UPREGULATION OF BRCA1 AND PPIP5K2 GENES AND PLATINUM DRUG RESISTANCE IN BOTH MILD AND SEVERE COVID-19 PATIENTS

Assist. Prof. Dr. Gizem AYNA DURAN
Izmir University of Economics, Faculty of Engineering, Biomedical Engineering Department,
Izmir, Türkiye, ORCID ID: https://orcid.org/0000-0002-2168-753X

ABSTRACT

Introduction: Covid-19 infection can lead to severe symptoms and diseases such as common cold, bronchiolitis, life-threatening pneumonitis, and pneumonia. Beside its well-known life-threatening affects, it can also trigger carcinogenesis and worsen the anti-cancer treatment processes. The aim of the study is to investigate the common and most robust upregulated genes in both mild and severe Covid-19 patients and to identify related biological processes and pathways which may be affected upon Covid-19 infection.

Materials and Methods: Data of five mild- and five severe-Covid-19 patients and five healthy individuals were included. The data of these patients were obtained from the GEO database (GSE164805) from GPL26963 platform (Agilent-085982 Arraystar human lncRNA V5 microarray). T-tests were used for statistical analysis to examine the statistical differences between healthy and Covid-19 patient groups in the intensity of each 60.797 genes. $p<0.001$ is accepted as a significance level.

Results: Mean age of the patients is 56.4 ± 7.54 years. In addition to 5 healthy individuals (33%), totally 10 Covid-19 patients (67%) are included in this study. Out of Covid-19 patients, 5 of them have mild Covid-19 infection however 5 patients have severe Covid-19 infection. Totally there are 13 male (87%) Covid-19 patients and healthy individuals. Upon gene screening of 60.797 gene for each patient, 3152 common genes of both mild and severe Covid-19 patients are identified. However, according to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, only tumour-suppressor gene BRCA1 and inositol pyrophosphate kinase gene (PPIP5K2) genes are upregulated in both mild- and severe- Covid-19 patients and these genes are related to biological processes such as platinum drug resistance pathway and phosphatidylinositol signalling pathway, respectively.

Conclusion: The history of the Covid-19 pandemic is more recent and long-term effects of Covid-19 infection has not yet been clarified especially on cancers. According to our results, platinum drug resistance is triggered via Covid-19 infection in both mild- and severe- Covid-19 patients which is associated with the upregulation of BRCA1 and PPIP5K2 gene. Overexpression of BRCA1 and PPIP5K2 genes are related to not only breast and ovarian cancers but also certain cancer types such as pancreas, stomach, colorectal and prostate cancers. However, these results should be validated with in vitro and in vivo studies.

Keywords: Covid-19, platinum-drug resistance, BRCA1, PPIP5K2, upregulation, cancer

INTRODUCTION

Mankind has faced various pandemics over the years throughout the world and severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) disease (named as Covid-19 disease) has been the fifth one since 1918 Spanish flu (H1N1 Influenza A Virus) pandemic. Similarly, Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) have been experienced before Covid-19 syndrome arise. Compared to other pandemics, which was first observed in December 2019 at Wuhan province of China, Covid-19 is a global threat with high mortality rate, and it has been more severe effects on human health, and it has affected larger populations of people from every country in the world (Yen-Chin Liu et al., 2020). According to WHO data, totally 494,587,638 confirmed cases have been recoded until 9 April 2022. Among these cases, there have been reported 6,170,283 deaths (https://covid19.who.int/) (Accessed 9 April 2022).

Covid-19 is a highly infectious and contagious respiratory disease may lead to severe symptoms and diseases such as common cold, bronchiolitis, life-threatening pneumonitis and pneumonia, and its transmission occurs from human to human via direct contact through air droplets (Saira Baloch et al., 2020) (Nikhil Kirtipal et al. 2020).

As SARS-CoV-2 is a non-retroviral ssRNA virus, it can persist in the host for a long term which can cause effects on many diseases, including cancer (Miguel Alpalhao et al. 2020) (Randall Re et al. 2017).

For instance, it has been shown that coronavirus can suppress the tumour suppressor proteins such as p53 and pRb which are commonly shown as mutated in cancer cells such as breast and pancreas cancers (Bhardwaj K et al. 2012) (Ma-Lauer et al., 2016) (Liz J Hernández Borrero et al. 2021) (Shunsuke Kitajima et al. 2020). Furthermore, SARS–CoV–2 infection can delay the acquired immune system response via lowering the level of lymphocytes and inhibiting the DNA repair mechanisms. According to *in vitro* studies, Hui Jiang et al. have shown that e SARS–CoV–2 spike protein can prevent the DNA repair proteins such as tumour suppressor gene Breast Cancer Susceptibility Gene 1 (BRCA1) which binds to tumour suppressor protein p53 (Hui Jiang et al. 2021) (Quin C et al. 2020).

The number of publications showing the effect of Covid-19 infection on carcinogenesis and cancer progression is quite limited. Therefore, we aim to investigate the common and most robust upregulated genes in both mild and severe Covid-19 patients and to identify related biological processes and pathways which may be affected upon Covid-19 infection.

METHODS

Acquisition of Dataset

Gene expression profiles of microarray data for COVID-19 (GSE164805) were obtained from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164805). Samples from GSE164805 dataset (GPL26963 (Agilent-085982 Arraystar human lncRNA V5 microarray) platform) were composed of five healthy people, five mild Covid-19 patients, and five severe Covid-19 patients.

Identification of Common Genes in Both Severe and Mild Covid-19 Patients

Initially, patients are grouped into three classes; healthy individuals, patients had mild Covid-19 and patients had severe Covid-19. Then, t tests are applied for each 60,797 genes to test the statistical significance between healthy group and the patients that had experienced Covid-19 infection. In addition, 2 more t tests are applied which examines the same context between healthy group-mild Covid-19 cases and healthy group-severe Covid-19 cases. Totally 3152 genes appear as an outcome and significant in all three tests. Group means for significant genes are compared to understand whether the gene is upregulated or downregulated with respect to healthy individuals.

Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) web tool was used to construct Venn diagram for determining the common upregulated genes in both mild and severe Covid-19 patients.

Enrichment Analysis of Common Genes in Both Mild and Sever Covid-19 Patients

Gene set enrichment analysis process was performed to determine the main affected biological pathways as well as common upregulated genes in both mild and severe Covid-19 patients. For this reason, ShinyGO v0.741: Gene Ontology Enrichment Analysis was performed (http://bioinformatics.sdstate.edu/go74/). ShinyGO v0.741 is a web-based gene enrichment analysis platform and KEGG database was selected to define the molecular interaction and relation networks of common upregulated genes in both mild and severe Covid-19 patients.

Statistical Analysis

T-tests were used for statistical analysis to examine the statistical differences between healthy and Covid-19 patient groups in the intensity of each 60.797 genes. $P<0.001$ is accepted as a significance level.

The statistical details of the applied tests are as follows:

$Ho: GI_{healthy} = GI_{Covid-19}$

$Ha: GI_{healthy} \leq$ or $\geq GI_{Covid-19}$

The null hypothesis states that gene intensity (GI) is equal across health and Covid-19 group. Alternative hypothesis indicates the statistically significant difference in gene intensities between the two groups (healthy and Covid-19) In the additional two t tests, Covid-19 groups are defined as mild Covid-19 and severe Covid-19. Corresponding p values are reported along with means of gene intensities for different groups.

Ethics Committee Approval

Ethics committee approval was not necessary in this study because publicly open information from the Gene Expression Omnibus (GEO) database was used.

RESULTS

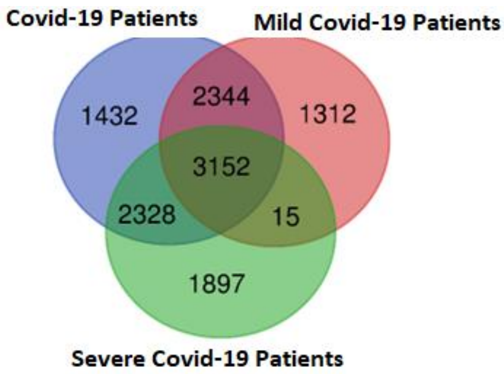
The characteristics of Covid-19 patients and healthy individuals are detailed in Table 1. In addition to 5 healthy individuals (33%), totally 10 Covid-19 patients (67%) are included in this study. Out of Covid-19 patients, 5 of them have mild Covid-19 infection however 5 patients have severe Covid-19 infection. The median age of the both Covid-19 patients and healthy individuals is 56.4 ± 7.54 and there are 13 male (87%).

Table-1: Demographic Data of Covid-19 Patients

	Number of patients/healthy individuals (% of patients)
Healthy Individuals	5 (33 %)
Patients with covid	10 (67%)
Patients with mild covid	5 (33 %)
Patients with severe covid	5 (33 %)
Male	13 (87 %)
Female	2 (13 %)
Age	Mean: 56.4 (SD: 7.54)

As a result of t-test analysis, totally 60.797 genes are screened, and we have shown that there are 3152 common genes of both mild and severe Covid-19 patients (Figure 1).

Figure-1: Common Genes in Both Mild and Severe Covid-19 Patients



By using 3152 common genes of both mild and severe Covid-19 patients, we have performed gene ontology enrichment analysis by using ShinyGO v0.741: Gene Ontology Enrichment Analysis platform. According to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, only BRCA1 and PPIP5K2 genes are found to be related to biological processes. In case of BRCA1 gene, platinum drug resistance pathway is pointed out. On the other hand, Phosphatidylinositol signalling pathway was related to PPIP5K2 gene (Table-2, Table 3a and Table 3b) (Figure-2). In addition to these pathways, related biological processes such as homologous recombination, ubiquitin mediated proteolysis, breast cancer, microRNAs in cancer are also related to common genes of Covid-19 patients.

Figure-2: KEGG Pathway Analysis

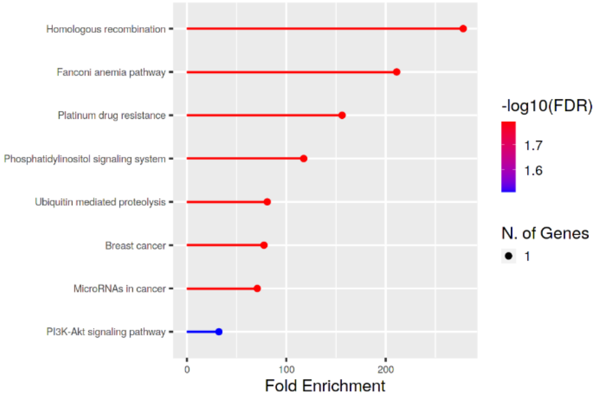


Table-2: BRCA1 and PPIP5K2 genes and related pathways due to Covid-19 infection

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathway	Genes
0.016086528	1	73	156.1369863	Platinum drug resistance	BRCA1
0.016086528	1	97	117.5051546	Phosphatidylinositol signalling system	PPIP5K2

Table-3a: BRCA1 gene may significantly related to Covid-19 infection in both severe and mild patients

Gene ID: BRCA1 (A_23_P207400)	Mean (Healthy Group)	Mean (Covid)	T-Test (P-value)- Inequal Variance Assumed	T-Test (P-value)- Equal Variance Assumed
All patients	6.09983542	7.59679018	1.72217E-05	2.7485E-06
Patients with mild covid	6.09983542	7.57494388	0.000467769	0.000314819

Patients with severe covid	6.09983542	7.61863648	3.80581E-05	2.96775E-05

Table-3b: PPIP5K2 gene may significantly related to Covid-19 infection in both severe and mild patients

Gene ID: PPIP5K2 (A_23_P213661)	Mean (Healthy Group)	Mean (Covid)	T-Test (P-value)- Inequal Variance Assumed	T-Test (P-value)- Equal Variance Assumed
All patients	9.4629745	10.7346445	3.56314E-06	1.01167E-05
Patients with mild covid	9.4629745	10.895597	0.00028186	0.000111798
Patients with severe covid	9.4629745	10.573692	0.000146882	0.000125185

DISCUSSION

Covid-19 infection is a global threat with high mortality rate and the history of the Covid-19 pandemic is more recent. Therefore, long-term effects of Covid-19 has not yet been clarified especially on cancers. In addition to life threatening diseases such as common cold, bronchiolitis, life-threatening pneumonitis, and pneumonia, Francescangeli et al. have hypothesized that Covid-19 dependent activation of several factors (innate immune cells activation and uncontrolled production of pro-inflammatory cytokines) may provide the favourable tumour microenvironment which can lead to tumorigenesis and metastatic relapse (Francescangeli et al. 2020). Even though possible contributions of coronavirus to carcinogenesis mechanisms and treatment processes have been shown, there have been limited number of studies explaining the exact and direct effects of the virus on specific cancer mechanisms. So far, there has not been any study which indicates the effects of Covid-19 infection on BRCA1 and PPIP5K2 expression patterns and related platinum-drug resistance mechanism. Therefore, we aim to determine the overexpressed genes in both mild and severe Covid-19 patients to identify the common and most robust gene patterns and related mechanisms particularly in cancers. To achieve this goal, we have screened 60.797 gene for each patient and healthy individual and 3152 common genes of both mild and severe Covid-19 patients have been identified (Figure 1). According to KEGG pathway analysis, only tumour-suppressor gene BRCA1 and inositol pyrophosphate kinase gene (PPIP5K2) genes are determined as upregulated in both mild- and severe- Covid-19 patients (Table 3) and these genes are related to biological processes such as platinum drug resistance pathway and phosphatidylinositol signalling pathway, respectively (Table 2, Figure 2).

BRCA1 gene involves in various processes such as DNA repair, transcription, and cell cycle checkpoint control (Venkitaraman 2002). As a well-known tumour-suppressor gene, it has been indicated that BRCA1 inherited germ-line mutations can lead to a predisposition of breast and ovarian cancers (Y Miki et al. 1994). Besides germ-line mutations of BRCA1 gene involvement in carcinogenesis, high expression of BRCA1 gene may trigger the early distant metastasis in estrogen-positive (ER+) breast cancer (Chang et al. 2022). In our study, only 3% of both mild- and severe- Covid-19 patients are female (Table 1) and the BRCA1 gene overexpression may be important in terms of breast and ovarian cancer predisposition upon Covid-19 infection.

While breast and ovarian cancers are the cancer types in which BRCA1 mutations are observed most, there are publications showing that BRCA1 mutations are associated with the carcinogenesis process in prostate, stomach, colorectal, and pancreatic cancers (Cavanagh et al. 2015) (Sopik et al. 2015). Similarly, it has been shown that non-small cell lung cancer patients with high BRCA1 gene expressions have shorter overall survival rates compared to ones with less low BRCA1 gene expression (Sadraei et al. 2018). Most of our (87%) of Covid-19 patients is male (Table 1) and according to our results, it is possible that Covid-19 infection in these male patients may cause genetic changes (BRCA1 overexpression) that may play a role in susceptibility to many cancers.

BRCA1 mutations and overexpression does not only lead to differences in tumour development and metastatic processes, but it can affect the anti-cancer treatment processes. For instance, Quinn et al have been shown that both BRCA1 overexpression and secondary mutations of BRCA1 are associated with platinum drug resistance in ovarian cancer patients (Quinn et al. 2007) (Dhillon et al. 2011). In our study, we have further indicated that BRCA1 upregulation is linked to platinum-drug resistance according to KEGG pathway analysis results (Figure 2, Table 2, Table 3a).

Platinum-based anticancer drugs such as cisplatin, carboplatin, oxaliplatin, nedaplatin, and lobaplatin are the most used agents to cure various cancers. However, some patients develop drug resistance against these agents which can lead to dramatic outcomes and poor cancer prognosis. In addition to effects of BRCA mutations on anticancer drug resistance (Quinn et al. 2007) (Dhillon et al. 2011), it has also been shown that phosphatidylinositol 3-kinase (PI3K) cascades also induce the platinum-drug resistance (Ohmichi et al. 2005). In our study, we have indicated that PPIP5K2 gene upregulation is related to phosphatidylinositol signalling process according to KEGG pathway analysis (Figure 2, Table 2).

Like BRCA1 upregulation, the high expression of Diphosphoinositol Pentakisphosphate Kinase 2 (PPIP5K2), inositol pyrophosphate kinase, gene has been related to poor prognosis of cancers such as colorectal cancers (Chen-Hui Cao et al. 2021). In our study, PPIP5K2 gene has been detected as upregulated in only and both mild- and severe- Covid-19 patients (Table 2, Table 3b). The upregulation of PPIP5K2 gene may be an indicator and prognosis marker not only for colorectal cancers but also in other types of cancers. This important point should be further investigated.

There are some limitations of this study. Covid-19 and patient data at the genetic level have not reached high numbers since the disease has yet to find a new place in the literature. The number of patients in our study can be considered as a limitation. In addition, despite the small number of patients, high number of genes (60.797 genes) were examined for each patient. Therefore, we can claim that the results are robust statistically.

CONCLUSION

According to our results, platinum drug resistance is triggered via Covid-19 infection in both mild- and severe- Covid-19 patients which is associated with the upregulation of BRCA1 and PPIP5K2 gene. Overexpression of BRCA1 and PPIP5K2 genes are related to not only breast and ovarian cancers but also certain cancer types such as pancreas, stomach, colorectal and prostate cancers. However, these results should be validated with high number of patient data and *in vitro* and *in vivo* studies.

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