A systems biology analysis of protein-protein interaction of digestive disorders and Covid-19 virus based on comprehensive gene information

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ABSTRACT

Aim: Analysis of networks of digestive disorder and their relationship with Covid-19 based on systems biology methods, evaluation similarity, and usefulness of networks to give a new treatment approach.

Background: Digestive disorders are typically complex diseases associated with high treatment costs. They are related to the immune system and inflammation. With the outbreak of Covid-19, this disease was shown to have signs like diarrhea. Some signs of Covid-19 are similar to those of digestive disorders, like IBD and diarrhea. Both of them are accompanied by inflammation and induce disorders in the digestive system.

Methods: DisGeNET and STRING databases were sources of disease genes and constructing networks and were used to construct the network of digestive diseases and Covid-19. Three plugins of Cytoscape software, namely ClusterONE, ClueGO, and CluePedia, were used to analyze cluster networks and enrichment pathways. To describe the interaction of proteins, information from KEGG pathway and Reactome was used.

Results: According to the results, IBD, gastritis, and diarrhea have common pathways. The CXCL8, IL-6, IL-1β, TNF-α, TLR4, and MBL2 molecules were identified as inflammatory molecules in all networks.

Conclusion: It seems that detecting genes and pathways can be useful in applying new approaches for treating these diseases.

Keywords: Digestive system disorders, Covid-19, IBD, Systems biology, Diarrhea, Gastritis.

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Introduction

Coronavirus from the large Coronaviridae family can cause diseases in humans and birds. SARS-CoV2 (Covid-19) was detected for the first time in 2019 in Wuhan, China, and three months later, it was widespread in the world and led to a pandemic (1). To date, physicians have reported many signs and symptoms of Covid-19, such as its effects on the digestive tract, nervous system, blood circulation, heart, lung, and kidney (2, 3). Studies have also reported that the coronavirus implicates 1844 genes, some of which are common among other diseases. The genes involved in digestive disorders like cholelithiasis, diarrhea, gallstone, gastritis, irritable bowel disease, stricture, and acid reflux also are common for Covid-19 (4).

Cholelithiasis is inflammation of the gallbladder; this disease is not well understood, but some similarities between cholelithiasis and gall stone disease have been discovered (5). Diarrhea is a disease defined by abnormal fluidity in stools, and this is common in developing countries, specifically Africa, shown by the high mortality rate in children (6). A
recent study done by Li et al. (2020) described diarrhea as the most common symptom (fifth main symptom) in Covid-19 patients (7). Gastritis is known as the acute or chronic inflammation of the stomach lining (8). There are many reasons for gastritis, such as alcohol abuse and the long-term intake of non-steroidal anti-inflammatory drugs (9). Recently, studies have shown patients with gastritis and inflammatory bowel disease (IBD) are at high risk of Covid-19 because of the high activity of ACE2 in their plasma (10-12). Sometimes Covid-19 can weaken patients with IBD, and it may lead to their death (13, 14).

A stricture occurs in intestinal fibrosis and collagen accumulation (15). The main reasons for stricture are cancer and inflammatory bowel disease; Crohn’s disease is also a cause of stricture in the small bowel (16). Based on recent findings, there are some common genes between stricture and Covid-19 (4). Gastroesophageal reflux is a common digestive disorder that affects millions of people worldwide (17). In addition to risk factors like diet, smoking, and body mass index, it has been suggested that some genes are important for acid reflux (18, 19). This study aims to investigate common genes and protein-protein interactions among the mentioned diseases and Covid-19 infection to find the similarities between gene pathways. It seems that finding the same gene pathway in those diseases can help find an approach to treating Covid-19 infection.

**Methods**

DisGeNET is an available platform that includes one of the largest collections of genes and variants associated with human disease (20). The related genes of digestive disorders (mentioned in the introduction) and Covid-19 were exported from the DisGeNET database and used to construct the PPI network. The Search Tools for Detecting Interacting Genes/Proteins (STRING), a database for predicted protein-protein interactions at EMBL, clusters the elicited results from many protein-protein interactions databases, like Mint, BioGrid, etc. To describe the interaction of proteins, information from the KEGG pathway at the address www.genome.jp/kegg/ and Reactome at address www.reactome.org was used (21). We constructed digestive disorders and Covid-19 networks by submitting the list of genes to the STRING database at the address www.string-db.org and analyzed the networks by Cytoscape software at the address www.cytoscape.org (22).

A network includes nodes (e.g., genes or proteins) and links/edges (e.g., co-expression relationships or physical interactions). In biology, network terms, degree, and betweenness are central parameters for analyzing network topology. Edges/links of a node are named the degree of that node. Nodes with high degrees are called hubs, and nodes that achieve top-ten or top-five percent of betweenness centrality are called bottlenecks (both based on the researcher’s definition) (23). Therefore, the nodes that simultaneously have hubs and bottlenecks are called hub-bottlenecks (24). The standard deviation (SD) and average degree (AD) were calculated, and nodes with more than two *SD + AD were selected as hub proteins in each network. In addition, the top five percent of betweenness centrality measures were selected as bottleneck proteins. Common genes, hubs, and bottleneck proteins of these Covid-19 and digestive disorder gene networks were extracted and used for more analysis. The common networks were constructed by importing common genes in the STRING database and clustered by the ClusterONE plugin of Cytoscape software (25). This software found overlapping protein complexes in a protein interaction network uploaded into Cytoscape (overlap threshold = 1, node penalty = 0, haircut threshold = 0) (26). By ClueGO and CluePedia plugins of Cytoscape software, pathway enrichment and the relation between pathways were accomplished (27, 28) (table 1).

**Table 1. The enrichment of three modules for diseases.**

| Disease                | Adjusted p-Value          | Module   |
|------------------------|---------------------------|----------|
| Immunodeficiency       | 4.07077294660061E-4       | Blue module |
| Macular Degeneration   | 2.447422407186003E-6      | Green module |
| Anemia                 | 5.473352213598934E-9      | Red module |
**Results**

Using the DisGeNET database, we extracted 844 genes for Covid-19, 56 genes for cholecystitis, 631 genes for diarrhea, 52 genes for gallstone, 293 genes for gastritis, 428 genes for irritable bowel disease (IBD), 29 genes for stricture, and 51 genes for acid reflux; 219 genes were shared among all diseases. Three diseases, i.e. IBD, gastritis, and diarrhea, showed some common genes with Covid-19, but the other three diseases showed no common gene. Using the STRING database, the common genes network was constructed. The Covid-19 network showed 219 nodes and 4932 edges (Figure 1).

![Figure 1](image1.jpg)

**Figure 1.** Common gene network containing 219 nodes and 4932 Edges. This network includes three modules that are shown by blue (cluster one), red (cluster two) and dark green (cluster three).

The nodes have been colored in green; Covid-19 genes and some of those were colored red, because they were common with IBD, gastritis, and diarrhea genes.

In the next step, the general network of Covid-19 genes was illustrated in which common genes with diarrhea are shown in red (Figure 2). The network of Covid-19 genes showed that some of these genes were common with gastritis. The nodes colored green are Covid-19 genes, and common genes with gastritis are colored in red (Figure 3). The association of Covid-19 genes and IBD has been shown. The nodes in green color are Covid-19 genes, and common genes with IBD are in red (Figure 4). The genes that are common between the three diseases are shown by a triangular shape.

**Discussion**

In systems biology science, PPI network analysis and pathway enrichment have been broadly used for discovering main proteins and pathways underlying complex diseases (29). Different types of disorders such as neurodegenerative and many cellular conditions have been analyzed (30, 31, 32, 33, 34, 35).
In this study, we obtained the gene list of six diseases (mentioned above) that seemed to have a common molecular mechanism. Among those, three diseases (IBD, gastritis, diarrhea) had a common mechanism with Covid-19 based on risk factors and studies (36, 37, 38, 39).

CXCL8 (IL-8) molecule is a member of the chemokine family that causes inflammation and pathogen elimination. Based on the PPI network, six genes, comprising CXCL8, IL-6, IL-1β, TNF-α, TLR4, and MBL2, were common in the inflammation network. Many studies have suggested that CXCL8 may also have a role in tissue injury, fibrosis, angiogenesis, and tumorigenesis. Thus, many cells like epithelial cells, fibroblasts, and neurons express CXCR1 and CXCR2 as receptors for CXCL8, and CXCL8 can cause neutrophil and macrophage migration. In inflammation diseases, they, mostly neutrophils, function as attractive chemokines to leukocyte migration. Studies have shown that the blockade of CXCL8 can prevent inflammatory diseases (40). In addition, TNF-α, IL-1β, IL-6, vascular endothelial growth factor (VEGF), and CXCL8 can play important roles in inflammatory diseases such as osteoarthritis, IBD, and infections like Covid-19 (41).

Wojdasiewicz et al. (2014) described the inflammatory potential of IL-1β, TNFα, IL-6, IL-15, IL-17, and IL-18, stating that they disturb tissue homeostasis and then, with the attraction of leukocyte cells, induce inflammation (42).

According to recent studies, the digestive disorders caused by Covid-19 have inflammatory signs and the same genes involved in digestive diseases that patients suffer from immune deficiency and mutation disorders.

The Sars-cov2 virus usually fuses to cells by the ACE2 receptor. Typically, the expression rate of this receptor in the digestive system is high, so cells in this region are reservoirs for the Sars-cov2 virus and cause inflammation and stimulation of immune responses. In stimulating an immune response, IL-1β, both independently and combined with other inflammatory cytokines, can affect the expression of adhesion molecules and induce signals to activate inflammatory molecules such as TLRs. Therefore, IL-1β can act as a helper to activate immune responses (43).

In addition, many studies have described the function of TNF-α like IL-1, and both of them affect the hepatic cells to induce the expression of complement peptides such as MBL2 (44).

TNF-α and IL-1 affect respiratory cells and thus decrease the efficiency of respiration. TNF-α, IL-1, and IL-6 also affect the hypothalamus, induce fever, and stimulate an immune response (45, 46). TNF-α is responsible for the expression of IL-6, IL-8 (CXCL8), RANTES (CCL5) and VEGF (47, 48). The study results showed that TNF-α, IL-1β, IL-6, TLR4, and MBL2 are common among Covid-19, IBD, gastritis, and diarrhea. These molecules induce inflammation and an immune response (49, 50, 51, 52).

It seems that methods used to treat diarrhea, IBD, and gastritis can be effective in treating Covid-19, according to their similar pathway and gene involvement. Moreover, identifying similarities in pathways and genes can give us a new approach to applying genes for therapy and for use as markers for diagnoses, albeit more studies are needed.

**Conflict of interests**

The authors declare that they have no conflict of interest.

**References**

1. Al-Rohaimi AH, Al Otaibi F. Novel SARS-CoV-2 outbreak and COVID19 disease; a systemic review on the global pandemic. Genes Dis 2020;7:491–501.
2. Jawaid SA, Jawaid M. Challenges faced by the Medical Editors in COVID19 Pandemic era. Pakistan J Med Sci 2020;36:855–6.

3. Mao R, Liang J, Shen J, Ghosh S, Zhu L-R, Yang H, et al. Implications of COVID-19 for patients with pre-existing digestive diseases. Lancet Gastroenterol Hepatol 2020;5:425–7.

4. Debmath M, Banerjee M, Berk M. Genetic gateways to COVID-19 infection: Implications for risk, severity, and outcomes. FASEB J 2020;34:8787–95.

5. Pak M, Lindseth G. Risk factors for cholelithiasis. Gastroenterol Nurs 2016;39:297–309.

6. Yilgwan C, Okolo S. Prevalence of diarrhea disease and risk factors in Jos University Teaching Hospital, Nigeria. Ann Afr Med 2012;11:217–21.

7. Klopfenstein T, Kadiane-Oussou NJ, Royer P-Y, Toko L, Gendrin V, Zayet S. Diarrhea: An underestimated symptom in Coronavirus disease 2019. Clin Res Hepatol Gastroenterol 2020;44:282–3.

8. Kishikawa H, Ojiro K, Nakamura K, Katayama T, Arahata K, Takarabe S, et al. Previous Helicobacter pylori infection–induced atrophic gastritis: A distinct disease entity in an understudied population without a history of eradication. Helicobacter 2020;25:e12669.

9. Elseweidy M. Brief review on the causes, diagnosis and therapeutic treatment of gastritis disease. Altern Integr Med 2017:6:2.

10. Yamamoto N, Ariumi Y, Nishida N, Yamamoto R, Bauer G, Gojobori T, et al. SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with ACE1 I/D genotype. Gene 2020;758:144944.

11. Mirzaei R, Karampoor S, Sholeh M, Moradi P, Ranjbar R, Ghasemi F. A contemporary review on pathogenesis and immunity of COVID-19 infection. Mol Biol Rep 2020;47:5365–76.

12. Garg M, Christensen B, Luelb JS. Gastrointestinal ACE2, COVID-19 and IBD: Opportunity in the Face of Tragedy? Gastroenterology 2020;159:1623–4.

13. Chao C-Y, Battat R, Al Khoury A, Restellini S, Sebastaini G, Bessisow T. Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: A review article. World J Gastroenterol 2016;22:7727.

14. Asadzadeh-Aghdaee H, Shahrokhi S, Norouzimina M, Hosseini M, Keramatinia A, Jamalan M, et al. Introduction of inflammatory bowel disease biomarkers panel using protein-protein interaction (PPI) network analysis. Gastroenterol Hepatol from bed to bench 2016;9:S8.

15. Crespi M, Dulbecco P, De Ceglie A, Conio M. Strictures in Crohn’s disease: from pathophysiology to treatment. Dig Dis Sci 2020;65:1904–16.

16. Mohan HM, Coffey JC. Surgical treatment of intestinal stricture in inflammatory bowel disease. J Dig Dis 2020;21:355–9.

17. Richter JE, Rubenstein JH. Presentation and epidemiology of gastroesophageal reflux disease. Gastroenterology 2018;154:267–76.

18. Clarrett DM, Hachem C. Gastroesophageal reflux disease (GERD). Mo Med 2018;115:214.

19. Argyrou A, Legaki E, Koutserimpas C, Gazouli M, Papacostantinou I, Gkiokas G, et al. Risk factors for gastroesophageal reflux disease and analysis of genetic contributors. World J Clin cases 2018;6:176.

20. Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, et al. DisGeNET: A comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res 2017;45:D833-D839.

21. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguetz P, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2010;39:D561–8.

22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13:2498–504.

23. Safaei A, Tavirani MR, Oskouei AA, Azodi MZ, Mohhebbi SR, Nikzamir AR. Protein-protein interaction network analysis of cirrhosis liver disease. Gastroenterol Hepatol from bed to bench 2016;9:114.

24. Zamanian-Azodi M, Mortazavi-Tabatabaei SA, Mansouri V, Vafaee R. Metabolite-protein interaction (MPI) network analysis of obsessive-compulsive disorder (OCD) from reported metabolites. Arvand J Heal Med Sci 2016;1:112–20.

25. Wu H, Gao L, Dong J, Yang X. Detecting overlapping protein complexes by rough-fuzzy clustering in protein-protein interaction networks. PLoS One 2014;9:e91856.

26. Bhosle A, Chandra N. Structural analysis of dihydrofolate reductases enables rationalization of antifolate binding affinities and suggests repurposing possibilities. FEBS J 2016;283:1139–67.

27. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plugin: pathway insights using integrated experimental and protein complex by rough-fuzzy clustering in protein-protein interaction networks. Bioinformatics 2009;25:1091–3.

28. Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics 2013;29:661–3.

29. Hunter DJ. Gene-environment interactions in human diseases. Nat Rev Genet 2005;6:287–98.

30. Safari-Alighiarloo N, Taghizadeh M, Rezaei-Tavirani M, Goliaei B, Peyvandi AA. Protein-protein interaction networks (PPI) and complex diseases. Gastroenterol Hepatol Bed Bench 2014;7:17.

31. Zamanian-Azodi M, Rezaei-Tavirani M, Rahmati-Rad S, Hasanzadeh H, Tavirani MR, Seyyedi SS. Protein-Protein Interaction Network could reveal the relationship between the...
breast and colon cancer. Gastroenterol Hepatol Bed Bench 2015;8:215.

32. Rezaei-Tavirani M, Zamanian-Azodi M, Rajabi S, Masoudi-Nejad A, Rostami-Nejad M, Rahmatirad S. Protein clustering and interactome analysis in Parkinson and Alzheimer’s diseases. Arch Iran Med 2016;19:0.

33. Safari-Alighiarloo N, Taghizadeh M, Tabatabaei SM, Shahsavari S, Namaki S, Khodakarim S, et al. Identification of new key genes for type 1 diabetes through construction and analysis of protein-protein interaction networks based on blood and pancreatic islet transcriptomes. J Diabetes 2017;9:764–77.

34. Zali H, Rezaei Tavirani M. Meningioma protein-protein interaction network. Arch Iran Med 2014;17:262-72.

35. Abbaszadeh H-A, Peyvandi AA, Sadeghi Y, Safaei A, Zamanian-Azodi M, Khoramgah MS, et al. Er: YAG laser and cyclosporin A effect on cell cycle regulation of human gingival fibroblast cells. J lasers Med Sci 2017;8:143.

36. Harcourt-Brown F. Digestive disorders. Textb Rabbit Med 2002;249-291.

37. Antonio N, Andrea T, Claudio T, Beatrice P, Pamela C, Chiara M, et al. Digestive disorders and Intestinal microbiota. Acta Bio Medica Atenei Parm 2018;89:47.

38. Organization WH. Coronavirus disease 2019 (COVID-19): situation report. 73. Geneva, Switzerland: WHO; 2020.

39. Singhal T. A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr 2020;87:281–6.

40. Russo RC, Garcia CC, Teixeira MM, Amaral FA. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. Expert Rev Clin Immunol 2014;10:593–619.

41. Nurul AA, Azlan M, Ahmad Mohd Zain MR, Sebastian AA, Fan YZ, Fauzi MB, Mesenchymal Stem Cells: Current Concepts in the Management of Inflammation in Osteoarthritis. Biomedicines 2021;9:785.

42. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014;2014:561459.

43. Dinarello CA. Overview of the interleukin-1 family of ligands and receptors. Semin Immunol 2013;25:389-93.

44. Bodmer J-L, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. Trends Biochem Sci 2002;27:19–26.

45. Henderson B, Pettipher ER. Arthritogenic actions of recombinant IL-1 and tumour necrosis factor alpha in the rabbit: evidence for synergistic interactions between cytokines in vivo. Clin Exp Immunol 1989;75:306.

46. Guerne PA, Carson DA, Lotz M. IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones in vitro. J Immunol 1990;144:499–505.

47. Alaaeddine N, Olee T, Hashimoto S, Creighton-Achermann L, Lotz M. Production of the chemokine RANTES by articular chondrocytes and role in cartilage degradation. Arthritis Rheum 2001;44:1633–43.

48. Honorati MC, Cattini L, Facchini A. VEGF production by osteoarthritic chondrocytes cultured in micromass and stimulated by IL-17 and TNF-α. Connect Tissue Res 2007;48:239–45.

49. Hunt RH, East JE, Lanas A, Malfertheiner P, Satsangi J, Scarpignato C, et al. COVID-19 and Gastrointestinal Disease: Implications for the Gastroenterologist. Dig Dis 2021;39:119–39.

50. Baryah ANS, Midha V, Mahajan R, Sood A. Impact of Corona Virus Disease-19 (COVID-19) pandemic on gastrointestinal disorders. Indian J Gastroenterol 2020;39:214–9.

51. Lam S, Lombardi A, Ouanounou A. COVID-19: A review of the proposed pharmacological treatments. Eur J Pharmacol 2020;886:173451.

52. Prescott HC, Rice TW. Corticosteroids in COVID-19 ARDS: Evidence and Hope During the Pandemic. JAMA 2020;324:1292–5.