Alpha-1 antitrypsin, retinol binding protein and keratin 10 alterations in patients with psoriasis vulgaris, a proteomic approach

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ARTICLE INFO	ABSTRACT
	Objective(s): Psoriasis is an autoimmune disease that appears on the skin. Although psoriasis is clinically and histologically well characterized, its pathogenesis is unknown in detail. The aims of this study were to evaluate the proteome of psoriatic patients' sera and to compare them with those of normal healthy human to find valuable biomarkers. ***Materials and Methods:*** In a case-control study, twenty cases of white patients with psoriasis vulgaris, 10 males and 10 females and sixteen healthy controls, 8 males and 8 females were enrolled in the study. The serum protein expression patterns obtained after depletion of albumin were compared by using two dimensional gel electrophoresis (2-DE) coupled to MALDI/TOF-TOF to identify disease associated proteins. ***Results:*** Differential expression of nine protein spots representing four unique proteins including alpha-1 antitrypsin, retinol binding protein, keratin 10 and an unknown protein (with pI 6.47 and molecular weight of 19941 Da), between psoriatic and healthy human serum were found. Furthermore, expression of four new alpha-1 antitrypsin isoforms with different molecular weight and isoelectric point were observed in psoriatic serums in this research for the first time. ***Conclusion:*** A unique proteomic profiling with abnormal expression of alpha-1 antitrypsin and presence of keratin 10 in sera of psoriasis patients were observed that may constitute new and useful findings of psoriasis and offer a clue to a better understanding of the inflammatory pathway.

Introduction

Psoriasis is an autoimmune disease that appears on the skin, transmits genetically, and affects approximately 2% of the world's population, with men and women being equally affected (1). It is a complex genetic disease with environmental and genetic components and in fact, the term represents a clinically heterogeneous group of diseases including psoriasis vulgaris, pustular psoriasis, psoriasis arthritis, erythrodermic psoriasis, nail psoriasis and psoriasis inversa (2). The most common phenotype is chronic plaque psoriasis with well-demarcated lesions typically located in the scalp and on the extensor surfaces (3). Although psoriasis is clinically and histologically well characterized, its pathogenesis is unknown in detail, and unlike other autoimmune diseases, does not have a generally accepted animal model. Our knowledge about its pathogenesis is derived exclusively from clinical studies and translational science performed in psoriatic patients.

Pathological conditions can induce some quality and quantity changes in sera and these characteristic changes provide a favorable biomarker to distinguish between healthy and sick states. Global gene expression (4-6) and protein analysis (7) in psoriasis have been investigated previously. Also, other aspects such as role of vitamins (8), immnunoglubolins (9), microRNAs (10), adipokines and cytokines (11) and methylglyoxal (12) in the pathophysiology of this autoimmune disease have been studied. Proteomic studies also are used in the evaluation of this disorder, but there are few published data about serum proteom alteration in psoriatic patients (13, 14) and more studies on this topic are needed. Proteome analysis can be regarded as a peptide screening approach aimed at documenting the overall distribution of proteins in cells, organs, or other samples (15). Therefore, the aim of this study was

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Table 1. Mean±SD of patients serum protein concentration and percentage of protein recovery obtained before and after treatment with TCA/acetone or Aurum kit

	Protein concentration (mg/ml)	Protein recovery (%)
Untreated	67.72±8.82	100
Aurum kit treated	29.25±7.15	43.1
TCA/acetone treated	8.27±3.14	12.2

to explore serum proteomics patterns of psoriatic patients by using two dimensional gel electrophoresis (2-DE) followed by MALDI/TOF-TOF.

Materials and Methods

A total of 36 patients (18 males and 18 females) were selected. Twenty cases of white patients with psoriasis vulgaris, 10 males and 10 females (mean ± SD age 41.42±12.7 years), and sixteen healthy control peoples, 8 males and 8 females (mean ± SD age 46.90±16.7 years) were enrolled in the study. All patients were diagnosed as having psoriasis vulgaris and had no prior history of other diseases or any medications which may complicate the analysis since two months ago. The study was approved by the local Ethics Committee. Fasting blood samples were taken from the cubical vein into sterile vacutainers without anticoagulant and after centrifugation (2000g, 10min), serum stored at -20°C until used. Protein concentration was determined according to the method proposed by Bradford, using bovine serum albumin (BSA) as a standard protein (16). Albumin concentration was assayed by bromocresol green (BCG) method. TCA/acetone precipitation (17) and Aurum serum protein mini kit (18) were used to remove high abundant proteins. Processed serum samples were analyzed by SDS-PAGE in the presence of 2-mercaptoethanol (2-ME) according to the Laemmli method (19) using the running and stacking gel of 12% and 3.0% acrylamide (w/v), respectively. Gels were stained with Coomassie Brilliant blue (CBB) R-250. Two-dimensional gel electrophoresis was performed according to the previously reported methods and resolved proteins were detected by staining with either CBB R-250 or silver nitrate, but omitting the glutaraldehyde fixation (20). For each serum sample analysis, 2-D gels have been performed at least in triplicate, with independent protein preparations. 2-D gels were scanned and analyzed by Melanie software, version 6.0.2.0. For MALDI/TOF-TOF analysis, the protein spots of interest were excised carefully and analyzed by MALDI/TOF- TOF by the "The Technology Facility Proteomics & Analytical Biochemistry Laboratory" (The University of York, UK). Database interrogation was performed using the National Center for Biotechnology Information (NCBI) databases on a GPS workstation. In order to highlight the serum proteome changes in psoriasis patients, image analysis has been performed on three replicates, and variations on spots' area were confirmed by
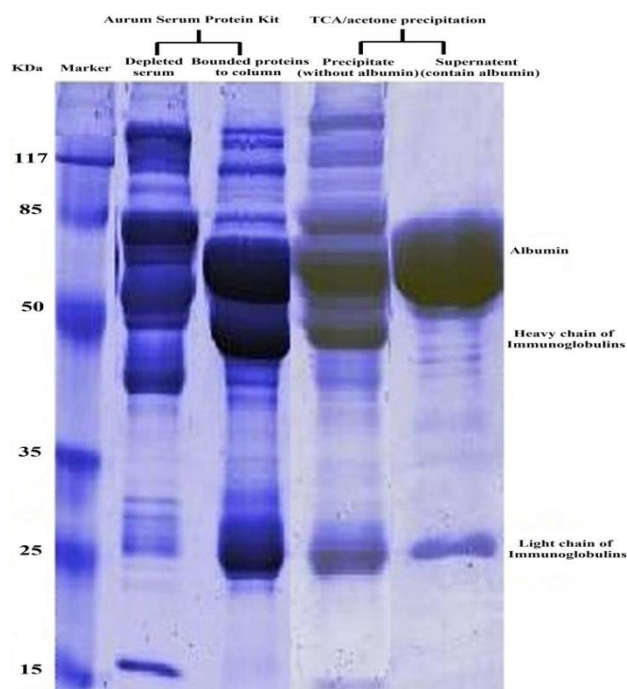

Figure 1. Human serum proteins stained with Commassie brilliant blue R-250 after depletion of high-abundant proteins using different depletion treatment on 12 % SDS-PAGE

statistical analysis.

Statistical analysis

Values were expressed as the mean±SD. Differences between the healthy and disease sera were determined by two independent sample T test. Using SPSS version 16, a *P*-value equal to 0.05 was considered statistically significant.

Results

The serum protein yield in untreated, TCA/acetone or Aurum kit treated serums are summarized in Table 1. Figure 1 depicts typical SDS-PAGE results of serum proteins obtained by different treatments, and in each sample protein in 2 μl of serum were loaded to the gel. A comparison of the proteomics patterns of health and patient serum gels, as well as both high protein depletion methods are shown in Figure 2.

Patient serum treated with TCA/acetone has four spots more than patient serum depleted with Aurum serum protein mini kit (Figure 2B and 2D, spot No. 6-9). As shown in Figure 2, nine protein spots were subjected to MALDI/TOF-TOF analysis. Table 2 lists the identities of the proteins which were analyzed in this experiment using MALDI/TOF-TOF and summarizes the differences in existence, absence or alteration in expression of spots between the two depletion methods and between patient and health control serums.

Table 2. MALDI/TOF-TOF analysis of selected protein spots from 2-Dimentional gel electrophoresis that differently expressed in sera from psoriatic and healthy human between two depletion methods

Spot no[*].	Protein name	MW (Da)	*P- value*	NCBI ID	TCA/aceton[†]		Aurum kit	
					H	P	H	P
1	Unknown protein	19941	6.47	gi|119569581	–	+	–	–
2	Keratin 10	39832	4.72	gi|186629	–	+	–	+
3	Chain A, The S Variant of human α1-Antitrypsin	39099	5.27	gi|231240	–	+	–	+
4	Chain A, Crystal structure of cleaved antitrypsin	37622	5.43	gi|7546268	–	+	–	+
5	Retinol binding protein 4	23371	5.76	gi|18088326	++	+	++	+
6	α1-AT (isoform 1)	22871	6.11	gi/28637	–	–	–	+
7	α1-AT (isoform 2)	22871	6.11	gi/28637	–	–	–	+
8	α1-AT (isoform 3)	22871	6.11	gi/28637	–	–	–	+
9	α1-AT (isoform 4)	22871	6.11	gi/28637	–	–	–	+

α1-AT: Alpha1- antitrypsin; H: healthy; P: patients.
[*] Spot no. related to the annotation in Figure 2. † + existence; - absence; ++ increased concentration

Discussion

It was found that three distinct proteins including different isoforms of α1-AT (according to a previous unpublished report by Wieland *et al*), retinol binding protein (RBP) and keratin 10 suffer changes in psoriatic serum against control healthy human serum. In contrast with our results, in a previous study conducted by Williamson *et al* (2013) it has been reported that abundance of more than 50 proteins was altered in psoriatic patent versus health ones. Between these proteins, profilin 1 is described as a candidate plasma biomarker of psoriasis (13). There are several reasons for this dissimilation such as differences in sample preparation, protein enrichment and detection.

Several investigators have emphasized the role of proteolytic enzymes, especially serine proteases in the pathogenesis of psoriasis (2, 21, 23). In the psoriatic lesion, MC$_{TC}$ cells (mast cells that contain several serine protease) are predominantly responsible for the mast cell infiltration into the papillary dermis (21). Alpha 1-AT is a glycoprotein with serine protease inhibition activity that interacts with and inhibits elastases, cathepsin G and chymase, and plays a role in reducing tissue inflammation. There are several investigations about the role of α1-AT in the pathogenesis of psoriasis (22, 23). In our study, two variants of α1-AT were identified that were only detected in the serum of the psoriatic patients, and were not detectable in healthy controls. One of these variants existed in four isoforms with Mw/pI about 22000/6-7 which contained 197 amino acids. All previously reported α1-ATs are different from this new variant in Mw and pI and there are no reports on previous studies about this variant. As shown in Figure 2, in patient serum
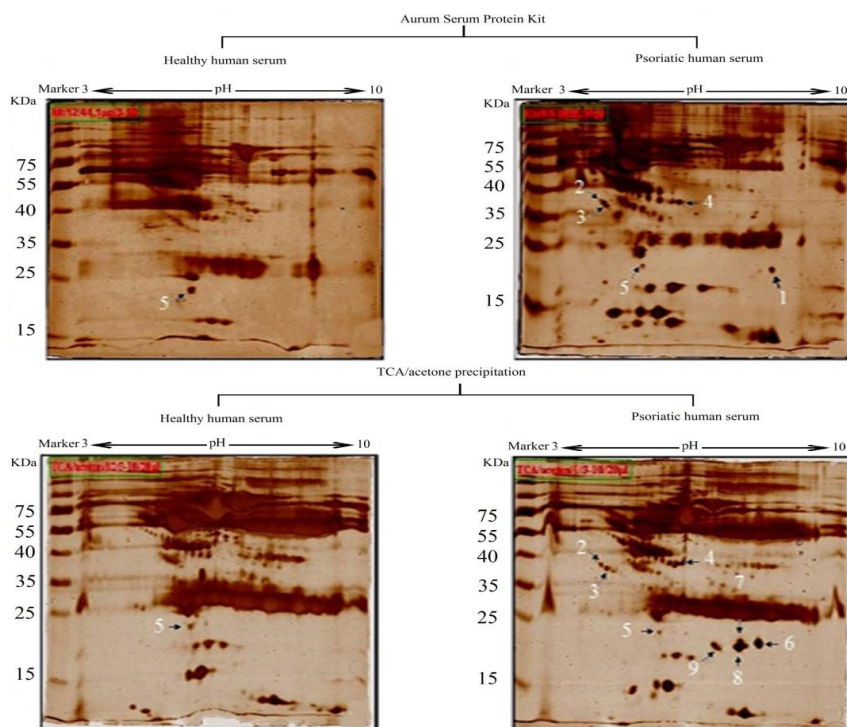

Figure 2. 2-Dimentioanl gel electrophoresis profile of different treated of human serum which were stained by silver staining (Spot number according to Table 2)

depleted with Aurum serum protein mini kit, compared to TCA/acetone depletion, four spots were eliminated. The main cause of this condition is the elimination of immunoglobulins from serum by using Aurum serum protein mini kit, and therefore the α1-ATs isoforms that create complexes with them are also removed from the serum. Thus, new complexes of Ig- α1-AT were found in psoriatic serum but the existence of Ig- α1-AT complexes were also reported in relatively longstanding studies in different joint diseases such as ankylosing spondylitis and rheumatoid arthritis (24).

Vitamin A (retinol) and its derivatives play different roles in the body (25, 26), and within the blood, free retinol binds to retinol binding protein (RBP), its serum transport protein. Some investigators have indicated that RBP is present in the intercellular spaces of the epidermis (27). Retinol can store in keratinocytes and convert to all-trans retinoic acid (ATRA) (28). RBP is involved in the regulation of intracellular retinoid concentrations and a decrease in patient serum RBP concentration, as seen in our study, causes psoriatic lesions.

One protein with pI/MW about 4.8/40000 existed in the serum of one patient, which was not observed in healthy human serum and by MS analysis, was identified as keratin 10 (K10). Keratins are intermediate filament-forming proteins that provide mechanical support and fulfill a variety of additional functions in epithelial cells (29). These proteins are grouped into two classes, the neutral-basic type II (K1-K8) and the acidic type I (K9-K20) (30). Proliferative basal cells express the K5-K14 pair. However, when keratinocytes begin terminal differentiation, they move upward, become postmitotic, and switch to the expression of a keratin pair K1-K10 (31). Under hyperproliferative conditions, such as psoriasis, downregulation of K10 occurs in keratinocytes, but there is one report of expression of K10 increase in lesional and symptomless skin of spreading psoriasis (32). In other previous studies, K10 was never found in the serum. However in this study, for the first time, K10 in the serum of patients with psoriasis vulgaris was found. This increase may be due to epidermal apoptosis that causes K10 leaking to the serum of psoriatic patients.

Conclusion

With an analysis of sera 2D maps, it has been possible to describe a pattern of proteins up- and down-regulation upon disease conditions. In conclusion, we here provide serum proteome analysis of patients with psoriasis. Nine proteins were differentially expressed in the psoriasis vulgaris group, as compared to the normal group of these proteins. Using specific ELISA or western blot assay for all of these proteins will provide a more complete evaluation of these valuable changes.

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