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Characterization and Serum Protein Profile of Rheumatoid Arthritis Subjects of Western Region of Mumbai

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ABSTRACT

Rheumatoid Arthritis (RA) a degenerative, inflammatory autoimmune disorder if undiagnosed can lead to mortality and morbidity. The diagnosis of RA is based on the scoring criteria of the 2010 ACR/EULAR classification for serological and acute-phase reactant measurements. Post-translational modifications of proteins are one of the reasons for RA. So far Synovial fluid-based proteomic studies have been done for protein characterization and studies on serum or urine samples to assess RA association proteins were minimal. Thus, the current study aimed to compare the serum samples of healthy and affected subjects using Orbitrap LC-MS and gene Ontology, Reactome & KEGG pathways to develop a profile. A study of 500 symptomatic patients was screened and found 46.6 % positivity of which 85 % were women & 15 % were men. A comparison of proteins between normal and affected individuals showed 27 differentially expressed proteins (DEP). A detailed study of protein functions and pathways using gene ontology was conducted. The studies indicated the 7 down-regulated proteins (Apolipoprotein B (112), Complement C3(89), CDNA FLJ75416 (30), Apolipoprotein A-I (24), Apolipoprotein A-IV (22), Complement C5(21), Prothrombin (14), and Heparin cofactor 2) may have good impact to initiate the Rheumatism associated Arthritis. Further studies on these proteins may help in their use for diagnostic purposes.

Keywords: Arthritis; LC-MS; Differentially expressed proteins; Autoimmune

NOMENCLATURE

ACR : American College of Rheumatism
EULAR : European League Against Rheumatism

1. INTRODUCTION

Rheumatoid Arthritis (RA) is complicated, systemic, chronic, inflammatory, and autoimmune illness that causes disability, including characteristic synovitis[1]. The causative agents could be gender, environmental conditions, genetic factors, and level of immunity[2]. The diagnosis of RA is based on criteria (ACR/EULAR 2010) that assess joints involved in the disease, serological and acute phase reactant measurements, and consequent symptoms. Serological tests of auto antibodies such as Rheumatoid factor (60-70 %) and Anti-Citrullinated Protein Antibody (ACPA) have acquired specificity and sensitivity (60-75 %). Acute-phase reactant proteins, such as ESR and CRP, are less sensitive because they are both specific for RA disease diagnosis and general markers of inflammation. Cytokines are associated with severe inflammation, thus making their diagnostic value difficult[3].

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Rheumatism is the result of an autoimmune disorder. The immune system, represented by B and T lymphocytes, attacks and removes antigens with the help of antibodies, which are protein molecules. In the case of rheumatism, the immune system misjudges the source and becomes involved in the protection steps that recruit proteins at different levels. Recent research on rheumatism has considered these proteins to be the main candidates for the triggering pathways of immunity. Proteins such as CRP, Serum Amyloid A, Calgranulin A, B, and C, and Vitamin D binding proteins are differentially expressed in Rheumatoid Arthritis.[1]

Previous studies of rheumatism were conducted internationally based on synovial fluids[4], adverse effects, and impact on females,[5,6] or genes associated with RA, such as the galectin-3 gene[7]. Multiplex assays[8], nano-liquid chromatography-tandem mass spectrometry (LC-MS/MS), and ELISA[1,9] were considered to study some of the sample profiles collected from synovial joints. The concentration of serum proteins may provide the systemic turnover of metabolites in a person, whereas synovial fluid markers can reflect the metabolic status at only one point[10]. The previous research in the Indian context included plasma-based studies that established a relationship between RA and lipid profile[11], isolation of pathogenic markers from

plasma[12], and identification of prognostic markers[13] or auto antigens from synovial fluid[14]. The current study profiled and identified proteins in the serum of patients with RA. Reference ranges for the Serological markers and Acute Phase reactant were established internationally from serum samples thus differentiating the abnormal and normal status of health. A simple procedure based on powerful tools such as LC-MS has been employed to identify and characterize proteins. Some of these proteins are indicators of changes in biological functions and interactions. Thus, they enable us to assess the problems and developments occurring during disease.

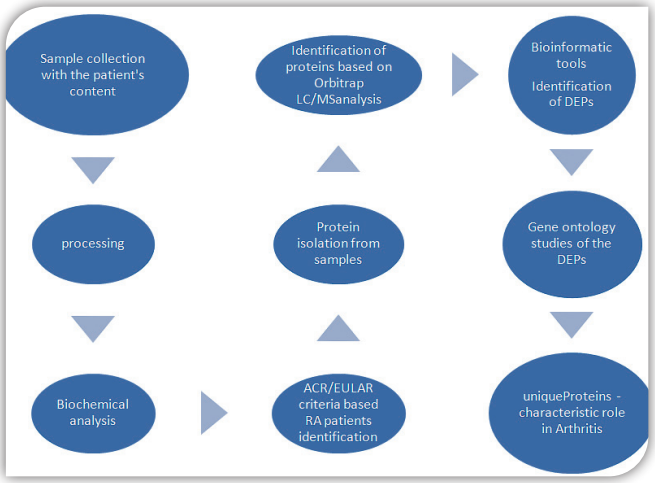

Figure 1. Characterization and serum protein profile of rheumatoid arthritis subjects.

2. MATERIAL AND METHODS

2.1 Serum Sample Collection

The samples were collected for one year from 3 different pathology laboratories in the western region of Mumbai. The blood samples were collected from the patients A. who suffer from symptoms of rheumatoidism with a suggested prescription for confirmatory tests by an orthopaedic/ general physician to assess the onset of disease, C. who have given their consent. The consent form taken from the patient who agreed for their serum samples to be used in the purpose of study to find protein pattern between normal and affected individuals. Only those who agreed can only participate and no compulsion on them. The antecubital region was preferred for blood collection during the study. Universal precautions were followed during blood collection. Clots formed after keeping blood at room temperature for 30 min. Centrifugation of the sample was performed f at 3000 rpm for 10-15 min. for serum sample preparation. A Chemi-Luminescent Microparticle Immunoassay(CMIA) was used to test for anti-CCP(Anti-Citrullinated Protein Antibody) with a sensitivity: of 70.6 % and specificity: of 98.2%. Rheumatoid factor and C-reactive protein levels were examined using immuno-turbidimetric methodology (sensitivity: 70.6 % and specificity: 98.2 %). Approximately 500 suspected cases of Rheumatoid Arthritis were screened for the presence of the disease based on the ACR/EULAR score. The criteria considered for RA disease confirmation was ACR. The EULAR score should be equal to or greater than 6 for all mentioned criteria[15]. Informed written consent was obtained from all subjects along with blood samples for proteomic analyses. Control samples were collected following the same procedure. The selection criteria for normal subjects were weight (>50 kg) and Hemoglobin (>12 g/dL) and no history of smoking or any other disease symptoms. The reason was to avoid the impact of smoking (external factor), anemic conditions (<12 g/dl hemoglobin), and possible underweight and other disease symptoms that may trigger or exist during the early stage of RA.

Anti-CCP (less than 5 U/ml), C-reactive protein (less than or equal to 5 mg/L), rheumatoid factor (less than 30 IU/ml), and matrix metalloproteinase protein-3 of 24-120 ng/ml range for women and 18-60 ng/ml range for men were considered as normal reference range for an average, healthy person. Individuals with parameter values above the normal range along with symptoms of affected joints were considered Rheumatoid Arthritis subjects. The serum samples of RA subjects and reference were further pooled into 3 samples of the affected and 1 normal (Control) subject. Serum samples with high values and with a range of anti-CCP >200 to 250; CRP->0.5 to 1.0; RF-30-50, and MMP3 >120 ng/ml were pooled and considered as sample -1, and those above the level of anti-CCP >250, CRP->1.0, RF50 and MMP3 >150 ng/ml were pooled as sample-2 representing the females and, sample-3 with higher values of the parameters such as anti-CCP >200, CRP->0.5 to 1.0,RF>30 and MMP3 >120 ng/ml of men were pooled. The fourth sample was a pooled serum sample from healthy subjects—the number of samples considered to pool up in proportion to the samples represented in the study.

2.2 Sample Preparation and Trypsin Digestion

A protocol for in-solution protein digestion was followed for processing native serum samples' processing.[16]Care was taken to avoid contamination of the samples and solutions. MilliQ water was used for sample preparation. 15µl of the serum sample was processed with 85mm Dithiothreitol (DTT), 55 mm Iodoacetamide (IAA), and 80 % Acetonitrile (ACN) solutions. This step was followed by Trypsin digestion for 3 h at 37 °C.

2.3 LC-MS/MS (Liquid Chromatography-Mass Spectrometry)

Samples of digested serum were examined using a Thermo EASY-NLC 1000 UPLC system. The system was attached to a Q Exactive-TM Quadrupole-Orbitrap instrument (Thermo Scientific, Waltham, MA, USA). The analytical column used was PepMap RSLC C18, 2 µm, 100A x 50 cm, and the pre-column was Acclaim Pep Map 100, 100 um x2 cm, a nanoviper. The mobile phase used was solvent A (0.1 % FA (formic acid) in milliQ water) and solvent B (80:20 ACN in milliQ water plus 0.1 % FA). The entire MS scan was collected in orbitrap at 70,000 resolutions for a scan range of 350-2000 m/z. The mixture was run for 120 min at a constant flow rate of 3.0 l/min

with an inner instrumental diameter of 2.303 mm. The chromatogram with the peaks was further studied based on the protein databases PDB[1,3,4].

2.4 Protein Identification Based on Peptide Sequencing

Each fraction of the protein chromatogram was analysed for peptide sequence identification and quantitation. The chromatograms of the proteins were further studied using the *Homo sapiens* database, NCBI (National Center for Biotechnology Information), and UniProt. Peptide and protein identification was performed using Thermo Proteome Discoverer 2.2 software and the *Homo sapiens* database.

2.5 Differentially Expressed Proteins (DEPs)

In the current study, the bioinformatics-based DEP identification procedure was used. The acquired MS data are submitted to a database search. The sequence of each analyzed peptide is identified and the abundances of identical light and heavy peptides are determined. Proteins obtained from healthy control and diseased subjects were compared using venny 2.1 software. Unique proteins were identified. Each protein was identified by two distinct peptides and a false discovery rate (FDR) of 1 %[17]. False Discovery Rate (FDR) of each protein was estimated through a decoy (reversed) DB search. A unique set of identified and quantified peptides determines the identity and abundance of the corresponding parent protein. Proteins with a false discovery rate of 0.05, > 2-fold change (upregulated), < 0.5 (downregulated). Proteins with > 2 unique peptides were considered as differentially expressed proteins (DEP) and used for further analysis.[18]

2.6 Gene Ontology (GO) Studies

GO studies were conducted to discover the various functions of DEPs at the cellular, molecular, and biological levels. Gene Ontology Enrichment Analysis was performed to determine the presence of genes on chromosomes.

2.7 Biological Pathway Analysis

A pathway study was conducted using the Go profiler database, with a false discovery rate of 5 % and a significant p-value (0.05). The probability of each outcome was calculated using the binomial test, and the Benjamin-Hochberg technique was used to adjust the p-values for multiple testing. The Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and Wikipathway (WP) databases were used as pathway software.

3. RESULTS

3.1 Diagnosing Score

The ACR/EULAR criteria score was obtained based on the serological tests (anti-CCP & RF), acute phase reactant values (CRP), duration of rheumatoid symptoms, and level of affected joints of the subjects. Each category has 0 to 5 points which get added lastly to make a score and diagnose RA. The overall study indicated that 46.6 % of the subjects met the requirement of an ACR/EULAR score of 6. Among the Rheumatoid Arthritis-confirmed subjects (233), women represented 85 %, and men 15 %.

3.2 Protein Peptide Identification

Representative samples of 191 women and 42 men with high levels of C-Reactive Protein (CRP), Rheumatoid Factor(RF), anti-Cyclic Citrullinated Peptide (anti-CCP), and Matrix Metalloproteinases(MMP)-3 proteins and the control group were considered for Q Exactive Orbitrap LC-MS analysis. Peaks of the controlled and human proteome data were compared and considered for peptide sequence identification and quantification. The overall observations indicated the representation of 641 proteins in the control, 393 proteins in the first, 470 proteins in the Second, and 336 proteins in the Third sample pool of subjects which will be herby referred to as samples.

The Venn plot developed using Venny software clustered the available protein data into groups of uncommon and common proteins (Fig. 2). The number of proteins restricted to the control sample pool was 364, followed by 180, 218, and 228 in samples 1, 2, and 3 respectively. A comparison of proteins shared by controls and samples revealed the presence of 277 proteins exclusively. Overall, 49 proteins were exclusively observed in the Arthritis samples. However, there was no indication of common proteins among the three pool samples. The distribution of 49 proteins is represented as 32 common proteins among samples 1 and 2, 8 common proteins among samples 2 and 3, and 9 common proteins between samples 1 and 3.

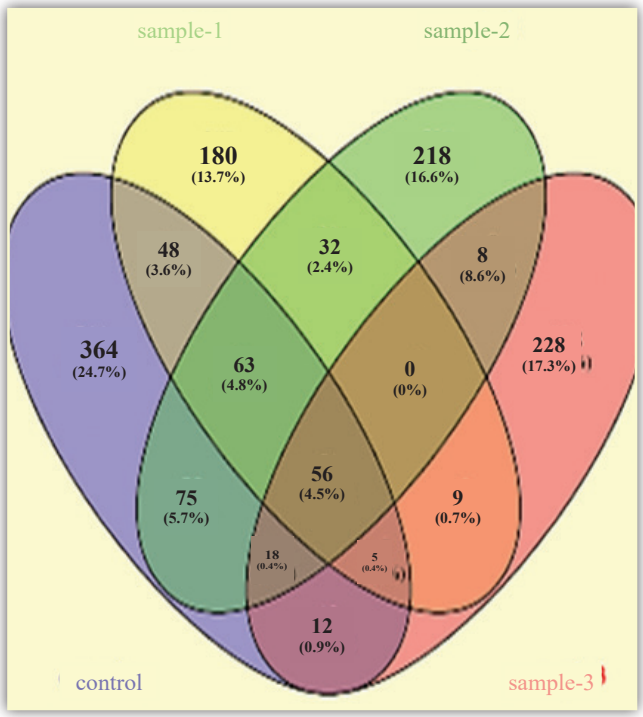

Figure 2. Venn diagram comparing protiens samples from RA patients.

3.3 DEPs Found in the Subject

Altogether, 27 proteins were identified as DEPs based on the standard procedure of proteins with a false discovery rate of 0.05, > 2-fold change(upregulated), < 0.5 (downregulated), and > 2 unique peptides[1,9,18]. In comparison to healthy controls, it was observed that 3 proteins indicated upregulated expression and, 24 with downregulated in the RA subjects (Table 1).

3.4 Gene Ontology Analysis Results

The gene ontology records showed gene types that expressed various molecular functions, biological processes, and cellular component roles. The significant functions and processes of each of the genes representing the protein were analyzed. The Pathway analysis predicted a significant value (Figs. 3, 4, 5).

Table 1. Upregulated and downregulated differentially expressed proteins

S. No .	Gene.	Accession	Description	Fold change	Expression (regulation)	P-value	Unique peptide
1	IGKV3-11	P04433	Immunoglobulin kappa variable 3-11	6.45	Up	0	3
2	C8G	P07360	Complementary component C8 gamma chain	5.43	Up	0	3
3	IgH	A0A2U8J9D6	Ig heavy chain variable region	2.79	Up	0	3
4	-	A8K1K1	cDNA FLJ76342	0.01	Down	0	3
5	HBD	P02042	Hemoglobin subunit delta	0.105	Down	0	3
6	APOC4-APOC2	K7ER74	APOC4-APOC2	0.116	Down	0	4
7	HBB	P68871	Hemoglobin subunit beta	0.118	Down	0	7
8	KRT9	P35527	Keratin, type I cytoskeletal 9	0.123	Down	0	4
9	GLNC1	A0A1K0GXZ1	Globin C1	0.14	Down	0	6
10	F13B	P05160	Coagulation factor XIII B chain	0.199	Down	0	3
11	APOC3	B0YIW2	Apolipoprotein C-III variant 1	0.258	Down	0	3
12	KRT1	H6VRG2	Keratin 1	0.292	Down	0	9
13	C5	P01031	Complement C5	0.29	Down	0	21
14	PPBP	P02775	Platelet basic protein	0.322	Down	0	6
15	APOA4	P06727	Apolipoprotein A-IV	0.357	Down	0	22
16	-	B0AZL7	cDNA, FLJ79457	0.371	Down	0	5
17	SERPIND1	P05546	Heparin cofactor 2	0.395	Down	0	10
18	APOB	C0JYY2	Apolipoprotein B	0.4	Down	0	112
19	PON1	P27169	Serum paraoxonase 1	0.423	Down	0	9
20	APOC1	P02654	Apolipoprotein C-I	0.425	Down	0	4
21	SERPING1	E9PGN7	Plasma protease C1 inhibitor	0.432	Down	0	5
22	F2	P00734	Prothrombin	0.437	Down	0	14
23	-	A8K5T0	cDNA FLJ75416	0.454	Down	0	30
24	APOA1	P02647	Apolipoprotein A-I	0.478	Down	0	24
25	KRT10	P13645	Keratin, type I cytoskeletal 10	0.481	Down	0	7
26	RBP4	P02753	Retinol-binding protein 4	0.49	Down	0	5
27	C3	P01024	Complement C3	0.492	Down	0	89

(Note: Up- Up-regulated, down- down-regulated)

Three proteins of 27 DEP were found to be upregulated and represented by Immunoglobulin kappa variable 3-11, Complement component C8 gamma chain, and IG heavy chain variable region. In contrast, 24 proteins were down regulated with >_3 unique peptides. The down-regulated proteins with >10 unique peptides, were considered to understand and assess their role in Rheumatism and pathways among the 24 differentially expressed proteins. These were Apolipoprotein B, Complement C3, CDNA FLJ75416, Apolipoprotein A-I, Apolipoprotein A-IV, Complement C5, Prothrombin, and Heparin cofactor 2, represented by 112,89,24,22,21,14 and 10 unique peptides respectively (Table-1).

Gene Ontology studies indicated the genes and respective proteins were associated with molecular, biological, and cellular component functions, The significant molecular functions include phosphatidylcholine-sterol O-acetyl transferase activator (p-value 5.816 x 10^{-6}), alcohol binding and lipid binding (p-value 9.112 x 10^{-6}) (Fig. 3). The prominent biological functions exhibited by proteins include cholesterol efflux (p-value 2.956 x 10^{-8}), plasma lipoprotein particle assembly (p-value 6.845 x 10^{-8}) (Fig. 4) and cell components represented by blood micro-particles (p-value 5.260x10^{-17}), extracellular space and extracellular region (p-values 2.819x10^{-16} and 6.239x10^{-14}) (Fig. 5). Blood micro-particles are also known as extracellular vesicles. These micro-particles or vesicles are released from various cells such as platelets, endothelial cells, and immune cells. Because RA is associated with the immune cell complex, an increase in blood micro-particles can be seen[19]. It can be regarded as a helpful marker if there is sufficient evidence to support its incidental relationship with RA.

Figure 3. Molecular funcation summary with significant value and gene association.

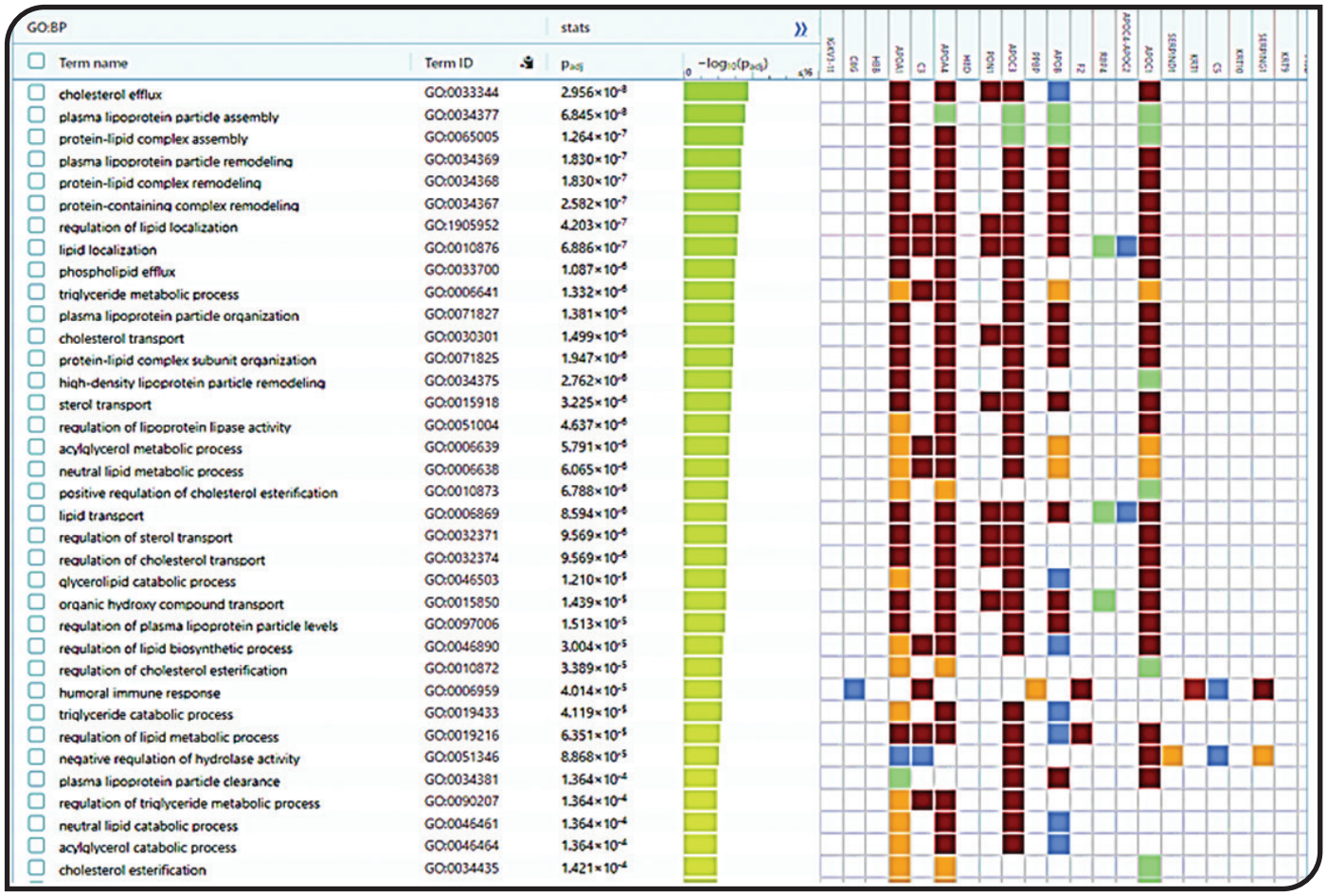

Figure 4. The Biological process with significant value and gene association.

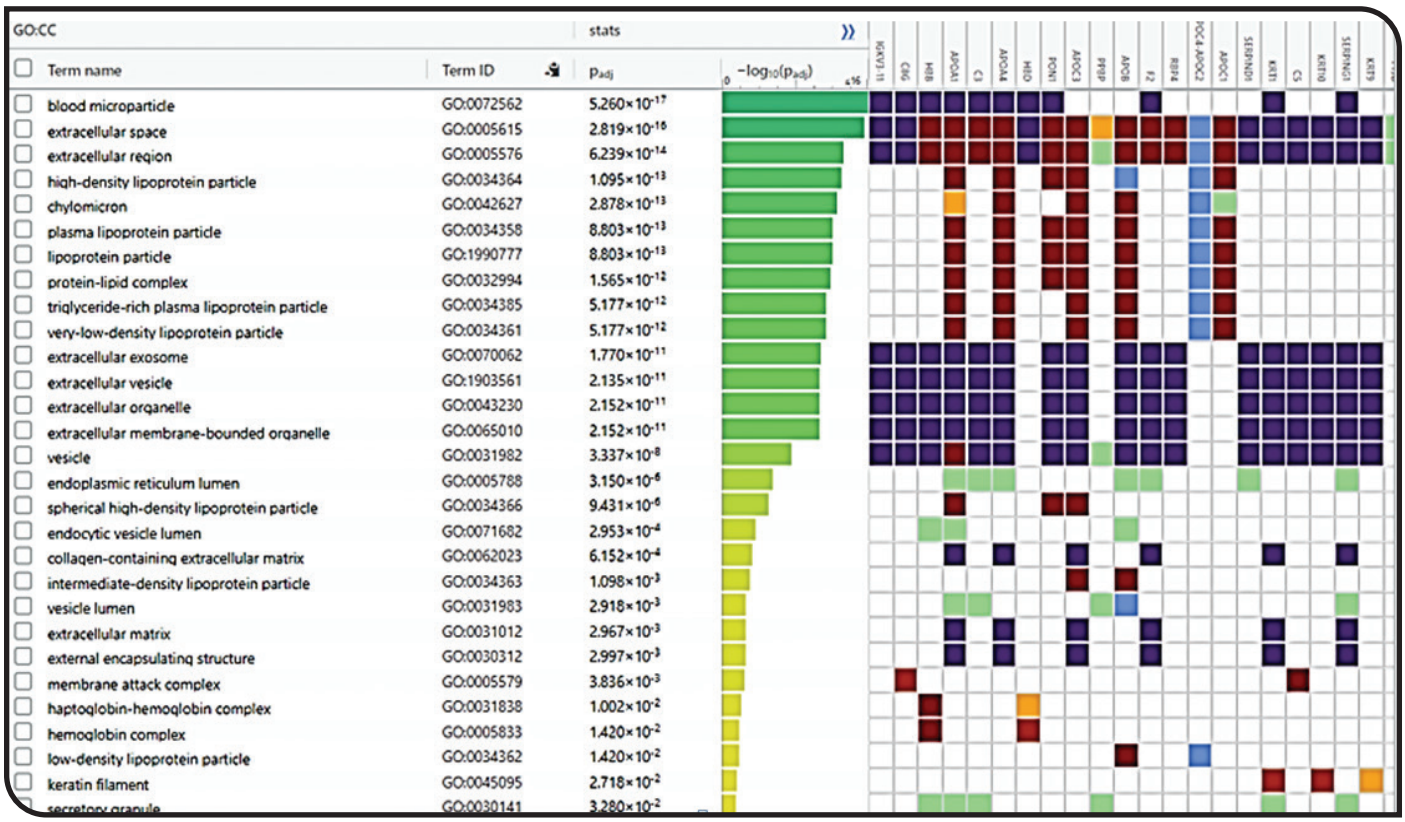

Figure 5. Cellular components with significnt value and gene association.

Pathway Analysis The 27 DEPs are involved in a total of 47 pathways. The KEGG pathway analysis indicated complement and coagulation cascades ($p = 7.146x10^{-9}$), cholesterol metabolism ($p = 2.057x10^{-6}$), plasma lipoprotein assembly ($p = 4.780x10^{-8}$), and vitamin digestion and absorption ($p = 7.583x10^{-4}$) as the most significant pathways. Each pathway comprises several proteins which play a prominent role in the function of a system.

A. Complement and Coagulation Cascades

The complement system is the most important component of the immune system. It plays a crucial role in protective immunological activities. The development of autoimmune disorders, including RA, is caused by an inadequate management of complement activation. Upregulation of complement C8 (fold change value: 5.43) and down regulation of complement C5 (fold change value: 0.299) and complement C3 (fold change value: 0.4920) hamper the complement activation process. The regulation of complement activation involves C3 protein activation and is regulated by a protein known as the Regulator of Complement Activation (RCA). RCA is present in healthy host cells. Pathogens do not express RCA on their surfaces but evade the complement system by binding to RCA circulating in human blood[20].

RCA is present in bacteria, viruses, fungi, and parasites[21]. The complement system and RCA deposition are crucial for maintaining tissue homeostasis, removing waste products and dead cells, and guarding against oxidative stress damage[22]. The activation of complement generates enzymatically active molecules (C3 and C5) and biological effectors, including opsonin (C3b, C3d, and C4b), anaphylatoxins (C3a and C5a), and C5b, initiating the assembly of the lytic Membrane Attack Complex (MAC). Thus, complement activation pathways (classical, lectin, and alternative) are activated[23]. Target cell opsonization to promote phagocytosis, Membrane Attack Complex (MAC) assembly on the cell surface, production of anaphylatoxins C3a and C5a involved in the host inflammatory response, C5a-mediated leukocyte chemotaxis, and clearance of antibody-antigen complexes are all caused by complement system activation. Thus, regular monitoring of C3 and C5 levels during a disease state can help prevent damage to the joints and synovial membrane.

Down regulation of DEPs [coagulation factor XIII chain (fold change: 0.099), platelet basic protein (fold change: 0.322), and heparin cofactor 2 (fold change: 0.395)] leads to dysregulation of the coagulation cascade. Coagulopathies and inflammatory diseases are associated with host defence mechanisms against physical injury. The coagulation cascade has several proteolytic reactions that activate thrombin. Thrombin cleaves fibrinogen to fibrin, which is the main constituent of blood clots. Apart from coagulation, thrombin is also involved in the regulation of the inflammatory response. Thus, dysregulation affects various illnesses, which in turn result in high levels of inflammation and thrombosis. Monitoring these proteins can aid in the identification of novel diagnostics and strategically designed treatments[24].

B. Cholesterol Metabolism and Plasma Lipoprotein Assembly

Downregulation of all apo lipoproteins (A to C, fold change value from 0.258 to 0.478) leads to a malfunction of fat digestion and absorption from the endoplasmic reticulum, leading to the exocytosis of chylomicrons released into the bloodstream. Apolipoproteins are crucial plasma lipoproteins because they make it easier for the body to absorb and secrete fat from the gut. It also plays a role in the activation of enzymes involved in lipoprotein metabolism. Plasma lipid concentration changes in response to modifications in the apolipoprotein amount or composition. In Rheumatoid Arthritis, disorders of lipoproteins can occur and can be corrected by measuring their levels. Thus, it has become a critical tool for diagnostic and prognostic aspects.

Lipoprotein A (LPA) is a modified Low-Density Lipoprotein (LDL) with an additional apo lipoprotein (A). Increased levels of LPAs in plasma can lead to CardioVascular Disease (CVD) and Peripheral Arterial Disease (PAD)[25,26,27]. The physiological role of LPAs is not clear and requires further studies. Individuals without diseases or any deficiency syndromes have extremely low values of LPA. Although it contributes to wound healing and tissue repair[2], LPA is susceptible to oxidative modification. It forms pro-inflammatory and pro-atherogenic oxidized phospholipids, oxysterols, and oxidized lipid-protein adducts known as "Oxidation Specific Epitopes" (OSEs)[25,26]. Various OSEs with LP(A) are considered "danger-associated molecular patterns" (DAMPs), which in turn cause local inflammation, apoptosis, tissue disintegration, and trigger innate immunity.[27] A modified LP(A) binds and transports pro-inflammatory molecules such as monocyte chemoattractant protein-1. It also acts as a major chemokine that induces and maintains vascular inflammation and atherosclerosis. The OSEs of LP(A) play a role in pathogenesis. This study revealed the down regulation of 24 proteins, including apolipoproteins A (I and IV), B, and apolipoprotein C (I and III). Every lipoprotein participates in lipid metabolic activities and lowers the risk of developing cardiovascular disease. Apolipoprotein B causes LDL to attach to specific receptors on the surface of liver cells and, transports LDLs into cells for breaking down to release cholesterol. Apolipoprotein C is secreted by the liver and is found in triglyceride-rich lipoproteins. It enhances the triglyceride hydrolysis of VLDL for energy delivery or storage. Thus, any downregulation of lipoproteins can lead to Coronary Vascular Disease (CVD).

C. Vitamin Digestion and Absorption

Previous studies indicated the role of vitamin D binding proteins as differentially expressed proteins and their association with Rheumatoid Arthritis[1]. In the current study, the down regulated expression of Retinol Binding protein 4 (RBP-4) with 5 unique peptides was observed in the serum samples of subjects. Retinol binding protein 4 (RBP-4) belongs to the lipocalin family and is a major

transport protein of the Retinol molecule or vitamin A. It has a prominent role in the sensitivity of insulin as well as in the pathogenesis of cardiovascular disease.

KEGG pathway analysis showed Staphylococcus aureus infection ($p = 1.249x10^3$) and corona virus disease (COVID-19) ($p = 3.736x10^3$). Patients with RA are at a higher risk of any type of bacterial or viral infection. *Staphylococcus Aureus* can produce various toxins that lead to septic shock or disseminated infection[29]. Keratin 10 protein is manufactured from the KRT10 gene. Keratin, a fibrous protein that forms the structural framework of cells known as keratinocytes, is the makeup of the skin, hair, and nails. It is also present in the outer layer of the skin and epidermis. Down regulation of keratin affects the interactions between intermediate filaments to form networks and their function. Thus, with hampered function, skin cells become fragile and easily damaged, leading to blistering in response to friction or mild trauma that causes infection. The position of DEPs on chromosomes has been studied, and it was observed that chromosomes 11 and 19 contained the most genes. In total, 18 of the 24 genes were found on chromosomes.

4. DISCUSSION

Serum protein profiles have been created to understand the protein composition of diseases such as colon cancer and psoriasis, self-antigen microarrays, and myocardial infarction[30,31,32,33] in the Indian context. Similarly, previous studies on protein analytical studies related to RA patients concentrated on the synovial fluid,[5] plasma, venous blood, or serum at the global level. Most of them concentrated more on the identification of biomarkers[9]. Glycolated proteins prepared by fluorescence-polyacrylamide gel electrophoresis (Flu-PAGE) with mass spectrometry were studied using 2-D Electrophoresis and SWATH-MS to identify DPs in the plasma of RA subjects[12]. Other studies included the synovial fluid of patients with RA using MALDI/TOF or 2D PAGE and immune-proteomics to identify the proteins[13,14].

The current study is unique in the Indian context, as it enlists and compares the spectrum of serum proteins based on the direct trypsinization of serum proteins followed by LC-MS Orbitrap and bioinformatics-based tools to identify DEPs. Protein patterns revealed the involvement of up regulated and down regulated proteins and their association with numerous pathways. These protein measurements can help in the diagnosis, prognosis, and therapeutic aspects of the disease.

The previous studies highlighted the increase of proteins including coronin-1A (CORO1A), fibrinogen-like-2 (FGL2), and macrophage capping protein (CAPG) in the synovial fluid of Rheumatoid Arthritis patients in earlier research.[5] Similar research on synovial fluid proteins conducted in Korea revealed a correlation between RA synovial fluid antibodies and fibronectin, semaphorin 7A precursor, growth factor binding protein (GRB7), and immunoglobulin chain[7]. The investigations showed that metabolic proteins such as adipocyte fatty acid binding protein, galectin-1, apolipoprotein A1 precursor, and peroxide, peroxy 2, were over expressed in the synovium and that vimentin expression was suppressed in synoviocytes of RA[34]. Several proteins were expressed differently in the serum of RA patients, including selenium protein P, C4b-binding protein beta chain, apolipoprotein M, N-acetyl muramyl-L-alanine amidase, catalytic chain, carboxypeptidase N subunit 2, apolipoprotein C-I, and apolipoprotein C-III[15].

In the Indian context, TTR from RA palsma[12], α-Taxilin (AlphaTaxilin)[13] form synovial fluid, and, GFAP and A1BG from the synovial fluid[14] were identified as biomarkers. The current serum profile indicated that regular monitoring of complement C3 and C5 levels during the disease stage can help prevent damage to the joints and synovial membrane. The current study indicated a down expression of RBP-4 protein. It supports the earlier study of RBP-4 as a promising biomarker for RA as the increased level of RBP4 is associated with the presence of atherosclerosis in patients with RA[35]. Monitoring proteins such as coagulation factor XIII, platelet basic protein, and heparin cofactor 2 can help identify novel diagnostics and strategically designed treatments. Apolipoprotein A, B, and C measurements can help prevent RA-associated cardiovascular diseases.

5. CONCLUSION

Serum protein analysis of patients with Rheumatoid Arthritis indicated a different profile and expression of proteins from those of the control and synovial fluid samples. Simple analytical procedures of direct trypsin digestion followed by Orbitrap-LC analysis are part of the unique approach considered in this study. Similarly, differentially expressed proteins were not observed in the previous studies, which may be because the samples were not of synovial fluid. 3 up regulated and 24 down regulated protein expressions were well noted in disease subjects compared with healthy controls. The 27 protein panels or quantitative analyses can aid in early diagnosis with accurate prognostic indications.

6. LIMITATIONS OF THE STUDY

The samples are of Suspected subjects having ACR/EULAR scores more than or equal to 6 only. The study has no extensions concerning diagnostics of the disease, medication, or follow-up after medication.

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