Evaluation of serum proteome from Indian psoriasis patients

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Psoriasis is a polygenic chronic skin condition, associated with many systemic disorders. Though it is most studied dermatological condition, molecular mechanism leading to its pathogenesis is still unclear. An insight into its proteome may help unrevealing some biomarkers and therapeutic targets. In this study, we carried out mass spectrometry based quantitative proteomic analysis of serum from psoriasis patients by employing Tandem Mass Tags (TMT) approach. We identified 861,887 MS/MS spectra corresponding to 493 proteins. These dysregulated proteins were further classified by Gene Ontology and protein-protein interaction of dys-regulated proteins revealed networks in psoriasis patients.

Keywords: Psoriasis, LC-MS/MS, Orbitrap, biomarkers, serum

Materials and Methods:

Sample collection
Whole blood samples were collected from patients diagnosed with psoriasis after obtaining approval from the Institutional Ethics Committee at Yenepoya University, Mangalore, India. For control cases, blood samples were collected from voluntary donors. Prior to collection, written informed consent were obtained from both healthy volunteers and patients with psoriasis. Serum was separated using standard centrifugation techniques and samples were stored at −80°C until further use.

Protein extraction
Depletion of abundant proteins in serum samples were carried out using a Human-14 Multiple Affinity Removal Column (Agilent Technologies) as per manufacturer’s protocol. Post depletion, protein concentration was estimated using Bicinchoninic acid (BCA) assay (Thermo Scientific Pierce, Rockford, IL, USA).
equivalent amount of protein from each of patients and control serum samples were taken for further analysis.

**Sample preparation for LC-MS analysis**

An equal amount of proteins from each condition was subjected to reduction, alkylation and trypsin digestion. Reduction and alkylation of the proteins was done by using 5mM of dithiothreitol and 20 mM of iodoacetamide, respectively. Trypsin was used as a proteolytic enzyme, in a concentration of 1:20 (enzyme: protein) and digestion was confirmed on SDS-PAGE. Digested peptides were subjected labeling using TMT tags. The labelled peptides were then subjected to fractionation and LC-MS/MS analysis. Data was acquired in technical triplicates.

**Mass spectrometry data analysis**

LC-MS/MS analysis of the samples was carried out using Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) using Orbitrap as mass analyser. Acquired raw data were processed on Proteome Discoverer (2.1) Suite with SEQUEST and Mascot as search algorithms against RefSeq human protein database. Search parameters included oxidation of methionine and acetylation of protein N-terminus as variable modification, whereas, carbamido methyl of cysteine as dynamic modification. False discovery rate was calculated using percolator with a reverse database. Mass error window of 10 ppm and 0.05 Da was allowed for MS and MS/MS, respectively. The peptide and protein data were extracted using high peptide confidence (1% FDR). Quantitation was carried out using the reporter ion quantifier node for TMT.

**Bioinformatics analysis**

Differential expression ratios were calculated where a fold change of ≥ 1.35 was considered to be upregulated and fold change of ≤ 0.74 was considered to be down regulated. Data was analyzed using unpaired t-test and p-value <0.05 was considered to be statistically significant. Gene Ontology (GO) analyses for both up- and down-regulated proteins were carried out using PANTHER (www. http://www.pantherdb.org/). Protein-protein interaction (PPI) network of dysregulated proteins were done using STRING software (www.string-db.org).

**Results and Discussion:**

In post genomics era, proteomics has become an important tool to study and identify proteins using mass spectrometry [16]. Further bioinformatics analysis is beneficial for analysing the proteins identified in a broader extent [17]. For such investigations, depletion of major abundant proteins such as albumin, keratin is favourable prior to proteomics analysis. Proteomics data leads to a better knowledge of disease biology, physiology, and pathogenic pathways and can further aid in biomarker discovery and identification of novel targets [18, 19].

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**Figure 1:** Workflow for the study

**Figure 2:** Protein-protein interaction (PPI) network. The PPI network was created based on STRING analysis of dys regulated proteins (A) Up regulated and (B) Down regulated proteins identified in the serum of psoriasis patients compared to healthy individuals
We identified significant overexpression of LPA (Lipoprotein (a)) in the serum of psoriasis patients as compared to the serum from healthy individuals (Fold change +2.16, p=0.01). Studies have demonstrated association of increased level of LPA with disease severity in psoriasis patients resulting in complications [20]. Higher levels of LPA in serum indicate that psoriasis is linked with oxidative stress, further resulting in disease severity. Increased LPA levels may potentially impact the level of LPA with disease severity in psoriasis patients resulting in complications more likely [21]. Similarly, we also identified increase expression of C-reactive protein (CRP) which is a well known inflammatory marker. Inflammatory nature of psoriasis makes higher expression of CRP more evident in serum of psoriatic patients. Studies have also indicated increased expression of CRP in psoriasis patients, suggesting its crucial role as a diagnostic marker for monitoring disease severity and activity [22-24]. Our study also revealed higher expression of peroxiredoxin 2 (PRDX2) which is involved in Redox balance system in proliferating cells. Similar

| Category name (Accession) | # genes | Percent of gene hit against total # genes | Percent of gene hit against total # Function hits |
|---------------------------|---------|------------------------------------------|-----------------------------------------------|
| Biological process         |         |                                          |                                               |
| cellular process (GO:0009987) | 6      | 50.00%                                   | 26.10%                                         |
| biological regulation (GO:0065007) | 4      | 33.30%                                   | 17.40%                                         |
| localization (GO:003179) | 3       | 25.00%                                   | 13.00%                                         |
| metabolic process (GO:0008152) | 3      | 25.00%                                   | 13.00%                                         |
| interspecies interaction between organisms (GO:0044419) | 1      | 8.30%                                    | 4.30%                                          |
| response to stimulus (GO:0050896) | 1      | 8.30%                                    | 4.30%                                          |
| signaling (GO:0023052) | 1       | 8.30%                                    | 4.30%                                          |
| multicellular organismal process (GO:0032501) | 1      | 8.30%                                    | 4.30%                                          |
| Cellular component      |         |                                          |                                               |
| cellular anatomical entity (GO:0110165) | 1      | 8.30%                                    | 4.30%                                          |
| protein-containing complex (GO:0032991) | 7      | 25.00%                                   | 13.00%                                         |
| Molecular function        |         |                                          |                                               |
| binding (GO:005488) | 5       | 41.70%                                   | 23.10%                                         |
| catalytic activity (GO:003824) | 2      | 16.70%                                   | 8.70%                                          |

Protein-protein interaction (PPI) analysis of these dysregulated proteins were carried out using STRING software (Figure 2). The analysis was carried out for both up- and down-regulated proteins. For this analysis, we carried out PPI analysis using up- and down-regulated proteins to generate the PPI network. The network has been provided in Figure 2A. The interaction was derived at high level of confidence (≥ 0.7). We further narrowed the analysis using significantly altered proteins. The analysis of the upregulated proteins revealed that there were 8 nodes and 2 edges based on a high confidence score ≥ 0.7 in STRING analysis (Figure 2A). Similarly, for the down regulated proteins, the analysis revealed that there were 12 nodes with 2 edges. The network generated from the significantly upregulated proteins revealed high level of connectivity among 3 proteins such as Lipoproteins a (LPA), C-reactive protein (CRP) and lipopolysaccharide-binding protein (LBP) (Figure 2A). We identified significant overexpression of LPA (Lipoprotein (a)) in the serum of psoriasis patients as functions of the dysregulated protein, we carried out GO analysis. The upregulated serum proteins were mainly enriched in the cellular process, metabolic process, and biological regulation (Table 1), while the functional enrichment terms of significantly down regulated proteins were mainly correlated with binding activity and catalytic activity (Table 2).

Table 1: Gene Ontology (GO) analysis of the upregulated proteins identified in the serum of psoriasis patients compared to healthy individuals

| Category name (Accession) | # genes | Percent of gene hit against total # genes | Percent of gene hit against total # Function hits |
|---------------------------|---------|------------------------------------------|-----------------------------------------------|
| Biological process         |         |                                          |                                               |
| cellular process (GO:0009987) | 4      | 44.40%                                   | 18.20%                                         |
| metabolic process (GO:008152) | 3      | 33.30%                                   | 13.60%                                         |
| biological regulation (GO:0065007) | 3      | 33.30%                                   | 13.60%                                         |
| response to stimulus (GO:0050896) | 3      | 22.20%                                   | 9.10%                                          |
| immune system process (GO:002376) | 2      | 11.10%                                   | 4.50%                                          |
| interspecies interaction between organisms (GO:0044419) | 1      | 11.10%                                   | 4.50%                                          |
| developmental process (GO:0032502) | 1      | 11.10%                                   | 4.50%                                          |
| multicellular organismal process (GO:0032501) | 1      | 11.10%                                   | 4.50%                                          |
| Cellular component      |         |                                          |                                               |
| cellular anatomical entity (GO:0110165) | 7      | 77.80%                                   | 63.60%                                         |
| intracellular (GO:000622) | 4       | 44.40%                                   | 33.30%                                         |
| Molecular function        |         |                                          |                                               |
| binding (GO:005488) | 3       | 33.30%                                   | 33.30%                                         |
| structural molecule activity (GO:0035198) | 1      | 11.10%                                   | 11.10%                                         |
| catalytic activity (GO:003824) | 5      | 55.60%                                   | 55.60%                                         |

Identification of differentially expressed proteins:
In the current study, the TMT-labelled samples were analysed on high-resolution mass spectrometry to identify the differentially expressed proteins in psoriasis and control serum samples (Figure 1). The search resulted on the identification of 861,887 MS/MS spectra corresponding to 493 proteins. Based on the fold change cut off (±1.35), we found 12 proteins to be significantly (p≤0.05) down regulated and 8 to be upregulated. To further explore the biological
expression has been reported in various studies [25]. Lipo polysaccharide-binding protein (LBP) serves as reliable marker for serum lipopolysaccharide concentration where a higher level of LPS is known to elicit chronic inflammatory cytokines level. Studies have demonstrated its raised level to be associated with onset of psoriasis [26]. PRDX2 is also known to be a negative regulator of apoptosis and is a defence response-related protein. Despite its active role in psoriasis, the underlying molecular mechanism is yet to be deciphered and hence further studies are warranted. Similarly, the network generated from the significantly down regulated proteins revealed high level of connectivity among 3 proteins such as thymosin beta-4 (TMSB4X), actin (ACTB) and profilin (PFN1) (Figure 2B).

We observed significant down regulation of profilin-1 (PFN1), platelet basic protein (PPBP) and selenoprotein P (SELENOl). PFN1 plays a major role in cellular proliferation, motility, and cellular growth and thus any aberrant expression resulting in inflammatory diseases [27, 28]. Lower levels of TMSB4X have been identified in the serum of psoriasis patients which also has been reported previously [29, 30]. This protein has been known to play an active role in organization of the cytoskeleton and known to bind to and sequester actin monomers, thus, inhibiting actin polymerization. Past research had shown that both the basement membrane molecular composition and the polarised expression of integrins were altered in psoriatic lesions. In the current study, we observed similar observation where proteins such as ACTB and TMSB4X were observed to be altered in the serum of psoriasis patients as compared to healthy volunteers.

Conclusions:
Nonetheless, proteomics research in psoriatic disease is still in its early stages as there are still numerous gaps that need to be filled. Validation of putative protein biomarkers in a large cohort of patients is required in order to properly establish diagnostic and prognostic biomarkers that may be employed in clinical practise. Furthermore, proteomics in combination with other emerging approaches will enhance our knowledge of disease mechanisms and, in turn, will aid in identification of therapeutic targets for psoriasis therapy in the future.

Conflict of interest:
None

Acknowledgements:
The authors thank Yenepoya (Deemed to be University) for providing seed grant to complete this study and the Centre for Systems Biology and Molecular Medicine for providing the infrastructure to carry out this research. VM is a recipient of a Women Scientist-A from the Department of Science and Technology, Government of India.

Author Disclosure statement:
The authors declare that they have no competing financial interests.

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