Network-based identification genetic effect of SARS-CoV-2 infections to Idiopathic pulmonary fibrosis (IPF) patients

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Submitted: 8 July 2020; Received (in revised form): 10 August 2020

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Abstract
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is accountable for the cause of coronavirus disease (COVID-19) that causes a major threat to humanity. As the spread of the virus is probably getting out of control on every day, the epidemic is now crossing the most dreadful phase. Idiopathic pulmonary fibrosis (IPF) is a risk factor for COVID-19 as patients with long-term lung injuries are more likely to suffer in the severity of the infection. Transcriptomic analyses of SARS-CoV-2 infection and IPF patients in lung epithelium cell datasets were selected to identify the synergistic effect of SARS-CoV-2 to IPF patients. Common genes were identified to find shared pathways and drug targets for IPF patients with COVID-19 infections. Using several enterprising Bioinformatics tools, protein–protein interactions (PPIs) network was designed. Hub genes and essential modules were detected based on the PPIs network. TF-genes and miRNA interaction with common differentially expressed genes and the activity of TFs are also identified. Functional analysis was performed using gene ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway and found some shared associations that may cause the increased mortality of IPF patients for the SARS-CoV-2 infections. Drug molecules for the IPF were also suggested for the SARS-CoV-2 infections.

Key words: SARS-CoV-2; idiopathic pulmonary fibrosis; differentially expressed genes; gene ontology; protein–protein interactions; hub gene; drug molecule

Introduction
The current world is going through a rough patch for the outbreak of coronavirus disease (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for COVID-19. SARS-CoV-2 is a virus belonging to the Coronaviridae family [1]. Spike glycoproteins of coronavirus raise the entry of the virus into cells and ACE2 of the virus binds with human ACE2 [2]. Among the most serious risk factors of COVID-19, idiopathic pulmonary fibrosis (IPF) is considered to be the most vital one [3]. As viral infections can immensely enhance IPF risks so patients recovering from COVID-19 can face numerous complications because of having IPF [4, 5].

In late 2019, the COVID-19 case was first discovered in a city named Wuhan which is situated in China and at the end of 2019 World Health Organization (WHO) declared COVID-19 as a serious epidemic of 21st century [6]. The clustering result of the affected people in the early days of the pandemic was linked to the Wuhan seafood market and with the contact of wild animals [7]. In 2020, COVID-19 spread all over the world. As of 3 March 2020, the virus has already spread in most of the provinces in China including 80 151 numbers of confirmed cases and 10 566 numbers of confirmed cases in another 72 countries of the world [8]. Until 6 June 2020, the numbers of confirmed cases all over the world were 6 663 304 including 392 802 death (https://covid19.who.int/). According to Worldometer, United States, Brazil, Russia, Spain, UK are among the top five countries where SARS-CoV-2 has spread the most. The first confirmed case in the United States was in January 2020 and the female patient visited China a few days before she had pneumonia and hospitalized and finally got herself SARS-CoV-2 positive [9]. From January 2020 to June 2020 United States had to witness a lethal face of COVID-19. Until 7 June 2020 according to WHO (https://covid19.who.int/), 1 886 794 cases were confirmed as COVID-19 positive including 109 038 deaths. A current study shows that Brazil has the highest transmission rate among all the countries of the world which makes Brazil a hotspot for COVID-19 [10, 11]. The first patient of COVID-19 was identified on 25 February 2020 who came back from Italy where the epidemic was ever so serious than other countries [12].

IPF is a chronic lung disease that causes serious decay of lung functionality [13]. Breathing complexity and dry cough are the primary symptoms of IPF [13]. A pathological study on IPF suggests that continuous lung injury might be one of the reasons of IPF [14]. IPF results in lung failure and respiratory complexities and the survival time range is between 3 and 5 years starting from diagnosis time [15]. The current study exhibits that SARS-CoV-2 contains S protein that has higher interaction for ACE2 and IPF patients contain a significant level of ACE2 that proved IPF as a risk factor for COVID-19 [16, 17]. These researches raise concerns about a number of interconnection between IPF and COVID-19.

In the field of biomedical research, high throughput methodologies are becoming significant and microarray data analysis is one of the most prominent techniques of high throughput methodologies that are used for analyzing gene expression in large-scale [18]. Microarray study simultaneously assists genetic researchers to study in terms of genetic expression [19]. Previous research demonstrates high throughput sequencing analysis for SARS-CoV which shows the prominent result in the assessment of data quality and gene expression [20]. Microarray data analysis for SARS-CoV-2 and risk factor IPF is not presented yet. This study attempts to find biological pathways and the relationship between COVID-19 and IPF. Two datasets were selected for analysis of the research. GSE147507 was selected for SARS-CoV-2 infection in humans and GSE35145 was selected for IPF gene expression analysis. Both the datasets were collected from the Gene Expression Omnibus (GEO) database. The initial work was to identify differentially expressed genes (DEGs) for GSE147507 and GSE35145 and then find common DEGs for COVID-19 and IPF. The common DEGs are the prime data for the entire study. Based on the common genes, further analysis was accomplished including gene set enrichment analysis and pathway analysis to have an understanding of biological processes of genome-based expression studies. Identification of hub genes from common DEGs is the most essential work as finding drug molecules mostly depends on hub genes. To achieve this protein–protein interactions (PPIs) network is designed to gather hub genes from the PPIs network. The workflow of the present research is displayed in Figure 1.
Methodology

Collection of the dataset

Dataset (GSE147507) illustrates infections of SARS-CoV-2 in transcriptional responses and dataset (GSE35145) represents interchange of gene expression in IPF and both datasets were compiled from GEO database [21]. GEO database was introduced for gene expression analysis using high throughput methodology under the National Center for Biotechnology Information platform [22]. Illumina NextSeq 500 platform was used for the GSE147507 dataset for extracted RNA sequence analysis and the GPL10558 (Illumina HumanHT-12 V4.0 expression bead chip) platform is used for GSE35145 dataset. GSE147507 dataset was contributed by Blanco-Melo D et al. [23]. Dataset for IPF (GSE35145) was presented by Yan Y Sanders et al. [24]. COVID-19 dataset (GSE147507) provides samples including SARS-CoV-2 infection in lung epithelium and lung alveolar cells of humans. The IPF dataset (GSE35145) contains eight samples including genetic expression alteration in IPF cells and normal tissue of the lung which is more suitable datasets for this study. GSE35145 dataset is a subset of the GSE35147 dataset and it indicates DNA methylation profile. For our analysis we have selected GSE35145 dataset because of microarray-based analysis for IPF samples.

Identification of DEGs and common gene identification between COVID-19 and IPF

Identification of DEGs for GSE147507 and GSE35145 datasets is the primary task of the research. To identify DEGs for GSE147507, the limma package of R programming language is implemented. Data that are produced from microarray analysis is retrieved through DESeq2 [25] and limma package [26]. Cut-off criteria was obtained for GSE147507 using adjusted P-value < 0.05 and log2-fold change (absolute) > 1.0. DEGs for the GSE35145 dataset were analyzed through GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) web tool which also uses limma package for identifying DEGs. Benjamini-Hochberg was applied for both the datasets for controlling of false discovery rate (FDR) [27]. The common gene identification between DEGs of GSE147507 and GSE35145 datasets was obtained using the R programming language.

Gene ontology and pathway finding in terms of Gene set enrichment analysis

Gene set enrichment analysis undertakes gene sets that have general biological functions and chromosomal locations [28]. For gene product annotation gene ontology (GO) term is used which is organized in three categories including biological process, molecular function and cellular component [29]. The principal reason for identifying GO terms is because of the understanding of molecular activity, cellular role and the location in a cell where the genes execute their functions. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway is usually used in the understanding of metabolic pathways and contains significant use over gene annotation [30]. In the purpose of significant pathway analysis WikiPathways [31], Reactome [32] and BioCarta databases were also used alongside the KEGG pathway. GO terms and all the pathways were obtained through web-based platform Enrichr (https://amp.pharm.mssm.edu/Enrichr/) for the common genes that were identified in the previous step. For experimented genome-wide genes Enrichr provides gene set enrichment analysis in web platforms [33].

Analysis of PPIs network

The activity of PPIs is considered to be the prime target of cellular biology study and works as a precondition for system biology [34]. Proteins perform their operation inside a cell with the interaction of another protein and information that is produced from a PPIs network raises perception about the function of
the protein [35]. Common DEGs are inserted in Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org/) for generating PPI network. STRING delivers experimental and predicted interaction-based information and the interaction produced through the web tool is defined with 3D structures, accessory information and confidence score [36]. The confidence score was also used for the current PPIs network with a medium confidence score of 0.400. The confidence score was set using the STRING platform that is considered to be a medium confidence score. For a superior visual representation of the network and the purpose of identifying hub genes, the obtained PPIs are analyzed through Cytoscape (https://cytoscape.org/). Cytoscape software acts as the most powerful one when it comes to integration with larger databases of genetic interactions, protein–protein and protein-DNA interactions [37].

Identification of hub genes and module analysis

PPIs network contains a number of nodes and edges and represents their interactions and among the nodes that have the most interaction is considered to be a hub gene. PPIs network analysis for the current research is attained through Cytoscape. Hub genes for the corresponding PPIs network are pointed out by using cytoHubba (http://apps.cytoscape.org/apps/cytohubba) which is also a plugin of Cytoscape software. The interface of cytoHubba is user-friendly which makes it the most prominent among all the hub detection plugins of Cytoscape and it contains 11 methods for topological analysis [38]. The hub genes for the current research are revealed by using the degree topological algorithm. The reason behind choosing degree algorithm rather than any other algorithm is that degree algorithm indicates the number of interactions for each gene in the PPIs network and it also assists the research by suggesting mostly dense modules in the PPIs network. Hub genes create concentrated areas that can be detected as an essential module from the PPIs network. Molecular Complex Detection (MCODE) (http://apps.cytoscape.org/apps/mcode) plugin of Cytoscape software is used to detect the most profound modules from the PPIs network. Highly interconnected portions are identified through MCODE clustering that assists the research in effective drug designing. For representing molecular complexes in the PPIs network MCODE is used by detecting the densely connected areas [39].

TF-gene interactions

TF-gene interaction with the identified common DEGs evaluates the outcome of TF on functional pathways and expression levels of the genes [40]. NetworkAnalyst (https://www.networkanalyst.ca/) platform is used to identify TF-gene interaction with identified common genes. NetworkAnalyst is a comprehensive web platform for performing gene expression for numerous species and also enables them to perform meta-analysis [41]. The network produced for the TF-gene interaction network is obtained from the ENCODE (https://www.encodeproject.org/) database which is included in the NetworkAnalyst platform.

TF-miRNA coregulatory network

Interactions for TF-miRNA coregulatory were collected from the RegNetwork repository [42] which assists to detect miRNAs and regulatory TFs that regulate DEGs of interest at the posttranscriptional and transcriptional level. TF-miRNA coregulatory network was visualized using NetworkAnalyst. NetworkAnalyst assists researchers in the easiest way to navigate complex datasets to identify biological features and functions which leads to effective biological hypothesis [43].

Identification of candidate drugs

Drug molecule identification is the key component of the ongoing research. Based on the common DEGs for COVID-19 and IPF diseases drug molecule is designed using the Drug Signatures database (DSigDB), which consists of 22 527 gene sets. The access of the DSigDB database is acquired through Enrichr (https://amp.pharm.mssm.edu/Enrichr/) platform. Enrichr is mostly used as an enrichment analysis platform that represents numerous visualization details on collective functions for the genes that are provided as input [44].

Results

Identification of DEGs and common gene identification between COVID-19 and IPF

GSE147507 dataset is used for the identification purpose of DEGs for COVID-19. One hundred and eight DEGs were obtained including 93 upregulated and 15 downregulated genes. For IPF dataset GSE35145 is used and a total of 359 DEGs were identified and among them 159 genes were upregulated and 200 genes were downregulated. Collected 108 genes for COVID-19 and 359 genes for IPF were compared using R programming language and identified 11 (SAA2, MMP9, SAA1, S100A8, ICAM1, P13, SOD2, C8orf4, SERPINA3, S100A12, S100A9) common DEGs. The comparing of common DEGs between two datasets is visualized through a Venn diagram in Figure 2. The results of the Venn diagram exhibit that the common DEGs are 2.4% among total 467 differentially expressed genes.

GO and pathway finding in terms of gene set enrichment analysis

Enrichr web tool was used for the analysis of gene set enrichment analysis. The current study analyzes GO terms and KEGG pathway for 11 (SAA2, MMP9, SAA1, S100A8, ICAM1, P13, SOD2, C8orf4, SERPINA3, S100A12, S100A9) common DEGs. The three most eminent GO terms include biological process, molecular
Table 1. GO terms, GO pathways and their corresponding P-values and genes for common differentially expressed genes

| Category                  | GO ID          | GO pathways                          | P-values       | Genes                      |
|---------------------------|----------------|---------------------------------------|----------------|----------------------------|
| GO biological process     | GO:0070486     | Leukocyte aggregation                 | 0.00000576     | S100A9, S100A8             |
|                           | GO:0050832     | Defense response to fungus            | 8.380e-8       | S100A12, S100A9, S100A8    |
|                           | GO:0030593     | Neutrophil chemotaxis                 | 1.430e-8       | SAA1, S100A12, S100A9, S100A8|
|                           | GO:0071621     | Granulocyte chemotaxis                | 1.792e-8       | SAA1, S100A12, S100A9, S100A8|
|                           | GO:1990266     | Neutrophil migration                  | 2.696e-8       | S100A12, S100A9, S100A8    |
|                           | GO:1900122     | Positive regulation of receptor binding| 0.003296       | MMP9                       |
|                           | GO:0002693     | Positive regulation of cellular        | 0.003296       | ICAM1                      |
|                           | GO:0032364     | Oxygen homeostasis                    | 0.003296       | SOD2                       |
|                           | GO:0003085     | Negative regulation of systemic        | 0.003296       | SOD2                       |
|                           | GO:0051549     | Positive regulation of keratinocyte    | 0.003296       | MMP9                       |
|                           |                | migration                             |                |                            |
| GO Molecular Function     | GO:0050786     | RAGE receptor binding                 | 1.038e-8       | S100A12, S100A9, S100A8    |
|                           | GO:0035325     | Toll-like receptor binding             | 0.000009879    | S100A9, S100A8             |
|                           | GO:0046914     | Transition metal ion binding           | 0.00001290     | S100A12, SOD2, MMP9, S100A9, S100A8|
|                           | GO:0008270     | Zinc ion binding                      | 0.00001547     | S100A12, MMP9, S100A9, S100A8|
|                           | GO:0004866     | Endopeptidase inhibitor activity       | 0.001625       | SERPINA3, P13              |
|                           | GO:0030145     | Manganese ion binding                  | 0.01909        | SOD2                       |
|                           | GO:0030414     | Peptidase inhibitor activity           | 0.01963        | P13                        |
|                           | GO:0061135     | Endopeptidase regulator activity       | 0.01963        | P13                        |
|                           | GO:0005507     | Copper ion binding                     | 0.02233        | S100A12                    |
|                           | GO:0046872     | Metal ion binding                      | 0.00006865     | S100A12, SOD2, S100A9, S100A8|
| GO Cellular Component     | GO:0062025     | Cytoplasmic vesicle lumen              | 4.624e-9       | SERPINA3, SAA1, S100A12, S100A9, S100A8|
|                           | GO:0071682     | Endocytic vesicle lumen                | 0.009585       | SAA1                       |
|                           | GO:0034774     | Secretory granule lumen                | 0.00001872     | SERPINA3, S100A12, S100A9, S100A8|
|                           | GO:0005881     | Cytoplasmic microtubule                | 0.02071        | SAA1                       |
|                           | GO:1904724     | Tertiary granule lumen                 | 0.02984        | MMP9                       |
|                           | GO:0030193     | Platelet alpha granule lumen           | 0.03625        | SERPINA3                   |
|                           | GO:0045111     | Intermediate filament                  | 0.03837        | S100A8                     |
|                           | GO:0005856     | Cytoskeleton                           | 0.002467       | S100A12, S100A9, S100A8    |
|                           | GO:0031091     | Platelet alpha granule                 | 0.04841        | SERPINA3                   |
|                           | GO:0035578     | Azurophil granule lumen                | 0.04841        | SERPINA3                   |

functions and cellular component. The ongoing study illustrates the top 10 GO terms for each of the subsections (biological process, molecular functions and cellular component), which is presented in Table 1. The data in Table 1 justify that the common DEGs are highly enhanced in neutrophil chemotaxis and granulocyte chemotaxis for the biological process subsection. Molecular function subsection data indicate a transition metal ion binding factor splendidly involved in the common DEGs. Cellular component study exhibits significant involvement of cytoplasmic vesicle lumen factors in common DEGs. KEGG, WikiPathways, Reactome and BioCarta pathway analysis is produced in Table 2. The information attained from Table 2 shows the IL-17 signaling pathway and TNF signaling pathway interaction with the most number of genes according to the KEGG pathway database. A collection of GO terms and pathways according to the combined score is depicted in Figure 3(A and B). A combined score is performed by the Enrichr web tool, which depends on the log of the P-value and z-score. Figures 3(A and B) represents GO terms and pathway analysis results from various pathway databases, respectively.

PPIs network to identify hub genes and module analysis

The common DEGs were provided as an input in STRING and the file produced from the analysis is reintroduced into Cytoscape software for visual representation. The PPIs network is created for further analysis of this study including hub gene detection for identifying drug molecules for COVID-19 and IPF. Eventually the results of the PPIs network connect for suggesting drug compounds that establish the PPIs analysis as a center point of this research. The PPIs network contains 60 nodes and 403 edges, which is picturized in Figure 4.
Table 2. Top pathways from KEGG, WikiPathways, Reactome and BioCarta databases and their corresponding P-values and genes for common differentially expressed genes

| Databases | Pathways                                      | P-value         | Genes                      |
|-----------|----------------------------------------------|-----------------|----------------------------|
| KEGG      | IL-17 signaling pathway                      | 0.00001563      | MMP9, S100A9, S100A8       |
|           | TNF signaling pathway                        | 0.001596        | MMP9, ICAM1                |
|           | Leukocyte transendothelial migration         | 0.001654        | MMP9, ICAM1                |
|           | African trypanosomiasis                     | 0.02017         | ICAM1                      |
|           | Bladder cancer                               | 0.02233         | MMP9                      |
|           | Fluid shear stress and atherosclerosis       | 0.002531        | MMP9, ICAM1                |
|           | Malaria                                      | 0.02663         | ICAM1                      |
|           | Viral myocarditis                            | 0.03198         | ICAM1                      |
|           | Staphylococcus aureus infection              | 0.03678         | ICAM1                      |
|           | Peroxisome                                   | 0.04473         | SOD2                       |
| WikiPathways | Vitamin B12 Metabolism WP1553              | 3.630e-11       | SERPINA3, SAA1, SAA2, SOD2, ICAM1 |
|           | IL1 and megakaryocytes in obesity WP2865    | 2.489e-7        | MMP9, S100A9, ICAM1        |
|           | Folate Metabolism WP176                     | 1.525e-10       | SERPINA3, SAA1, SAA2, SOD2, ICAM1 |
|           | Selenium Micronutrient Network WP15         | 5.914e-10       | SERPINA3, SAA1, SAA2, SOD2, ICAM1 |
|           | Mammary gland development pathway - Involution (Stage 4 of 4) WP2815 | 0.005488 | MMP9 |
|           | Photodynamic therapy-induced NF-kB survival signaling WP3617 | 0.0001620 | MMP9, ICAM1 |
|           | Osteopontin Signaling WP1434                | 0.007129        | MMP9                      |
|           | Platelet-mediated interactions with vascular and circulating cells WP4462 | 0.009313 | ICAM1 |
|           | Cells and Molecules involved in local acute inflammatory response WP4493 | 0.009313 | ICAM1 |
|           | Extracellular vesicles in the crosstalk of cardiac cells WP4300 | 0.01040 | MMP9 |
| Reactome  | DEx/H-box helicases activate type I IFN and inflammatory cytokines production Homo sapiens R-HSA-3134963 | 0.00002138 | SAA1, S100A12 |
|           | Advanced glycosylation endproduct receptor signaling Homo sapiens R-HSA-879415 | 0.00002138 | SAA1, S100A12 |
|           | Scavenging by Class B Receptors Homo sapiens R-HSA-3000471 | 0.002747 | SAA1 |
|           | RIP1-mediated NF-kB activation via ZBP1 Homo sapiens R-HSA-1810476 | 0.00005742 | SAA1, S100A12 |
|           | TRAF6-mediated NF-kB activation Homo sapiens R-HSA-933542 | 0.00007540 | SAA1, S100A12 |
|           | ZBP1(DAI) mediated induction of type I IFNs Homo sapiens R-HSA-1606322 | 0.00008873 | SAA1, S100A12 |
|           | TAK1 activates NF-kB by phosphorylation and activation of IKKs complex Homo sapiens R-HSA-445989 | 0.00008873 | SAA1, S100A12 |
|           | Formyl peptide receptors bind formyl peptides and many other ligands Homo sapiens R-HSA-444473 | 0.004392 | SAA1 |
|           | Cytosolic sensors of pathogen-associated DNA Homo sapiens R-HSA-1834949 | 0.0005787 | SAA1, S100A12 |
|           | TRAF6 Mediated Induction of proinflammatory cytokines Homo sapiens R-HSA-1834949 | 0.0006883 | SAA1, S100A12 |
| BioCarta  | Inhibition of Matrix Metalloproteinases Homo sapiens h | 0.004392 | MMP9 |
|           | rectxPathway                                 | 0.006035        | SOD2                       |
|           | Cardiac Protection Against ROS Homo sapiens h flumazenilPathway | 0.007129 | SOD2 |
|           | Erythropoietin mediated neuroprotection through NF-kB Homo sapiens h eponfkbPathway | 0.008767 | SOD2 |
|           | The IGF-1 Receptor and Longevity Homo sapiens h longevity pathway | 0.008767 | SOD2 |

Identification of hub genes and module analysis for suggesting therapeutic solutions

To trace the hub genes from the PPIs network which is highlighted in Figure 3, cytohubba is used which is a plugin of Cytoscape software. The hub genes were sorted by their degree value, which indicates the number of interactions of the genes in the PPIs network. Top five identified hub genes are VEGFA, AKT1, MMP9, ICAM1 and CD44. Hub protein interactions with other protein in the PPIs network are demonstrated in Figure 5. The network consists of 53 nodes and 378 edges. Highly dense modules are designed from the PPIs network using MCODE which is also a plugin of Cytoscape software. MMP9 and ICAM1...
Figure 3. (A) Biological process, molecular function and cellular component related GO terms identification result according to combined score. The higher the enrichment score, the higher number of genes are involved in a certain ontology. (B) Pathway analysis result identification through KEGG, WikiPathways, Reactome and BioCarta. The results of the pathway terms were identified through the combined score.

are the two genes which are highlighted in the module network as these two genes are also the common DEGs between the two datasets. Module analysis is shown in Figure 6. The module analysis network contains 16 nodes and 106 edges. Topological analysis for the hub genes (VEGFA, AKT1, MMP9, ICAM1, PI3, SOD2, C8orf4, SERPINA3, S100A12, S100A9) is identified using cytohubba. The topological analysis result is presented in Table 3.

TF-gene interactions

TF-gene interactions were collected using NetworkAnalyst. For the common DEGs (SAA2, MMP9, SAA1, S100A8, ICAM1, PI3, SOD2, C8orf4, SERPINA3, S100A12, S100A9) the TF-genes were identified. TF regulators’ interaction with the common DEGs is visualized in Figure 7. The network contains 142 nodes and 180 edges.
Figure 4. Protein–protein interactions (PPIs) network for identified common differentially expressed genes that are shared by two diseases (COVID-19 and IPF). Nodes in orange color indicate common differentially expressed genes and edges specify the interconnection in the middle of two genes. The analyzed network holds 60 nodes and 403 edges.

Table 3. Topological result exploration for top five hub genes where the network density is 0.274, network diameter 3 and network radius 2

| Hub gene | Degree | Stress | Closeness centrality | Betweenness centrality | Distance | Eccentricity | Edge betweenness | Transitivity |
|----------|--------|--------|----------------------|------------------------|----------|--------------|-----------------|-------------|
| VEGFA    | 38     | 2248   | 48.1667              | 362.6209               | 1.269230 | 2            | 0.121695        | 0.34424     |
| AKT1     | 38     | 2502   | 48.1667              | 396.249                | 1.269230 | 2            | 0.133888        | 0.35277     |
| MMP9     | 34     | 2126   | 46.5                 | 343.5242               | 1.346153 | 2            | 0.095339        | 0.38324     |
| ICAM1    | 29     | 1594   | 44                   | 242.5716               | 1.442307 | 2            | 0.064545        | 0.42365     |
| CD44     | 27     | 1060   | 42.3333              | 189.3317               | 1.480769 | 2            | 0.064571        | 0.4359      |

edges. The network contains a total of 132 TF-genes. MMP9 is regulated by 22 TF-genes and ICAM1 is regulated by 69 TF-genes. These 132 TF-genes regulate more than one common DEGs of the network, which indicates high interaction of the TF-genes with common DEGs. Figure 7 represents the TF-gene interaction network.

TF-miRNA coregulatory network
TF-miRNA coregulatory network is generated using NetworkAnalyst. The analysis of the TF-miRNA coregulatory network delivers miRNAs and TFs interaction with the common DEGs. This interaction can be the reason for regulating the expression of the DEGs. The network created for TF-miRNA coregulatory network comprises 101 nodes and 131 edges. Thirty-nine miRNAs and 53 TF-genes have interacted with the common DEGs. Figure 8 dispenses TF-miRNA coregulatory network.

Identification of candidate drugs
Enrichr platform is used to identify drug molecules for 11 common DEGs. The data were collected from the DSigDB database. According to P-value and adjusted P-value, the results from the candidate drugs were generated. The analysis depicts that parthenolide CTD 00000087 and MIGLITOL CTD 00002031 are the two drug molecules that most genes are interacted with. As these signature drugs were detected for the common DEGs, these drugs represent common drugs for COVID-19 and IPF. Table 4 points out the candidate drugs from the DSigDB database for common DEGs.

Discussion
IPF is regarded as a risk factor for COVID-19. When the lung tissue of a person gets damaged and that is the time when
Genetic effect of SARS-CoV-2 infections to IPF patients

Figure 5. Detection of hub genes from the PPIs network of common differentially expressed genes. The highlighted five genes are VEGFA, AKT1, MMP9, ICAM1 and CD44. These five genes are considered as hub genes according to their degree value. The network has 53 nodes and 378 edges. According to topological analysis, the degree value of VEGFA and AKT1 was 38. The degree value of MMP9, ICAM1 and CD44 were 34, 29 and 27, respectively.

Table 4. Suggested top drug compounds for the IPF-2 infections

| Name of drugs                  | P-value       | Adjusted P-value | Genes           |
|--------------------------------|---------------|------------------|-----------------|
| MIGLITOL CTD 00002031          | 0.00001810    | 0.004285         | S100A12, S100A9 |
| CHEMBL55802 CTD 00003118       | 0.00002876    | 0.005514         | MMP9, ICAM1     |
| Hesperidin CTD 00006087        | 0.00004187    | 0.007024         | MMP9, ICAM1     |
| Cytochalasin D CTD 00007076     | 0.00005197    | 0.007472         | MMP9, ICAM1     |
| Prolinedithiocarbamate CTD 00002658 | 0.00007540  | 0.008928         | MMP9, ICAM1     |
| Parthenolide CTD 00000087      | 0.00002540    | 0.001705         | S100A12, S100A9 |
| FEXOFENADINE HYDROCHLORIDE CTD 00003191 | 0.00008193 | 0.009163 | MMP9, ICAM1 |
| Hydroxytyrosol CTD 00000267     | 0.00008193    | 0.009163         | S100A12, S100A9 |
| Antimycin A CTD 00005427        | 0.00008873    | 0.009401         | MMP9, ICAM1     |
| Anacardic acid C15:3 CTD 00003117 | 0.00008873  | 0.009160         | MMP9, ICAM1     |
the functionality of the lung cannot adjust properly to its task. People with lung disease are at higher risk of COVID-19. The study assists to narrate Bioinformatics lessons for meaningful analysis of SARS-CoV-2 affected lung epithelium and lung alveolar samples and IPF affected lung tissue of humans. Methodologies related to Bioinformatics are used for the study to identify 108 and 359 DEGs from GSE147507 and GSE35145, respectively. For establishing relationships and for detecting candidate drugs according to COVID-19 and IPF, common DEGs between GSE147507 and GSE35145 datasets were identified. After identification 11 (SAA2, MMP9, SAA1, S100A8, ICAM1, PI3, SOD2, C8orf4, SERPINA3, S100A12 and S100A9) common DEGs were found. The rest of the research study is continued with the analysis of GO, KEGG pathway analysis, PPIs, TF-gene interactions, TF-miRNA coregulatory network and candidate drug detection.

Identified 11 common DEGs were used for detecting GO terms. GO terms were selected according to the P-values. For biological process leukocyte aggregation, defense response to fungus, neutrophil chemotaxis, granulocyte chemotaxis and neutrophil migration are among the top GO term. For establishing relationships and for detecting candidate drugs according to COVID-19 and IPF, common DEGs between GSE147507 and GSE35145 datasets were identified. After identification 11 (SAA2, MMP9, SAA1, S100A8, ICAM1, PI3, SOD2, C8orf4, SERPINA3, S100A12 and S100A9) common DEGs were found. The rest of the research study is continued with the analysis of GO, KEGG pathway analysis, PPIs, TF-gene interactions, TF-miRNA coregulatory network and candidate drug detection.

The determination of the KEGG pathway is identified for 11 common DEGs. The analysis was achieved form the common DEGs because to find a similar pathway for both COVID-19 and IPF. Top 10 KEGG pathway includes IL-17 signaling pathway, TNF signaling pathway, Leukocyte transendothelial migration, African trypanosomiasis, bladder cancer, fluid shear stress and atherosclerosis, malaria, viral myocarditis, Staphylococcus aureus infection and peroxisome. IL-17 signaling pathway contributes cytokine storm basically in SARS-CoV-2 and also in pulmonary based viral infections [48]. Meanwhile results from WikiPathways show the most interacted gene pathways are Vitamin B12 Metabolism WP1533, Folate Metabolism WP176 and Selenium Micronutrient Network WP15. Results from the Reactome pathway produce DE/H-box helicases activate type I IFN and inflammatory cytokines production Homo sapiens R-HSA-3134963 pathway.

PPIs network analysis is the most prominent section of the study as hub gene detection, analysis of modules and drug identification thoroughly depends on the PPIs network. Analysis for PPIs was also generated for SAA2, MMP9, SAA1, S100A8, ICAM1, PI3, SOD2, C8orf4, SERPINA3, S100A12 and S100A9 genes, as these genes are common DEGs. According to the PPIs network VEGFA, AKT1, MMP9, ICAM1 and CD44 genes were declared as hub genes because of their high interaction rate or degree value. ICAM1 serum of the median level was higher in IPF patient’s serum samples compared to healthy samples [49]. To focus on the essential regions of the PPIs network, module analyses of the hub genes were achieved. The reason of focusing on highly concentrated area is a more effective drug compound suggestion.

TF-gene interaction was obtained with the common DEGs. TF-genes work as regulators according to genetic expressions which may result in creating cancer cells. From the network, ICAM1 shows a high interaction rate with other TF-genes. The degree value of ICAM1 in the TF-gene interactions network is 69. Among the regulators, STAT3 and KLF16 have significant interaction. The degree value of STAT3 and KLF16 are 5 and 4, respectively in the TF-gene interactions network. The upregulated STAT3 gene is found in lung carcinomas of human and can be a contributing factor for regulation in lung diseases [50].

Regulatory biomolecules act as potential biomarkers in numerous complex diseases. Keeping this part in memory, the activities of miRNAs and TF-genes that are analyzed for the regulation of common DEGs are visualized in the TF-miRNA coregulatory network. Thirty-nine miRNAs and 53 TF-genes are found in the study. Among the most interacted TFs, AR has the higher degree value of 4. Drugs based on androgen modulation can be contributed as treatment factor for SARS-CoV-2 [51]. Proof of changing the miRNA expression in IPF samples is established in various research and family members of miR-200 plays a vital role in the regulation of IPF samples [52]. TF-genes are reactors for the regulation of gene expression and the regulation is completed through binding with targeted genes and miRNAs on the other hand, able to regulate gene expression through mRNA degradation [53].

According to DSSigDB database drug molecules were suggested from 11 common DEGs. Among all the candidate drugs, the current study highlights the top 10 significant drugs. MIGLITOL CTD 00002031, CHEMBL55802 CTD 00003118, hesperidin CTD 00006087, cytochalasin D CTD 00007076, proline/dithiocarbamate CTD 00002658, parthenolide CTD 00000087, FEXOFENADINE HYDROCHLORIDE CTD 00003191, hydroxytyrosol CTD 00000267, antymycin A CTD 00005427, anacardic acid C15:3 CTD 00003117 are the peak drug candidates for COVID-19 and IPF. Parthenolide demonstrates role of anti-inflammatory activities against IPF [54] that proves the efficiency of the proposed drugs. The current study uses a number of Bioinformatics methodologies in GSE147507, which indicates
SARS-CoV-2 infection in human lung epithelium cell and GSE35145 compare sample between affected and normal IPF tissue of humans. This study hopefully integrates COVID-19 and risk factor IPF treatment. These drugs can be considered for further verification by chemical experiments. As SARS-CoV-2 is a new virus, less research has been done so far. This is the reason for collecting less number of samples for analyzing the results. In future, if more samples are available, the current study would be more effective in the context of the SARS-CoV-2 pandemic.

**Conclusions**

In the context of transcriptomic analysis, no other research has been done so far on SARS-CoV-2 and IPF. We have accomplished DEGs analysis between two datasets and filtered the materials through common gene identification and attempted to find infection responses between SARS-CoV-2 and IPF affected lung cells. Analyses regarding SARS-CoV-2 and IPF predict the way of detecting infections for various diseases. The drug targets are suggested logically as they are derived through the identification of hub genes and it possibly plays an active preface for already sanctioned drugs. As SARS-CoV-2 is a recent discovery, there has been little research on its risk factors and infections. Unique research on SARS-CoV-2 will become more and more important with the availability of exceeding datasets.

**Key Points**

- Protein–protein interactions network-based analysis assists to find out only the definite genes related to both SARS-CoV-2 and IPF and the preconditioning step of Systems biology is fulfilled through protein–protein interactions analysis.
- Gene set enrichment based analysis predicts Gene ontology terms for both SARS-CoV-2 and IPF affected lung cells and hub gene identification makes the prediction of drug compounds even more effective.
- Computer-aided drug suggestion significantly brings out drugs like parthenolide that produces numerous actions including anti-inflammatory based actions. And various pathway-based analyses highlight the usefulness of the biological system for both SARS-CoV-2 and IPF in the context of molecular-based information.
- Module analysis focuses on concentrated regions of the protein–protein interactions network that justifies the high involvement of hub nodes and eventually establishes the drug prediction even more logical and efficient.
Figure 8. The network presents the TF-miRNA coregulatory network. The network consists of 101 nodes and 131 edges including 53 TF-genes, 39 miRNA and nine differentially expressed genes. The nodes in pink color are the differentially expressed genes, a yellow node represents miRNA and other nodes indicate TF-genes.

Acknowledgment

This manuscript has not been published yet and not even under consideration for publication elsewhere. The authors are grateful who have participated in this research work.

Funding

There is no funding for this work.

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