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Proteomic plasma profile of psoriatic patients

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Highlights

- In psoriatic plasma increase in proinflammatory and signaling molecules was found.
- Psoriatic patients plasma is characterized by decrease in, inter alia, apoM.
- In psoriatic patients plasma increase in 4-HNE-protein adducts was observed.
- Psoriasis specific proteins may contribute to more specific pharmacotherapy design.

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ABSTRACT

Background: Psoriasis is a chronic, immune-mediated inflammatory skin disease with severe consequences for the whole organism. The lack of complete knowledge of the main factors predisposing an individual to the appearance of psoriatic lesions, has recently led to the search for modifications in biochemical pathways participating in the development of this disease. We therefore aimed to investigate changes in the plasma proteomic profile of patients with psoriasis.

Material and Methods: A proteomics approach was used to analyze the expression of proteins in plasma from psoriatic patients and healthy controls (sex- and age-matched individuals). The analysis was performed using gel electrophoresis, followed by nanoflow LC-MS/MS using a Q-Exactive OrbiTrap mass spectrometer.

Results: Proteomic data indicated a significant decrease in the level of proteins involved in lipid metabolism, such as apolipoprotein M, and proteins involved in the management of vitamin D levels in psoriatic patients' plasma. These changes were accompanied by the expression of proteins involved in immune response and signal transduction. This was particularly evident by the level of transcriptional factors, including AT motif binding factor 1, which regulates excessive cellular proliferation and differentiation. It was also suggested that psoriasis development was associated with increased expression of proteins directly involved in signaling molecule secretion [biotinidase and BAI1-associated protein 3]. In addition, the lipid peroxidation product - 4-hydroxynonenal (4-HNE) generates higher level of adducts with proteins in the plasma of psoriatic patients. Moreover, plasma proteins from healthy subjects creating with 4-HNE adducts were mainly characterized as structural, while in the plasma of psoriatic patients, increased levels of 4-HNE-protein adducts with catalytic activity were observed.

Conclusion: The results presented herein confirm the current knowledge about the profile of proteins responsible for the immune response and management of vitamin D in the plasma of psoriatic patients. However, several new proteins were also identified, which are involved in signal transduction and lipid metabolism as well as catalytic activity. The expression or structure of these proteins was shown to change through the course of the development of psoriasis. This knowledge may help contribute to the design of more specific pharmacotherapy.

Abbreviations: Ps-psoriasis; Ctr-control.

Keywords: Psoriasis; Proteome; 4-HNE-protein adducts; Plasma

1. INTRODUCTION

Psoriasis, a chronic, immune-mediated inflammatory skin disease, is estimated to affect approximately 2–4% of global the population [1]. Symptoms of psoriasis may affect the body's entire surface, causing considerable psychosocial disability and majorly affecting a patients' quality of life [2]. Psoriasis prevalence may be associated with depressive illness, psoriatic arthritis, and even cardiovascular disease [3].

It is well recognized that psoriasis prevalence is associated with chronic inflammatory reactions *in vivo*, resulting in the increased expression of proinflammatory factors in both human and rat skin cells, as well as human plasma [4–6]. Changes in the signal transduction pathways in psoriatic skin tissue, including the activation of mitogen-activated kinases (such as p38, ERK1/2, JNK) [7,8]. and transcription factors involved in the inflammatory response (e.g. NFκB, Foxp3, HIF-1α), cause cells to proliferate (STAT3) and an antioxidant response (Nrf2) occurs [9–12]. In addition, the level of proteins involved in the antioxidant defense (including superoxide dismutase) are significantly increased in psoriatic skin tissue [13]. In psoriatic skin biopsies, there exists a strong imbalance between pro- and anti-apoptotic proteins, with a significant shift toward the pro-apoptotic direction [14].

As a consequence of the local changes described above, psoriasis development is associated with increased inflammatory and oxidative conditions, resulting in oxidative modifications to the structure and function of certain plasma antioxidant proteins and lipids [15]. Moreover, low molecular weight electrophilic lipid peroxidation products may also interact with proteins and, via adduct formation, alter protein structure and function further [16]. To date, increased levels of 4-hydroxynonenal [4-HNE] and 4-HNE-protein adducts were observed in the blood of patients with another skin disease – vitiligo [17]. The influence of lipid peroxidation products on the proteomic profile of psoriatic patients' plasma remains to be elucidated. It is therefore assumed that psoriatic patients' cellular metabolic disorders may affect the proteomic profile, including structural modifications and 4-HNE-protein adduct formation. Evaluating protein profile changes will help identify metabolic pathways that could be useful for early disease diagnosis and improving or selecting appropriate pharmacotherapy.

Therefore, the aim of this study was to analyze the proteomic profile of plasma from psoriatic patients, with special attention paid to protein modifications caused by lipid peroxidation products.

2. MATERIAL AND METHODS

2.1. Plasma samples

Blood samples were collected from 6 untreated psoriatic patients (3 men and 3 women; age range 27–54 years, mean 40) and 6 healthy people (sex- and age-matched individuals forming a control group; age range 28–55 years, mean 40). Eligible patients were those who were given a diagnosis of plaque psoriasis for at least 6 months with at least 10% of the total body surface area affected. The severity of psoriasis was assessed using the Psoriasis Area and Severity Index (PASI) score (median 17; range 10-25). None of the patients or healthy subjects had received topical or oral medications during the 4 weeks before the study. Individuals whose history indicated any other disorders were excluded from the study. None of the participants were smokers. The study was approved by the Local Bioethics Committee Medical University of Bialystok (Poland), No. R-I-002/502/2015. Written informed consent was obtained from all the patients.

Blood samples were taken into ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3,000g (4°C) to obtain the plasma for analysis.

2.2. SDS-PAGE and in-gel digestion

To partially reduce albumin level in plasma samples, the ProteoExtract Albumin Removal Kit (Calbiochem, San Diego, CA) was used. Total protein content in samples was measured using the Bradford assay [18]. The volume of the sample containing 30μg of protein was mixed in a ratio 1:2 with sample loading buffer (Laemmle buffer containing 5% 2-mercaptoethanol), heated at 95°C for 7 min and separated on 12% Tris-Glycine SDS-PAGE gels. Following electrophoretic separation, gels were fixed in methanol: acetic acid: water (4:1:5; for 1 h) and stained with Coomassie Brilliant Blue R-250

(for 4 h). All lanes were cut out of the gel and sliced into 10 sections. Proteins in each section were reduced with 10mM DTT, alkylated by incubation with 50mM iodoacetamide, and in-gel digested with sequencing grade trypsin (Promega, Madison, WI, USA). The resulting peptide mixture was extracted from the gel fractions, pulled into 5 sections for each line (figure 1), and dried using vacuum centrifugation [19].

2.3. LC-MS/MS analysis

The dried peptides were dissolved in 50 µL 5% ACN + 0,1% formic acid (FA) and 2 µL of this mixture was separated using an Ultimate 3000 (Dionex, Idstein, Germany). Peptide mixture was trapped on a 300 µm id x 5 mm long C18 µ-precolumn (Dionex, LC Packings) and then loaded onto a 150 mm x 75 µm PepMap RSLC capillary analytical C18 column with 2µm particle size (Dionex, LC Packings) at a flow rate of 0.300 µl/min. The gradient started at 3 min and ramped to 60% Buffer B (90% acetonitrile + 0.03% FA) over a period of 60 min [20]. The eluted from the column peptides were analyzed using a Q Exactive HF mass spectrometer with an nanoelectrospray ionization source (ESI) (Thermo Fisher Scientific, Bremen, Germany). Obtained data were acquired with the Xcalibur software (Thermo Fisher Scientific, Bremen, Germany). The mass spectrometer was externally calibrated and operated in positive and data-dependent mode. Survey MS scans were conducted in the 200–2000 *m/z* range with a resolution of 120,000. In subsequent scans, the top ten most intense ions were isolated and fragmented on an HCD collision cell (collision energy was 30 eV), and the fragments were analyzed at 30,000 resolution. A 10 s dynamic exclusion window was applied, and an isolation window of 4 *m/z* and one microscan was used to collect suitable tandem mass spectra.

2.4. Protein identification, grouping, and label-free quantification

Processing of the raw data generated from LC-MS/MS analysis and protein identification and quantification were carried out using Proteome Discoverer 2.0 (Thermo Fisher Scientific, Bremen, Germany) and Sequest HT (SEQUEST HT algorithm, license Thermo Scientific, registered trademark University of Washington, USA). Protein label-free quantification was prepared according to the intensities of the precursor ions signal. For protein identification the following search parameters were used: peptide mass tolerance set to 10 ppm, MS/MS mass tolerance set to 0.02 Da, mass precision was set to 2 ppm, up to two missed cleavages allowed, minimal peptide length was set to six amino acids, cysteine carbamidomethylation, and carboxymethylation, methionine oxidation and 4-HNE – cysteine/lysine/histidine adducts formation set as a dynamic modification [21]. For each protein, minimal number of identified unique peptides was set to two peptides. Input data were searched against the UniProtKB-SwissProt database (taxonomy: *Homo sapiens*, release 2017-08). Protein grouping was performed according to molecular function using the Gene Ontology (GO) database available in the Proteome Discoverer 2.0 (Thermo Fisher Scientific, Bremen, Germany).

2.5. Statistical analysis

Analysis of each plasma sample was performed in three independent experiments. Data were analyzed in Stata® 13.0 (Stata Corp LP, College Station, TX, US) using principal component analysis (PCA). For determine significant differences between group the Tukey's honestly significant difference (HSD) post hoc test was used. The Shapiro-Wilk test was used for testing the normality of data distribution and the one-way Student`s *t*-test for multiple comparisons. Data visualization (clustering and heatmap for top 50) was performed using free available Perseus® 1.6.0.7 software (http://www.coxdocs.org). Results from individual protein label-free quantification are expressed as the mean ± standard deviation (SD) for n = 6. P-values less than 0.05 were considered statistically significant.

3. RESULTS

As a result of the proteomic analyzes have been identified and earmarked for further analysis 486 proteins meeting the set in methodology requirements, which ID numbers, names, selected two unique peptides, and intensity in each sample are presented in a Supplementary table 1.

Compared to healthy individuals, the proteomic profile from psoriatic patients' plasma displayed significant differences that were even visible at the level of electrophoretic separation. The

largest differences were particularly noticeable for low molecular weight proteins or their fragments, as specifically observed in section V (Figure 1). In addition, proteomic data tested with the principal component test shows clustering of data between psoriatic patients and healthy individuals (Component 1 - 29.4%; Component 2 - 20.4%; Figure 2). Table 1 shows the identity and function of the top 20 proteins that differentiated the test groups. Univariate statistical analysis of the individual proteins identified 14 proteins whose expression was significantly different in psoriatic plasma samples compared to those from healthy individuals (Figure 3). All of them, with the exception of coagulation factor XIII A (ID P00488), were included in the top 20 PCA loadings.

The heat map from hierarchical clustering analyses of the top 50 proteins that differentiates the plasma from psoriatic and healthy individuals (obtained by *t*-test) is shown in Figure 4. As seen in the figure, the groups of psoriatic and healthy individuals are clearly divided into two separate clusters. Observed variability within examined group is related to the individual variability of the patients including differences in age and sex. Moreover, the proteins clustered into four groups according to protein function:

Cluster 1 (pink): mainly proteins involved in the inflammatory response [e.g. immunoglobulin-like proteins: GCT-A3 (ID A0A109PW65), anti-factor VIII (ID A2KBC6), complement factor H (ID U5TZG9); interleukins 6 (ID P05231) and 23 (ID Q9NPF7); and proteins with peptidase activity: serine-type endopeptidase V3-2 (ID Q5NV80), metallocarboxypeptidase (ID B1AP58), and α1-antichymotrypsin (ID A0A087WY93)]; expression of these proteins was increased in psoriatic samples.

Cluster 2 (blue): mainly proteins involved in signal transduction [e.g. proteins responsible for the control of cellular proliferation and death, such as POTE ankyrin domain family member F (ID A5A3E0), AT motif binding factor 1 (ID Q6TCJ2), trimethylguanosine synthase (ID Q96RS0); and proteins directly involved in the secretion of signaling molecules: BAI1-associated protein (ID O94812) and biotinidase (ID P43251)]; expression of these proteins was increased in psoriatic samples.

Cluster 3 (green): a small group of proteins responsible for vitamin D and calcium transport and metabolism [e.g. Vitamin D binding protein (ID Q6LDC6), EF-hand calcium-binding protein B (ID Q7Z6G3), calbindin (ID P05937), and 25-hydroxylase (ID O15528)]; expression of these proteins was decreased in psoriatic samples.

Cluster 4 (purple): mainly proteins partially connected with lipid metabolism [e.g. apolipoprotein M (ID I2D5J2), L1 (ID O14791), M (ID O95445), sulfated glycoprotein 2 (ID Q6LDQ3), lipopolysaccharide-binding protein (ID P18428), and lipoprotein lipase (ID P06858)]; expression of these proteins was decreased in psoriatic samples.

The analysis of 4-HNE-protein adducts identified differences between the plasma of psoriatic patients and healthy individuals (Figure 5). Our data shows approximately 2.5-times higher levels of 4-HNE-protein adducts in psoriatic samples compared to healthy individuals. These groups were also different in terms of the proteins forming adducts with 4-HNE (omitting albumin). In the case of plasma from healthy individuals, proteins that formed adducts with 4-HNE were mainly involved in binding and catalytic activity in addition to structural molecules. In the plasma of psoriatic patients, the number of structural molecules forming adducts with 4-HNE decreased in favor of binding an increased number of proteins with catalytic activity. The names of identified and quantified proteins that formed 4-HNE adducts are shown in Figure 5 and the selected example of MS/MS spectra containing 4-HNE-peptide adduct is presented in Figure 6.

4. DISCUSSION

Human plasma is regarded as a determinant of human health status because it contains data derived from all cells or tissues of the human body [22]. Therefore, studying the specific metabolic changes in skin from individuals with chronic and inflammatory diseases, such as psoriasis, can be conducted using this relatively easily available fluid [23]. This is further justified by the fact that psoriasis is recognized as a systemic disease associated with multiple organ dysfunction and an increased risk of cardiovascular disease [24].

An inseparable component of the development of psoriasis as a chronic immune-mediated inflammatory disease, is increased plasma levels of proteins responsible for immune reactions, such as

interleukins (e.g. IL-4, IL-6, or IL-10), chemokines (e.g. MCP-1), or angiogenic cytokines (e.g. PDGF) [25]. In this study, we identified the main factors that distinguished individuals with psoriasis from healthy people, which were primarily proteins involved in immune reactions, such as interleukins: IL-6 (ID P05231) and IL-23 (ID Q9NPF7), anti-factor VIII (ID A2KBC6), and immunoglobulin GCT-A3 (ID A0A109PW65). IL-6, is a pleiotropic proinflammatory cytokine produced by activated monocytes and keratinocytes in psoriasis, and is recognized as one of the mediators involved in regulating both local and systemic inflammatory reactions in psoriasis [26]. Increased IL-6 expression has therefore been observed in serum and skin lesions of patients with psoriasis [4]. IL-23 has been implicated in the pathogenesis of psoriatic lesions, with recent studies revealing that the dominant role of IL-23 involves the stimulation of a subset of CD4 T-cells (IL-17 T cells) to produce IL-17A, a critical component for establishing autoimmune inflammation [27]. Moreover, IL-17A induces the generation of other proinflammatory cytokines, predominately by endothelial cells and macrophages.

An organism's immune response included components of the complement system, C3 and C5, whose elevated levels in psoriasis modulate immune system activity by activating B and T lymphocytes, and increasing the proliferation and suppression of T cell apoptosis [28]. It has previously been shown that these complement factors mediate psoriasis [29,30]. Our results show that another complement factor-H (ID U5TZG9), which binds the C3 component and regulates an alternative pathway of the complement system, is also involved in body's reaction during the development of psoriasis [31,32]. The activation of complement factors is regulated, among others, by serine-type endopeptidase V3-2 (ID Q5NV80), which is involved in regulating leukocyte migration [33]; however, its metabolic function and mechanism of action have not yet been described in psoriasis. We found that level of this peptidase is increased in psoriasis. Increased levels of other serine-type endopeptidases were also previously shown in psoriasis and other chronic immune-mediated inflammatory diseases, such as allergies [34]. However, V3-2 has not been previously described as a protein involved in the development of psoriasis. Independent of the above, other proteins with peptidase activity also participate in the inflammatory response detected in plasma from psoriatic patients. Metallocarboxypeptidases, whose expression levels increase in plasma from psoriatic patients [35], cut the terminal peptide bonds of the C-terminal amino acid [36]. As a result of this activity, inactivation of anaphylatoxins, such as complement-derived C3a and C5a, is observed [37]. In addition, carboxypeptidases inhibit fibrinolysis by preventing the binding of plasminogen to fibrin clots, which further contributes to the development of psoriasis [38].

The infiltrating immune cells, including lymphocytes and neutrophils, dramatically alter the cellular composition of the epidermis in psoriasis, whose extracellular matrix (ECM) stability is provided by metalloproteinases that are crucial for both tissue homeostasis and functioning of the skin under extreme and pathological conditions. During ECM remodeling, various exo- and endo-peptidases cooperate. Collagenases, including MMP8, which shows increased expression in plasma from psoriatic patients, cleaves fibrillary collagen types I, II, III, V, and IX, as well as several other matrix and non-matrix proteins, including cell growth factors [39]. MMP8 is mainly expressed in granulocytes, which form a part of the psoriatic infiltrate and preserve MMP8 within special granules for subsequent release into the intercellular space upon granulocyte activation [40]. However, elastolytic MMP12, whose levels are also significantly increased in psoriatic patients in our study, is probably secreted by inflammatory macrophages [41] and is involved in macrophage mediated proteolysis and matrix invasion. MMP12 degrades a broad spectrum of extracellular matrix components, including type IV collagen, gelatin, fibronectin, laminin-1, entactin, vitronectin, and the protein components of proteoglycans [42]. The highest MMP12 expression is associated with immune cells that infiltrate the papillary dermis, with 17-fold higher levels of MMP12 mRNA shown in psoriatic lesions compared to healthy skin [43]. One of the main substrates for matrix MMP8 and MMP12, namely α1-antichymotrypsin, may be released into the blood and the increased level is observed in plasma from psoriatic patients', in this study.

Chronic inflammation and increased skin flaking promote the release of α1-antichymotrypsin, resulting in cell migration and activation of other matrix metalloproteinases as well as angiogenesis [44]. Enabling increased immune cell migration into skin tissue causes psoriatic peeling [45].

In the development of disease, particularly in the case of a chronic, immune-mediated disease, proteins involved in signal transduction play an important role. Data from our study shows that in plasma from psoriatic patients, highly expressed signal transduction proteins, such as POTE ankyrin domain family member F (POTEF) (ID A5A3E0), AT motif binding factor 1 (ATBF1) (ID Q6TCJ2), and trimethylguanosine synthase (ID Q96RS0), are mainly responsible for the control of cells proliferation and death. Under physiological conditions, POTEF participates in the maintenance of retina homeostasis and the control of cellular proliferation [46]. However, in the case of chronic inflammation, POTEF induces apoptosis through Bak/Bax activation [46]. ATBF1 participates in regulating gene transcription, mainly those involved in myogenic and neuronal differentiation, but also acts as a tumor suppressor in several cancers [10,47]. Moreover, this transcription factor, with its multiple homeodomains and zinc finger motifs, is also associated with atrial fibrillation and the negative regulation of c-Myb [48,49]. This causes excessive cells proliferation, which may be the reason for its increased levels in the plasma of psoriatic individuals.

In this study, we also showed that the development of psoriasis is associated with the increased expression of proteins directly involved in signaling molecule secretion, e.g. biotinidase (ID P43251) and BAI1-associated protein 3 (BAP3) (ID O94812). Increased biotinidase activity in psoriatic patients has previously been shown [50]; however, increased BAP3 level in plasma from psoriatic patients is shown for the first time. BAP3 is a G-protein coupled receptor and is mainly expressed in the brain in association with neurotransmitter secretion [51]. Its increased secretion into the blood might be the result of the disorders in the signaling at the level of the nervous system.

Psoriasis is also suggested to be associated with a decrease in the level of proteins involved in vitamin D metabolism. Vitamin D deficiency has also been previously observed in psoriatic patients [52], indicating disturbances in the synthesis and transport of this vitamin. This mainly owes to a significant decrease in the expression of 25-hydroxylase (ID O15528), which converts vitamin D3 into its active form – calcitriol [53], which inhibits keratinocyte proliferation by upregulating elastase protease inhibitors [54]. Therefore, observed in this study decreased level of serine protease inhibitors such as serpin 1 (C5J0G2) or PRO2275 (Q9P173) in serum of psoriatic patients contributes to the excessive skin exfoliation [55,56]. In addition, vitamin D deficiency is caused by the decreased expression of vitamin D binding and transport proteins, such as calbindin and vitamin D binding protein - VDBP (ID Q6LDC6), which in contrast to calbindin binds and transports active vitamin D only [57]. Vitamin D deficiency in the plasma of patients with psoriasis is often accompanied by calcium deficiency [58]. In turn, this has been suggested to be connected with decreased level of EF-hand calcium-binding protein B (ID Q7Z6G3), which belongs to a group of calcium binding proteins. EF-hand calcium-binding protein B has also previously been shown to play a role in calcium transport [59] therefore its decreased expression might disturb calcium flux in the human body.

Chronic inflammation in psoriasis causes impaired lipid metabolism, which is observed as a decrease in the expression of proteins partially connected with these metabolic processes. Psoriasis has also been described as being associated with disorders in plasma lipid metabolism, which is specifically associated with changes in the expression of apolipoprotein E or A (ApoE, ApoA) [60,61]. However, in the present study, the most significant decrease was seen for apolipoprotein M (ApoM) (ID I2D5J2), which is predominantly enriched with high-density lipoprotein (HDL), and in smaller quantities of low- and very low-density lipoprotein (LDL and VLDL) [62]. ApoM reduces the migration and proliferation of vascular endothelial cells, thereby inhibiting the activation and proliferation of T lymphocytes and lipid oxidation. As such, an ApoM deficit might contribute significantly toward an inflammatory reaction and psoriatic lesions. In addition, the expression of lipoprotein lipase (LPL) (ID P06858) has been shown to decrease, which affects the hydrolyzation of the triglycerides in lipoproteins into free fatty acids and glycerol, and consequently the conversion of VLDL into IDL and then to LDL, thereby resulting in an imbalance in the ratio of VLDL/LDL in psoriatic patients [63]. LPL deficiency also leads to an elevated level of triglycerides in the bloodstream, which further enhances the inflammatory reactions [64]. Furthermore, decreased level of sulfated glycoprotein 2 (ID Q6LDQ3) in psoriatic patients indicate an impairment of the hydrolysis of certain glycolipids [32,65].

A high similarity in action exists between apolipoproteins and lipopolysaccharide-binding protein (LBP) (ID P18428). These small molecules are suggested to control the response to LPS under physiological conditions, by forming high-affinity complexes with LPS that bind monocytes and macrophages [66]. Lower levels of LBP have been seen in psoriatic patients, which may disturb an organism's reaction to LPS. LPS levels in serum and keratinocytes from psoriatic patients have been described as being overexpressed [67,68].

Disorders in lipid metabolism also result in changes in the level of lipid mediators, specifically generated during lipid peroxidation. As a result, lipid peroxidation electrophilic aldehydes, including 4-HNE, are generated. 4-HNE is described as a signaling molecule having a wide range of functions [16]; however, the role of 4-HNE-protein adduct formation in psoriasis development has never been investigated. Results from the present study show 4-HNE–protein adduct levels were approximately 2.5-times higher in the plasma of psoriatic patients compared to healthy people. As a result, these changes are suggested to affect the activity and functions of these proteins. In the plasma of psoriatic patients, 4-HNE forms adducts with Rho GTPase-activating protein 12 (ID Q8IWW6), adenylate kinase 7 (AK7) (ID Q96M32), and tryptophan 2,3-dioxygenase (ID P48775), which are mainly involved in the catalytic activity that was not observed in the plasma from healthy individuals. Rho GTPase-activating protein 12 converts the inactive GDP-bound state of Rho-type GTPases and activates GTP hydrolysis. The active form of Rho protein also interacts with several effectors to transduce signals that lead to diverse biological responses, including actin cytoskeletal rearrangements, regulation of gene transcriptions, cell cycle regulation, and negative control of apoptosis in the case of several types of cancer cells [69]. There is no data on the effect of 4-HNE adduct formation on Rho GTPase-activating protein; however, in the case of another GTPase-activating protein (Ral-binding GTPase-activating protein RLIP76), 4-HNE adduct formation triggers the general functions of this protein, binding and removing lipid peroxidation products [70]. It is therefore suggested that 4-HNE-Rho GTPase-activating protein 12 adduct formation also triggers the Rho GTPase activity and inhibits the process of apoptosis. Moreover, Rho GTPase launches the cascade of Rho kinases activity leading to neutrophil migration and disruption the intercellular connections between the skin cells [71,72]. As a result excessive exfoliation in the examined psoriatic patients is observed.

In the plasma of psoriatic patients, 4-HNE also creates adducts with adenylate kinase 7 (ID Q96M32) - a phosphotransferase that catalyzes the interconversion of adenine nucleotides (ATP, ADP, or AMP) and, by constantly monitoring phosphate nucleotide levels, plays an important role in energy homeostasis [73]. Generally, the formation of 4-HNE-kinase adducts at non-toxic concentrations may lead to the activation of kinase enzymatic activity [16]. Given the lack of data on the effect of 4-HNE on adenylate kinase, it is assumed that 4-HNE stimulates adenylate kinase 7 to increasingly monitor phosphate nucleotide levels in an effort to maintain energy homeostasis under chronic inflammatory conditions.

In the case of tryptophan 2,3-dioxygenase (ID P48775), which catalyzes the oxidation of L-tryptophan to N-formyl-L-kynurenine and plays a central role in the physiological regulation of tryptophan flux in the human body, formation of 4-HNE adducts is also suggested as an activating factor by analogy to other enzymes [74]. Tryptophan 2,3-dioxygenase activity also decreases the humoral immune resistance [75], therefore its increased activity in psoriasis could be explained as an attempt to reduce the sensitivity of skin cells to immune signals.

5. CONCLUSION

We herein confirm our current knowledge regarding changes in the proteomic profile of plasma from patients with psoriasis in relation to increased levels of proteins involved in the immune response and decreased levels of proteins responsible for vitamin D metabolism. However, we also identified several new proteins involved in signal transduction, lipid metabolism and catalytic activity whose expression or structure was modified in psoriatic patients. This group of proteins identified in this study should therefore be examined for a potential biomarker of psoriasis. Rapid diagnosis, connected with specific biomarker detection, as well as an improved understanding of the metabolic changes occurring in psoriasis development, could contribute to the design of targeted pharmacotherapy.

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Figure 1. SDS-PAGE separation and staining with Coomassie Brilliant Blue R-250 of plasma proteins from the psoriatic patients and control group. The grid indicates the borders of the protein migration zones. Data shown are from two independent gels with 12 samples each. Figure 2. Principal component analysis of plasma proteins from the psoriatic patients and control group. Abbreviations: Ps-psoriasis; Ctr-control.

Figure 3. Volcano plot of plasma proteins from the psoriatic patients and control group. Red dots indicate proteins of statistical significance among the tested groups. Only the names of these proteins have been given.

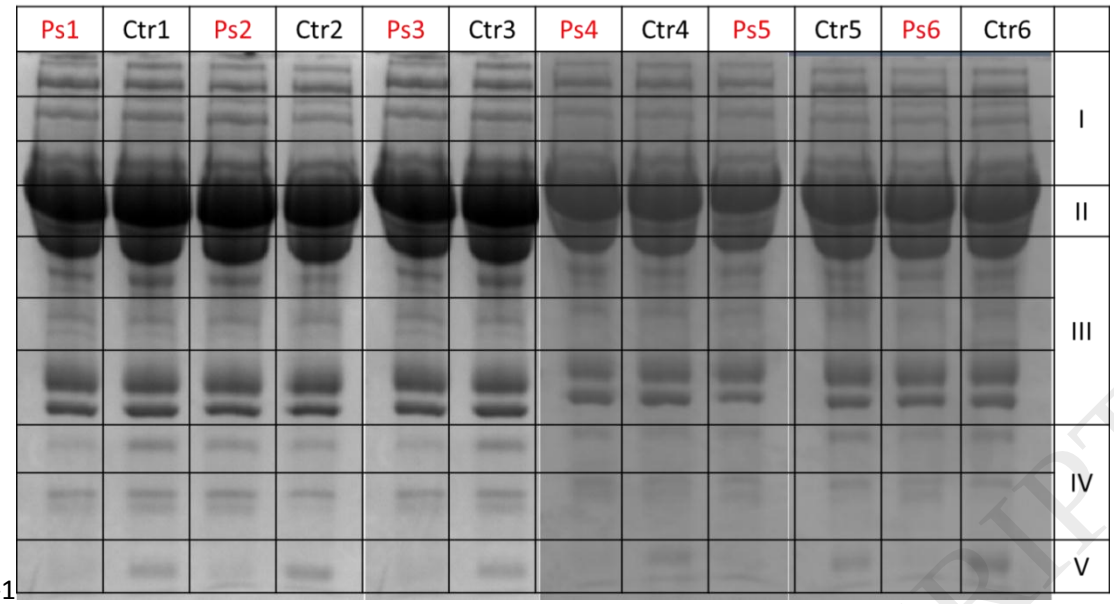
Figure 4. Heat map and clustering for the top 50 proteins from the psoriatic patients and control group. Protein expression levels (log transformed) were scaled to the row mean. The color key relates the heat map colors to the standard score (z-score), i.e. deviation from the row mean in units of standard deviation above or below the mean.

Figure 5. Analysis of 4-HNE-protein adducts in plasma samples from the psoriatic patients and control group: the level of 4-HNE-protein adducts vs. control plasma samples, and the molecular function of proteins that formed adducts with 4-HNE in the psoriatic and control samples.

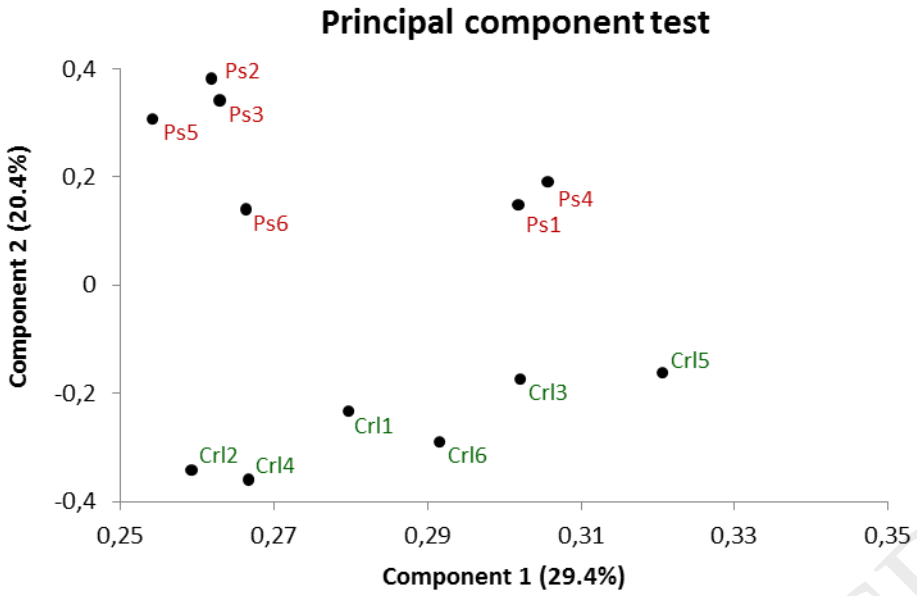
Figure 6. Peptide of adenylate kinase 7 (Q96M32) forming adducts with 4-HNE in selected psoriatic patient (A - Ps1) and control individual (B - Crt1). Obtained (MS/MS) spectrum and identified sequence; red arrows show b^+ ions and blue arrows show y^+. Only once charged ions has been marked.

Table 1. Top 20 loadings from the principal component analysis (grouped according to their functions) of plasma proteins from the psoriatic patients and control group.

Protein ID	Protein name	Identified unique peptides [position]	Function
I2D5J2	Apolipoprotein M	EFPEVHLGQWYFIAGAAPTK [38-57] EELATFDPVDNIVFNMAAGSAPMQLHLR [58-85]	lipid transport
Q6LDQ3	Sulfated glycoprotein 2	IDSLLENDR [57-65] LALDIEIATYR [357-367]	enzymatic activators that promote the hydrolysis of certain glycolipids
P06858	Lipoprotein lipase	ALLVLTLAVWLQSLTASR [5-22] SIHLFIDSLLNEENPSK [271-287]	triglycerides metabolism
Q6LDC6	Vitamin D binding protein	EDFTSLSLVLYSR [38-50] KFPSGTFEQVSQLVK [51-65]	vitamin D transport
O15528	25-hydroxylase	AVGSVFVSTLLTMAMPHWLR [224-243] WLGEGPTPHPFASLPFGFGK [433-452]	vitamin D metabolism
C5J0G2	Serpin 1	GTEAAGAMFLEAIPMSIPPEVK [13-34] AVLTIDEKGTEAAGAMFLEAIPMSIPPEVK [5-34]	serine proteases inhibition
Q9P173	PRO2275	AVLTIDEKGTEAAGAMFLEAIPMSIPPEVK [62-91] SPLFMGK [107-113]	serine proteases inhibition
B1AP58	Carboxypeptidase N catalytic chain	FPPEEELQR [111-119] EALIQFLEQVHQGIK [126-140]	metallocarboxypeptidase activity
A5A3E0	POTE ankyrin domain family member F	YPMEHGIITNWDDMEK [769-784] SYELPDGQVITIGNER [939-954]	control of cellular proliferation and death and control of metabolic function
Q96RS0	Trimethylguanosine synthase	GIGLDESELDSEAELMR [79-95] CDVVVDAFCGVGGNTIQFALTGMR [691-714]	transcriptional regulation
O94812	BAI1-associated protein 3	LQGAVDMDTLEPVDASSR [715-732] TLHPVYDELFYFSVPAEACR [1074-1093]	G-protein coupled receptor signaling, neurotransmitter secretion
Q6PYX1	Hepatitis B virus receptor binding protein	TPEVTCVVVDVSHEDPEVK [157-175] TTPPVLDSDGSFFLYSK [294-310]	signal transduction
Q6TCJ2	ATBF1	KPILMCFLCKLSFGYVR [107-123] NISAIIQGIGK [149-159]	the cell cycle inhibition
E7ETR7	γ-glutamyltranspeptidase 1	KPILMCFLCK [107-116] SFVTHAVHDHR [124-134]	glutathione hydrolise
B4DDG4	Galectin-3-binding protein	LASAYGAR [130-137] STSSFPCPAGHFNGFR [455-470]	scavenger receptor activity
P43251	Biotinidase	DVQIIVFPEDGIHGFNFTR [104-122] TSIYPFLDFMPSPQVVR [123-139]	release of biotin from biocytin
Q5NV80	V3-2 protein	ALIYSTSNK [48-56] FSGSLLGGK [64-72]	endopeptidase activity, regulation of immune response
A2KBC6	Anti-FactorVIII scFv	EVQLLESGGGLVQPGGSLR [1-19] ASQSVSSSYLAWYQQKPGQAPR [155-176]	complement compounds inhibitor
U5TZG9	Complement factor H	KGEWVALNPLR [68-78] RPCGHPGDTPFGTFTLTGGNVFEYGVK [83-109]	coagulation factor
P05231	Interleukin-6	YILDGISALR [59-68] EALAENNLNLPK [83-94]	regulation of immune response

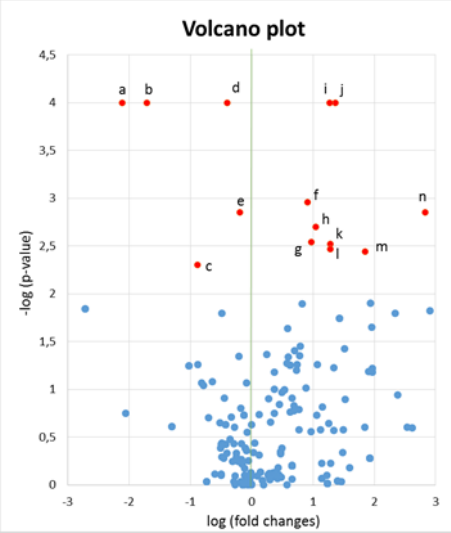


Figr-1

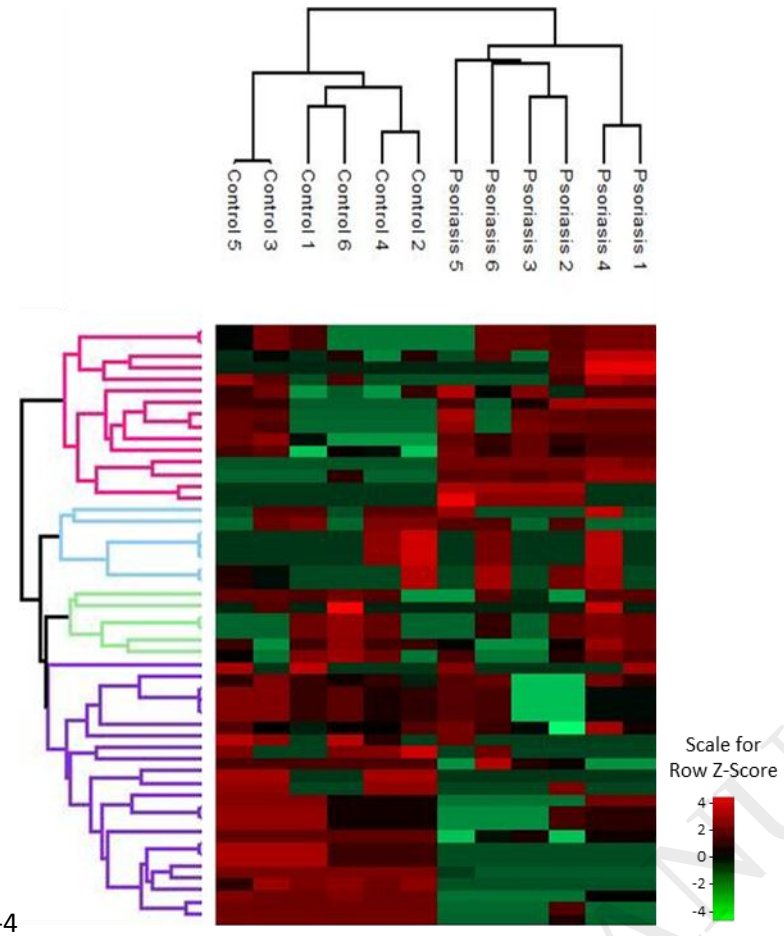


Figr-2

Figr-3

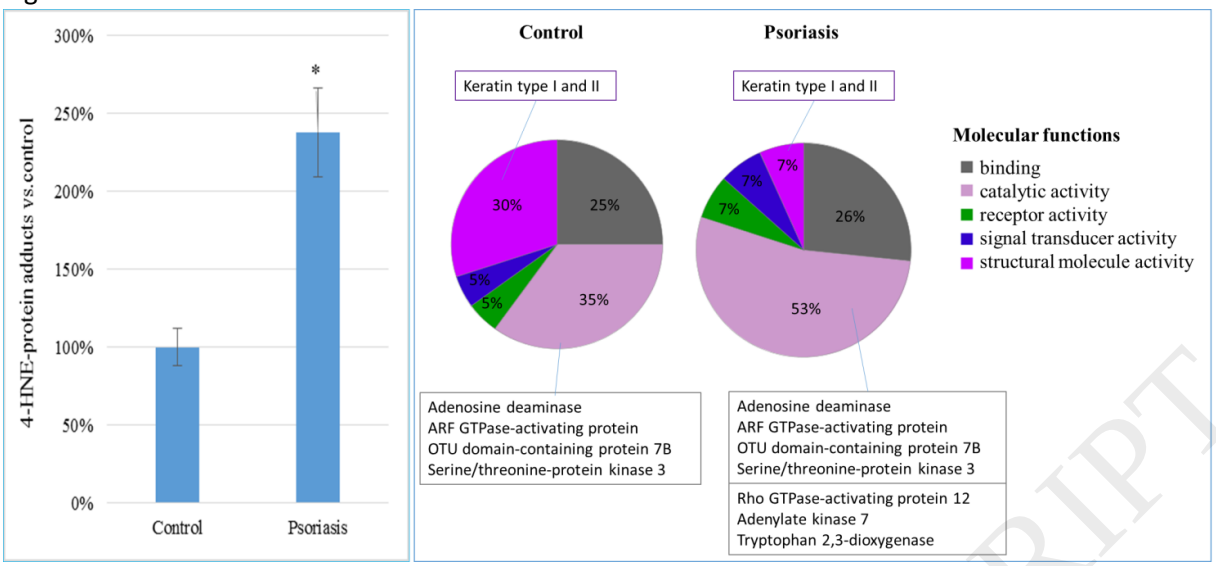


	Protein ID	Protein name	Fold change (Psoriasis/Control)
a.	I2D5J2	Apolipoprotein M	0,01
b.	Q6LDQ3	Sulfated glycoprotein 2	0,39
c.	Q6LDC6	Vitamin D binding protein	0,45
d.	Q6PYX1	Hepatitis B virus receptor binding protein	0,64
e.	P00488	Coagulation factor XIII A	0,83
f.	B1AP58	Carboxypeptidase N catalytic chain	8,08
g.	O94812	BAI1-associated protein 3	9,41
h.	P43251	Biotinidase	11,19
i.	Q96RS0	Trimethylguanosine synthase	12,54
j.	A5A3E0	POTE ankyrin domain family member F	12,91
k.	Q5NV80	V3-2 protein	13,05
l.	U5TZG9	Complement factor H	13,05
m.	A2KBC6	Anti-FactorVIII scFv	22,52
n.	Q6TCJ2	ATBF1	33,67



Figr-4

Figr-5



Figr-6