie Shiea:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2025.04.004.

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