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RESEARCH ARTICLE

DIFFERENTIAL ANALYSIS OF GENE EXPRESSION IN SEVERELY SICK COVID-19 PATIENTS

Rodríguez-Carrascal Daniela[1,3,5], Hernández Fernando[4,5] and Ramírez Carlos Darío[1,2]

1. Unit of Genetic and Forensic Studies (UEGF), Venezuelan Institute of Scientific Research (IVIC), Caracas, Venezuela.
2. Bolivarian University of Venezuela (UBV), Caracas, Venezuela.
3. Faculty of Pharmacy and Biochemistry, Department of Biological Chemistry, Chair of Pathological Biological Chemistry, University of Buenos Aires, Buenos Aires, Argentina.
4. Pathophysiology Laboratory, Center for Experimental Medicine"Miguel Layrisse", Venezuelan Institute of Scientific Research (IVIC), Caracas, Venezuela.
5. Equal contribution.

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Abstract

This study investigates differential gene expression in severely sick COVID-19 patients to elucidate molecular mechanisms driving disease progression. Using transcriptomic data from 44 severe COVID-19 patients and 10 healthy individuals (NCBI GEO: GSE171110), we analyzed whole blood samples via DESeq2, identifying 737 differentially expressed genes (DEGs). Of these, 662 were upregulated (e.g., inflammatory and antiviral pathways) and 75 downregulated. Principal component analysis (PCA) and heatmaps revealed distinct transcriptional signatures between groups, highlighting immune dysregulation. Key upregulated pathways included Jak-STAT, MAPK, PI3K-Akt, Toll-like receptor, and TNF signaling, associated with cytokine storms, neutrophil activation, and oxidative stress. MHC class I genes (linked to CD8+ T-cell and NK-cell cytotoxicity) were elevated, while MHC class II (involved in CD4+ T-cell cytokine production) was suppressed. Pro-inflammatory interleukins (IL-1β, IL-6, IL-12) and antiviral IFN-β were also upregulated, indicating heightened inflammation and antiviral responses. Conversely, DNA repair pathways were disrupted. The findings suggest severe COVID-19 involves hyperactivation of innate immunity and cytotoxic T-cell responses, coupled with impaired adaptive immunity (reduced CD4+ T-cell function). These mechanisms may contribute to tissue damage and cytokine release syndrome (CRS).The study highlights the role of transcriptional dysregulation in driving severe disease outcomes, providing insights into potential therapeutic targets for immune response modulation.

Corresponding author: Ramírez Carlos Darío
1. Address**:** Unit of Genetic and Forensic Studies (UEGF), Venezuelan Institute of Scientific Research (IVIC), Caracas, Venezuela.
2. Bolivarian University of Venezuela (UBV), Caracas, Venezuela.

Introduction:

Covid-19 caused by the novel coronavirus (SARS-CoV-2), Over 760 million cases and 6.9 million deaths have been recorded worldwide since December 2019, but the actual number is thought to be higher (1). Patients with this disease have very variable clinical pictures, ranging from the absence of symptoms to respiratory deficiency, which progressively leads to death. Studies have postulated that immune response is fundamental in the progression of this disease. When the immune system fails to control the virus efficiently in the acute stage, a macrophage activation syndrome can develop that causes the flow of a cascade of cytokines that lead the patient to a critical or severe state (2). However, it is still not completely known how these mechanisms are carried out, nor the variables that trigger the severe condition, which occurs in a small percentage of these patients (3). Therefore, for a greater understanding of the disease it is necessary to study the cellular pathways and the transcriptional response to infection in the host cells. To clarify the signaling pathways affected in the severe state of patients, the present work proposes the analysis of the transcriptome of whole blood samples from patients with severe COVID-19 in comparison with apparently healthy individuals.

Objective:

To analyze the transcriptional profile in whole blood samples from healthy individuals and patients with severe Covid-19 to compare and analyze the pathways involved in patients with this disease.

Methodology:

Transcriptomic data were obtained in the form of pre-processed count matrices (aligned and filtered readings, not normalized) from the NCBI Gene Expression Omnibus (GEO) platform (https://www.ncbi.nlm.nih.gov/geo/. Accession number: GSE171110) (4), which were represented by 54 samples, of which 44 were from patients with severe Covid-19 and 10 healthy individuals. The counting matrix was imported by the DESeqDataSetFromMatrix function of the DESeq2 package (v1.32.0), it contained 30,185 transcribed genes. All normalized transcripts with a maximum mean of all rows less than 10 were excluded, resulting in 24,099 transcripts present. And again, with DESeq2, the set of differentially expressed genes was calculated (severe COVID-19 versus Healthy Individuals), the p-value used was 0.05, and a FC of 1. The variance stabilization transformation (VST) and Log2 were used for the calculation of normalized counts of each transcript. With all the transcripts present, a principal component analysis was performed. The most variable transcripts were visualized on a heat map. And an analysis of differentially expressed pathways was performed using the KEGG package.

Results and Discussion:

The differential expression analysis for the 24,092, with p-value < 0.05 and FC = 1, yielded a total of 737 differentially expressed genes (Figure 1A), of which 662 (2.7%) were positively regulated (LFC > 1.00), while 75 (0.31%) were negatively regulated (LFC < -1.00). Both in the analysis of main components (Figure 1B) and in the heat map (Figure 1C) it can be observed that the samples of patients with "Severe COVID-19“present a differentially expressed gene signature different from that of the samples provided by the “Healthy Individuals “.

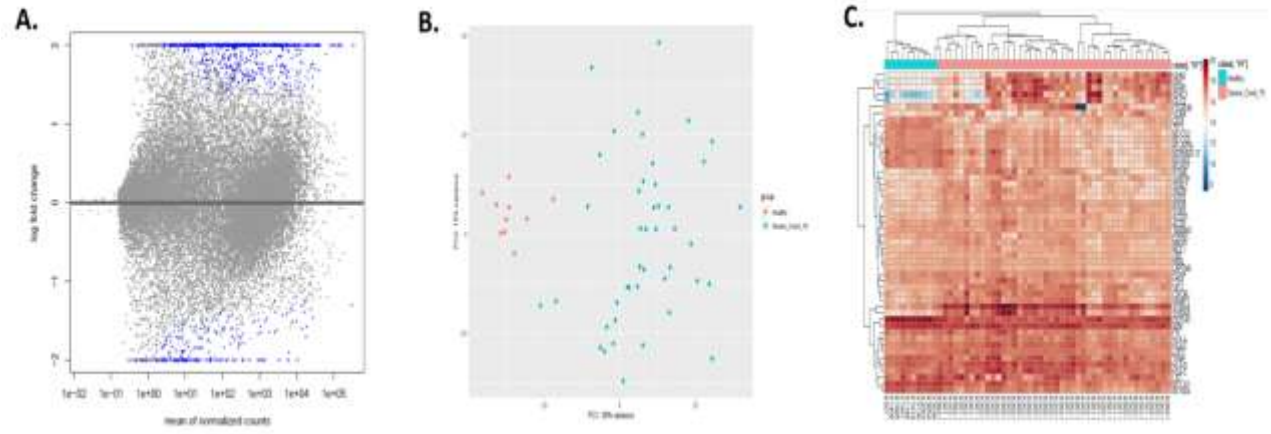

Figure 1: A. Log2 shift volcano diagram, differentially expressed genes are shown in blue color. B. Main component graph C. Heat map of differential gene expressions between COVID-19 patients and healthy donors

(each column represents a different sample; the rows represent different genes; the color represents the level of gene expression: red indicates a positive gene expression in the sample and blue indicates a negative gene expression in the sample.

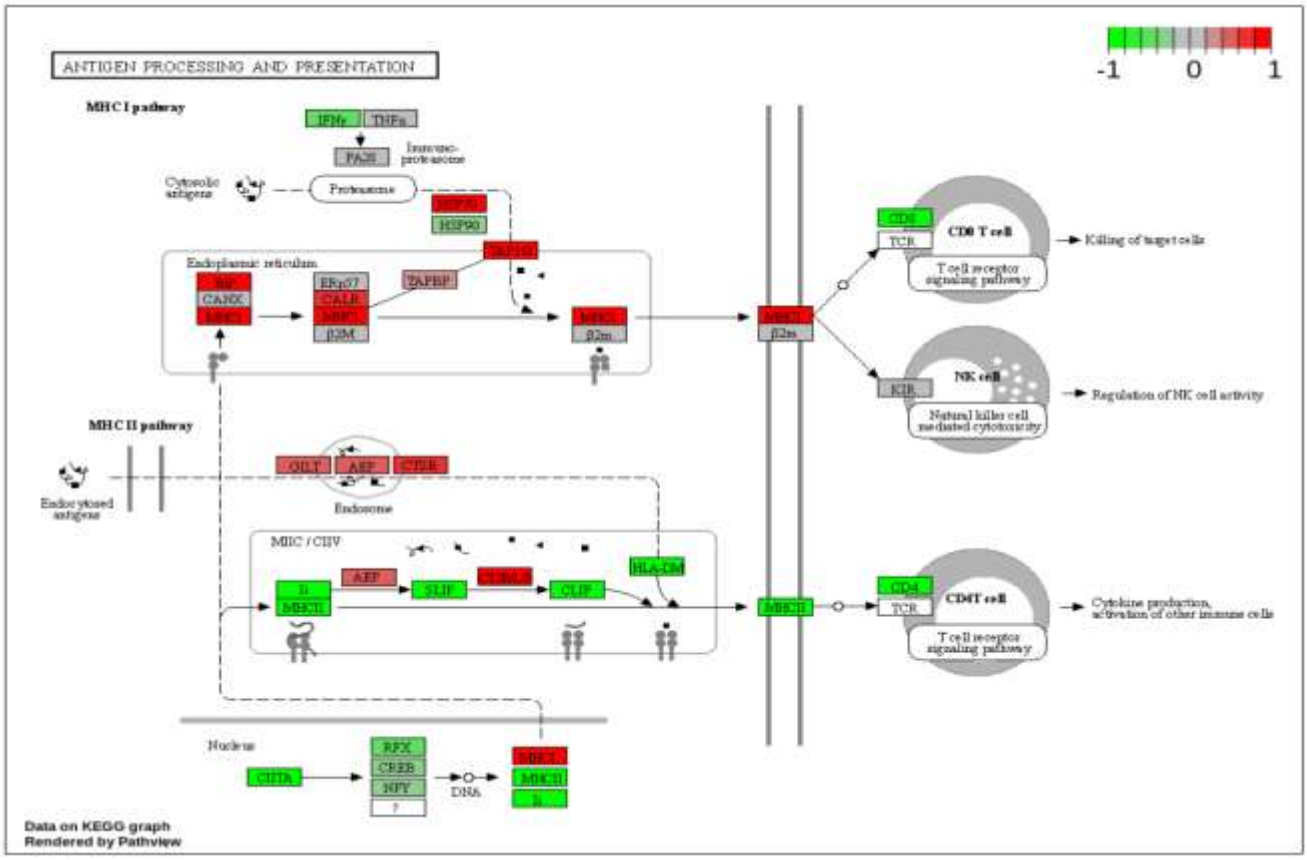

Figure 2: Antigen Processing and Presentation Pathways.

Through the analysis of differentially expressed pathways, it was observed that the Jak-STAT, MAPK, PI3K-Akt, and PLCγ signaling pathways were positively regulated, activated by chemokine/cytokine receptors. This leads to altered cellular functions such as proliferation, differentiation, apoptosis, migration, and ubiquitination-mediated proteolysis. There is production of reactive oxygen species and which in turn produces dysregulation of the DNA mismatch repair (MMR) system (5).

At the level of immunity, positively regulated expression of the MHCI was observed, which triggers the CD8 T lymphocyte response and regulates the activity of NK cells, which function as inducers of cell death. On the other hand, the MHCII showed negatively regulated expression, as well as the activity of CD4 T lymphocytes, which act in the production of cytokines (Figure 2). In addition, there is a strong regulation of positive expression in genes associated with the inflammatory response, which includes the Toll-Like receptor signaling pathway, the TNF signaling pathway, pro-inflammatory interleukins: IL-1b, IL-6, IL-12, which also plays an important role in the recruitment, function and survival of neutrophils (6). On the other hand, positive expression of IFN-β, which functions as a mediator of the antiviral response, was observed (Figure 3).

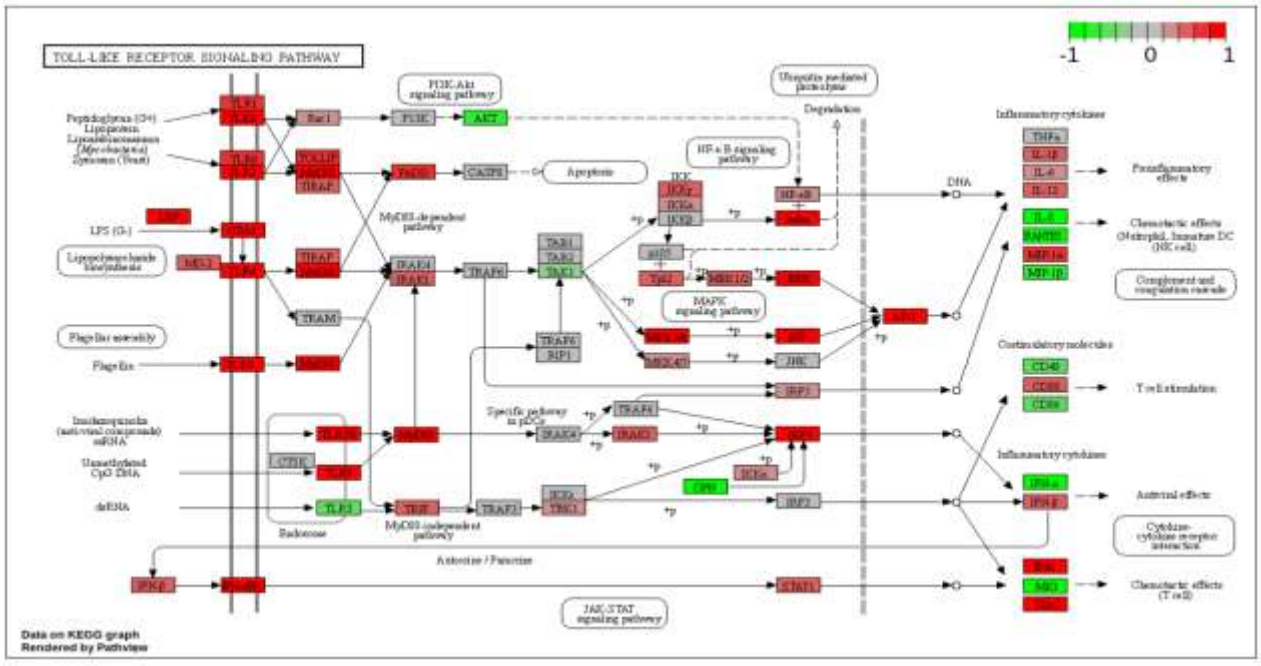

Figure 3: Signaling pathway of the Toll-Like receptor.

Gene expression studies conducted on blood samples from COVID-19 patients indicated that genes associated with inflammatory and hypercoagulability pathways (7) and the imbalance between innate and adaptive immune responses are the main factors responsible for severe disease course (8). Thair et al (9) found a differential transcriptomic pattern in blood that differentiated COVID-19 patients from other viral infections, suggesting that their top differentially expressed genes (DEGs) may be linked to new mechanisms of pathogen evasion from host immune response. Most recently, Aschenbrenner et al. (10) revealed neutrophil activation signatures in severe cases, coupled with an elevated expression of genes related to coagulation and platelet function, and absence of T-cell activation.

Considering the ability of coronavirus infections, particularly SARS-CoV-2 infections, to induce inflammation and lung injury and the similarity of this novel virus to MERS-CoV and SARS-CoV in its ability to infect lung epithelial cells, it is expected that this emerging life-threatening coronavirus utilizes some of the same cellular signaling pathways as MERS-CoV and SARS-CoV. As a therapeutic approach, the use of anti-inflammatory agents during COVID-19 to affect inflammatory signaling pathways might be beneficial for reducing the severity of the disease (11). The NF-κB, cytokine regulation, ERK, and TNF-α signaling pathways have been shown to be likely causes of inflammation in MERS-CoV and SARS-CoV infections, with neutrophilia and basophilia exacerbating the disease in SARS patients [210]. The increased levels of inflammatory cytokines in serum of COVID-19 patients have shed light on the involvement of such signaling pathways in the pathogenesis infection of SARS-CoV-2. Further studies are needed to clarify the exact roles of cellular signaling pathways once the SARS-CoV-2 initiates infection in its host cell. Also, researchers in molecular medicine should consider the roles of the most strongly up- and downregulated components of cellular signaling pathways during COVID-19 to identify and design better molecular drugs that will decrease the fatality rate of this novel pandemic coronavirus.

The findings suggest severe COVID-19 involves hyperactivation of innate immunity and cytotoxic T-cell responses, coupled with impaired adaptive immunity (reduced CD4+ T-cell function). These mechanisms likely contribute to tissue damage and cytokine release syndrome. The study underscores the role of transcriptional dysregulation in driving severe outcomes, offering insights into potential therapeutic targets to modulate immune responses.

Conclusion:

In the severe state of SARS-CoV-2 viral infection, differential gene expression is observed, primarily involving genes associated with signaling pathways linked to CD8+ T lymphocyte recruitment, pro-inflammatory responses, cell cycle disruption, and antiviral defense mechanisms.

Competing interests:

The authors declare that they have no financial or nonfinancial competing interests.

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