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ORIGINAL RESEARCH

# Fibrinogen Dysregulation is a Prominent Process in Fatal Conditions of COVID-19 Infection; a Proteomic Analysis

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**Abstract:** **Introduction:** Molecular pathophysiology of COVID-19 is not completely known. Expression changes in patients' plasma proteins have revealed new information about the disease. Introducing the key targeted plasma protein in fatal conditions of COVID-19 infection is the aim of this study. **Methods:** Significant differentially expressed proteins (DEPs) in the plasma of cases with a fatal condition of COVID-19 were extracted from an original article. These proteins were included in a network via STRING database along with 100 first neighbor proteins to determine central nodes of the network for analyzing. **Results:** Queried and added proteins were included in a scale free network. Three hub nodes were identified as critical target proteins. The top queried hub proteins were chains of fibrinogen; Fibrinogen Alpha chain (FGA), Fibrinogen gamma chain (FGG), and Fibrinogen beta chain (FGB), which are related to the coagulation process. **Conclusion:** It seems that fibrinogen dysregulation has a deep impact on the fatality of COVID-19 infection.

**Keywords:** SARS-CoV-2; Proteomics; Proteins; Protein Interaction Maps; Fibrinogen

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## 1. Introduction

Coronaviridiae family viruses possess a single RNA genome with a maximum of 32 kilobases (1). Coronaviruses have been found in many different animal cases (2, 3). Several coronaviruses are pathogenic to humans with mild clinical symptoms (1); however, in November 2002, severe acute respiratory syndrome (SARS), which was first reported in Guangdong (4), resulted in the death of 774 patients in 37 countries (5). Middle East respiratory syndrome (MERS) corona virus (MERS-CoV), detected in Saudi Arabia for the first time in 2012, led to 858 fatalities (6). Recently, in 2019, a new type of corona virus called SARS CoV 2 was discovered, which leads to COVID-19 (7). WHO has reported millions of confirmed cases and hundreds of thousands of deaths due to COVID-19 pandemic around the world (8). In addition to the lower respiratory tract, many other organs, such as nervous system, gastrointestinal tract, liver, kidney, and lymph node, have been infected by SARS-CoV 2 (9). There are many symptoms for COVID-19; including fever, pneumonia, and acute respiratory distress syndrome (10). Pathophysiological changes such as lymphopenia (11), microthrombosis (12), cytokine release syndrome (13), and vascular coagulation have been reported in severe COVID-19 cases (14). However the molecular pathogenesis of COVID-19 is poorly understood despite the extensive efforts of scientists (15). Pathophysiological changes during viral diseases and infections lead to alteration of plasma protein expression (16). Identification of differentially expressed proteins (DEPs) in the plasma during COVID-19 could help us understand the molecular pathophysiology of disease. Understanding the molecular mechanism of the viral infection could contribute

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to finding different treatment methods. Proteomics, as a high throughput method, is applied to assess the effects of SARS Cov2 on patients' plasma proteins. Proteomic findings could be assessed as an interactome unit, which is interesting for many investigators. In such studies, a limited number of critical proteins could be identified as critical DEPs (17, 18). There is a limited number of nodes, known as central nodes, which are discriminated from others by their connections to first neighbors or involvement in shortest pathways (19, 20). Identifying central proteins among the hubs, which are characterized by their connections with the first neighbors (21, 22) and central proteins, could assist us in gaining useful information for finding main disease biomarkers. In the present study, findings of a proteomic investigation by Ting Shu et al. (16), which was performed with the aim of identifying plasma biomarkers of COVID-19 in fatal cases were assessed using network analysis to find the main targets of SARS-CoV 2.

## 2. Methods

Considering fold change > 1.5 and p-value < 0.01, 42 differentially expressed proteins were extracted from data of the original article published by Ting Shu et al. (16). Since original data about differentially expressed proteins in serum of patients relative to the healthy controls have been previously published by Ting Shu et al. in Immunity (2020, 53 (5)), the details of data production and sampling are described in the authors report and here we only explain the methods of bioinformatic analysis. The data are related to the differentially expressed proteins of plasma in fatal cases of COVID-19. The queried proteins were included in a network via "protein query" of STRING database by Cytoscape software 3.7.2. Confidence score cutoff =0.4 was applied to construct the interactive network. Among the 42 queried proteins 32 were recognized by STRING. For better resolution the network was constructed by the 32 queried proteins and 100 first neighbors from STRING database. The main connected component of the constructed network was analyzed using "Network analyzer" application of Cytoscape. The analyzed network was visualized based on degree value and the identified hub nodes correspond to the degree value.

## 3. Results

A total of 32 differentially expressed recognized proteins were assessed to construct a network using Cytoscape software via protein query of STRING database. For better resolution, 100 first neighbor proteins extracted from STRING were added to construct the network (Fig1). Network analyzer considered topological properties including Degree, betweenness centrality (BC) and Stress (Table 1). Hub-bottleneck nodes are identified based on highest value of degree and BC.

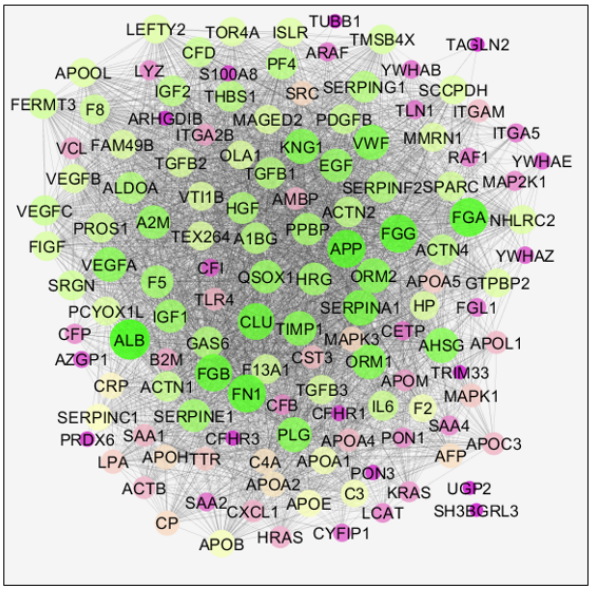


**Figure 1:** The 32 queried proteins recognized by STRING database plus 100 first neighbors from STRING are included in a network. The nodes are laid out based on degree value; color from green to red and size increment are corresponding to increase of degree value.

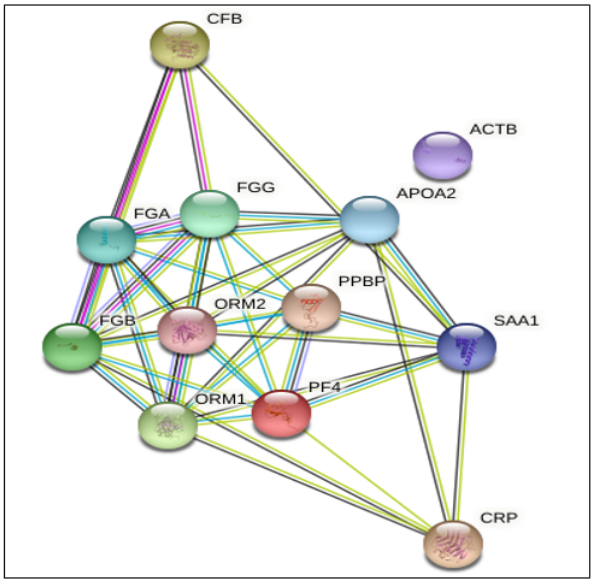


**Figure 2:** Network including top queried hub is extracted from STRING database.

As the queried and added proteins were included in a scale free network, 32 hub nodes were determined as central proteins (Table 1). The three top hub proteins (FGA, FGG and FGF) were members of fibrinogen family. Other hubs, arranged based on degree and BC, were ORM1, ORM2, PPBP, PF4, CRP, APOA2, SAA1, ACTB, CFB, and LCAT. Since centrality values of the hub nodes were highly dispersed, the three

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**Table 1:** The list of 32 top hub-bottlenecks of COVID-19 fatality-based network with their corresponding degree (K) and betweenness centrality (BC) values

| Row | Query proteins | K | BC | Stress |
|---|---|---|---|---|
| 1 | FGA | 108 | 0.032 | 7228 |
| 2 | FGG | 105 | 0.023 | 6398 |
| 3 | FGB | 102 | 0.021 | 5830 |
| 4 | ORM1 | 92 | 0.014 | 3850 |
| 5 | ORM2 | 91 | 0.016 | 3686 |
| 6 | PPBP | 79 | 0.007 | 1900 |
| 7 | PF4 | 75 | 0.003 | 1212 |
| 8 | CRP | 52 | 0.004 | 1152 |
| 9 | APOA2 | 49 | 0.003 | 898 |
| 10 | SAA1 | 38 | 0.002 | 642 |
| 11 | ACTB | 37 | 0.013 | 3174 |
| 12 | CFB | 27 | 0.004 | 1418 |
| 13 | LCAT | 25 | 0.001 | 172 |
| 14 | CETP | 23 | 0 | 114 |
| 15 | TLN1 | 22 | 0 | 96 |
| 16 | SAA2 | 21 | 0 | 32 |
| 17 | FGL1 | 20 | 0 | 38 |
| 18 | CFI | 17 | 0.01 | 892 |
| 19 | YWHAZ | 15 | 0 | 86 |
| 20 | YWHAE | 14 | 0.01 | 134 |
| 21 | AZGP1 | 13 | 0 | 2 |
| 22 | S100A8 | 10 | 0 | 56 |
| 23 | CFHR1 | 9 | 0 | 158 |
| 24 | CFHR3 | 7 | 0 | 8 |
| 25 | PON3 | 7 | 0 | 0 |
| 26 | PRDX6 | 7 | 0 | 44 |
| 27 | ARHGDIB | 4 | 0 | 6 |
| 28 | TAGLN2 | 3 | 0 | 0 |
| 29 | TRIM33 | 2 | 0 | 0 |
| 30 | TUBB1 | 2 | 0 | 0 |
| 31 | SH3BGRL3 | 0 | 0 | 0 |
| 32 | UGP2 | 0 | 0 | 0 |

The prioritized proteins are arranged based on degree values.

top queried fibrinogen hubs with highest degree values were determined as the central nodes of the analyzed network and discussed in the more detail (Table 1).

Degree and stress of fibrinogen chain hubs were more than others. ORM1, ORM2, and PPDP proteins followed fibrinogen chains, respectively. Neighbor proteins of queried hubs are shown in Figure 2.

## 4. Discussion

COVID-19 patients with severe condition, present with intense inflammation, which is induced by acute respiratory syndrome (23) and leads to cytokine storm development. One of the main distinct features of COVID-19 is coagulopathy (24), which is commonly observed among patients and is accompanied with severe thromboembolic conditions (25). Coagulopathy increases D-dimer levels and leads to thromboembolism (26). The guidelines of international society of thrombosis and haemostasis recommended anticoagulant therapy for COVID-19 patients (27). Several fold increase in fibrinogen level is reported in severe cases of COVID-19 (28). Our data analysis revealed that enough connections between the studied proteins, could form a scale free network. The first neighbors added to the queried proteins provide a scale free network (Figure 1). Assessments indicated that the scale free network could provide useful information to distinguish a limited set of proteins among a large number of proteins (Figure 2).

Our results demonstrated that fibrinogen chains of FGA, FGG and FGB are top hub proteins related to COVID-19 fatalities (Table 1). On the other hand, neighbor proteins related to fibrinogen chains are APOA2, ORM2, ORM1 and CFP (Figure 2). Considering molecular pathways of the coagulation process, in which fibrinogen chains are involved, may open a window to help in treatment of disease. The role of fibrinogen in acute COVID-19 cases and clot formation has been considered in researches. Fibrinogen is a glycoprotein that is produced in liver as an anti-infective organ. Liver overreacts during acute inflammatory phase in hospitalized COVID-19 patients and secretes several reactants such as fibrinogen, C reactive protein (CRP), ferritin and plenty of cytokines (29, 30). Secretion of those reactants is the body's defense mechanism against invading pathogens. In this regard, the dual function of fibrinogen is important, as it regulates antimicrobial activity of the immune cells and clot formation. Mac-1(CD11b/CD18) is a leucocyte integrin receptor, regulating inflammatory responses, and fibrinogen is a ligand for Mac-1, in addition to having coagulator functions (31). Mac-1 is a receptor for COVID-19 RNA strand and increase in fibrinogen secretion could impregnate Mac-1 to reduce the negative effects of the virus (32, 33). Ko YP et al. summarized several host defensive mechanisms of fibrinogen, in summary two main mechanisms are fibrin matrix barrier formation and immune protective functions of host (34).

Formation of thrombosis could limit pathogen spread as a defensive mechanism and the researchers believed that localized formation of lungs thrombosis could restrict SARS Cov-2 virus from spreading (35). D-dimer is a product of fibrin degradation in blood after clot fibrinolysis. Increase in D-dimer is accompanied by reduction of fibrinogen release from the platelets (25). A hypothesis states that in patients with COVID-19 and other infectious diseases, increase in D-dimer and decrease in the secretion of fibrinogen, lead to activation of immune responses. On the other hand, decrease in D-dimer and increase in the secretion of fibrinogen, activates the coagulation mechanism and thrombi formation (36). Among the key genes that interact with D-dimer, fibrinogen level, and coagulation process, FGA, FGG, and FGB are prominent. Although additional gene clusters help govern D-dimer and fibrinogen count and thrombosis

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in COVID-19 patients (37). High circulating level of fibrinogen has been linked to COVID-19 coagulopathy; however, Jecko Thachi et al. believed that in COVID-19 patients fibrinogen is probably increased to protect the host (36). C reactive protein (CRP) released by liver is anti-infective and increases in acute phase of COVID-19, along with ferritin and fibrinogen, as a defense mechanism against pathogens (30). Our results revealed the indirect connection of CRP with FGB via ORM2 protein (Fig2). CPR forms a complex with histones to protect them from endothelial damage resulting from edema and thrombosis in hosts suffering from COVID-19 (38). Researches believed that evaluating CRP or fibrinogen levels in addition to other conventional markers could be useful for prediction of cardiovascular disease in patients with intermediate risk factors (39).

Orosomucoid isoforms (ORM1 and ORM2) are inducers of M2 macrophages and increase in various infections (40)(41). Our results also revealed a connection between ORM1 and ORM2 and fibrinogen chains (Figure 2). This connection may be related to severe infection in fatal COVID-19 cases.

Overall, the role of acute infections in COVID-19 patients, with regard to the secretion of fibrinogen and other prominent proteins, can be evaluated in future investigations.

## 5. Conclusion

It can be concluded that activation of the clotting and embolism mechanisms along with fibrinogen secretion in patients with COVID-19 are the prominent processes in fatal cases.

## 6. Declarations

### *6.1. Acknowledgment*

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### *6.2. Authors' contributions*

All authors have contributed equally in the project administration. Final revision was done by Vahid Mansouri and Mostafa Rezaei Tavirani and verified by all authors.

### *6.3. Conflict of interest*

There is no conflict of interest.

### *6.4. Funding and supports*

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