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A study of the immunological and biochemical changes of COVID-19 patients and the gene expression of their Nrf2 gene in southern Iraq

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Abstract---This study was to determine the effect of the Coronavirus in samples (blood and serum) and its association with biochemical and immunological parameters and some factors including age, gender, clinical symptoms, and chronic diseases. 100 samples of COVID-19 patients (male and female) were collected in southern Iraq for 3 months (March 1 - 1 June). The results of this study showed (84% severe state and 16% critical state). High levels of ferritin in the severe case of COVID-19 patients, while D-dimer high levels in the critical cases of COVID-19 patients. CRP and WBC are at high levels in critical cases for COVID-19 patients. While the platelet levels of COVID-19 patients were low in critical cases. RT-PCR examined NRF2 gene expression to determine gene expression levels 5'-A/GTGACNNNGC, its relationship with COVID-19, and biochemical and immunological parameters. High levels of gene expression in severe cases and we did not find any effect of gender or age on gene expression levels NRF2. Parameter levels were not affected by the gender of the patient when infected with COVID-19. While platelet levels were high in females, due to some physiological factors. LDH and RBC levels are high in age groups (>70 years) and WBC high levels in age groups (40-59 years) of COVID-19 patients. While the HGB levels of COVID-19 patients were low in the age groups (>70 years). There was a high positive relationship between gene expression levels and ferritin, D-dimer, RBS, CRP, and WBC levels. The weak inverse relationship between gene expression levels and platelets (PLT).

Keywords---COVID-19, Nrf2, polymerase chain reaction (PCR).

Introduction

SARS-CoV-2 virus causing a series of acute atypical respiratory disorders has spread in Wuhan, Hubei Province, China. COVID-19 is the name given to the disease caused by this virus in December 2019¹. The SARS-CoV-2 virus primarily affects the respiratory system, although it also affects other organ systems. COVID-19 respiratory symptoms are now well acknowledged to be quite diverse, ranging from minor symptoms to severe hypoxia with ARDS². The virus was identified as a new coronavirus after sequence-based analysis of samples from the patients. Furthermore, the genetic sequence was made available to aid in the detection of viral infection³. Initially, it was thought that individuals infected with the Wuhan coronavirus in China may have visited a seafood market where live animals were sold, or that they may have eaten infected animals or birds. However, additional examination indicated that some people became infected even if they had no history of visiting a fish market, it soon became clear that efficient person-to-person transmission was also occurring⁴. The clinical spectrum of SARS-CoV-2 infection appears to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness, and severe viral pneumonia with respiratory failure and even death, with many patients being hospitalised with pneumonia in Wuhan². Inflammation is generally a pervasive response to disturbances in the tissue homeostasis due to a variety of stimuli such as pathogens, tissue injury, or contaminants which involves the activation of innate and adaptive immunity⁵.

Changes in the patients' biochemical markers, such as Ferritin, CBC, and D-dimer status, were documented on laboratory-confirmed coronavirus infections⁶. Another study found that individuals with COVID-19 had changes in inflammatory markers such as C-reactive protein (CRP) and elevated lactate dehydrogenase (LDH) in a higher number of COVID-19 patients⁷. Scientists have discovered a group of human genes that combat the virus that causes COVID-19, SARS-CoV-2. Knowing which genes aid in the management of viral infection can help researchers better understand the factors that influence illness severity and identify potential therapy alternatives⁸. NRF2 is a multifunctional transcription factor that protects cells from oxidative damage. It encodes a large number of genes that are involved in immunity and inflammation, as well as antiviral activity. The Nrf2 signaling pathway controls anti-inflammatory gene expression and prevents inflammation from progressing⁹. The physiological and pathological environment has a substantial impact on Nrf2 levels. As a result, effective treatment requires precisely timed and targeted modulation of the Nrf2 pathway¹⁰. The Nrf2 system is an important part of the body's natural defense against harm caused by hyperglycemia.

Aim of study

Investigation of gene expression of Nrf2 gene. To know the protective role of Nrf2 gene in covid-19 patients. Examination of immunological (CPR measurement and the differential count study of the WBC), biochemical parameters (Ferritin, D-

dimer, LDH and RBS results), and examination of hematological parameters CBC (RBC, HGB and PLT).

Method

Trial design and patients

All 100 patients were admitted to Al-Hussain hospital of Dhi-Qar and diagnosed with COVID-19 pneumonia from Apr 1 to Mar 30, 2021. Diagnosis of COVID-19 infection in patients was made by a positive test for viral RNA of respiratory secretions obtained by broncho-alveolar lavage, sputum, nasopharyngeal swab, or oropharyngeal swab. Demographic information, clinical characteristics (including medical history, severity, and comorbidities), and chest CT scan results of each patient were obtained from the medical record system of the hospital. This study included (60) person apparently healthy individuals as a control group, who have no history or clinical evidence of Covid-19 or any other chronic disease, and no obvious abnormalities. The control group is the group of healthy individuals, their number was 30, divided according to their ages into three groups.

Sample selection

Blood samples were collected by venipuncture from 100 patients and 60 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. Each blood sample was divided into two parts:

- Three milliliters were put directly in a sterile tube containing EDTA for DNA extraction, CBC test.
- Two milliliters were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -20 C° freezing. These sera (100 T1DM patients and 30 controls) were used for estimating:
 - The concentration of C-RP, D-dimer.
 - The concentration of Ferritin, LDH, RBS.

Statistical Analysis

The analyses of data were expressed as mean $\pm$ SD. The comparisons between each studied groups were performed with Least Significant differences (LSD). $P < 0.05$ was considered probability level using. All the statistical analyses were done by using Pentium-4 computer through the (SPSS program) Statistical Package for Social Sciences (version-21)

Results

A 100 samples were collected from COVID-19 patients, including 16 critical cases and 84 severe cases, also were collected and examined 60 samples are collected from healthy people as a control group. The results according to groups study showed an increase in the average of Ferritin, and D-dimer in covid-19 patients compared to the control group. An increase levels LDH and RBS in critical cases compared to the control group. Levels of C-reactive protein (CRP) and White blood

cells in critical cases and severe cases compared with the control group. Levels of red blood cells a high in severe cases compared with the control group, while a lower levels of HGB in severe cases, and a lower levels of PLT in critical cases compared with rate severe cases and control group. The gene load according to groups study the levels of NRF2 gene expression were high in covid-19 patients in severe cases compared with the critical cases and the control groups. Where the results showed the NRF2 gene expression rate in severe cases (3.28), and critical cases (1.70), while in the control group (1.21), with high statistical differences at a significant (0.05). All Parameters Results According to Gender there are no high significant differences in the Mann-Whitney test for the NRF2 gene expression for gender between Male(58) and Female(42), so the levels of P. value was more than (0.05) in the parameters. There are significant difference (>0.05) in LDH (0.064), but in PLT (0.025) there are high significant differences (0.05). Gene Load Results According to Gender there are no high significant differences (0.05) in the Mann-Whitney test for the NRF2 gene expression in all patients (Male 58 and Female 42). Ferritin, D-dimer, LDH and RBS Results According to Age Groups the results of the current study showed there are no significant differences between the levels of ferritin and D-dimer in covid-19 patients and age group. The results of the LDH examination showed a high-level LDH in the age group (710.30) >70 years, compared with other age groups (<40 , 40-59, and 60-79 years) respectively. There are no statistically significant differences (0.05). The results of the Random blood sugar examination showed a high-level RBS in the age group (>70 years) compared with another age group (<40 , 40-59, 60-79 years) respectively. There are no statistically significant differences. CRP and WBC Results According to Age Groups the results of the current study showed there are no significant differences in the levels of C-reactive protein in covid-19 patients compared with the age group (<40 , 40-59, 60-79, >70 years) respectively. This study's results showed significant differences (0.05), in the age group (40-59 years) compared with the age groups (60-79 years) and (>70 years). RBC, HGB and PLT Results According to Age Groups in this study, results showed there are no significant differences (0.05) in the levels of Red blood cells compared with age groups. This study results showed the significant differences (0.05) in the levels HGB from covid-19 patients between age group (<40 years) and age group (40-59 years) compared with age groups (>70 years). This study's results showed of the levels of platelet compared with age group in covid-19 patients there are no significant differences (0.05). Gene Load Results According to Age Groups the results of the current study showed that there are no significant differences (0.05) between NRF2 gene expression rate and age groups of covid-19 patients. Pearson Correlation between All Parameters in the Study The results of the current study showed that there are many highly statistically significant relationships between some criteria, where there was a moderately positive correlation (0.619) between ferritin rate and NRF2 gene expression rate, while the relationship between D-dimer and platelets was a weak inverse relationship (0.304), but the relationship between ferritin and D-dimer on the one hand and HGB, on the other hand, is a very weak inverse relationship. The results also showed that there are positive relationships between the level of RBS, D-dimer, LDH, and CRP, while there are inverse relationships between HGB and the count of RBC.

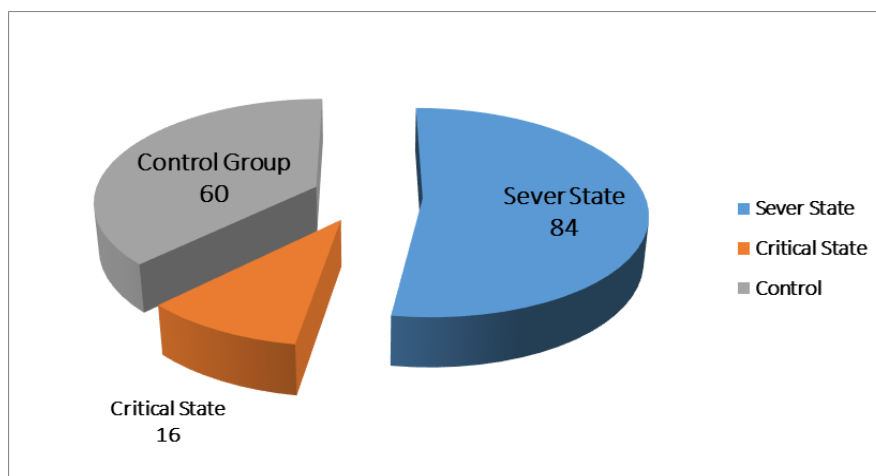


Figure 1: Distribution of Samples to Study Groups

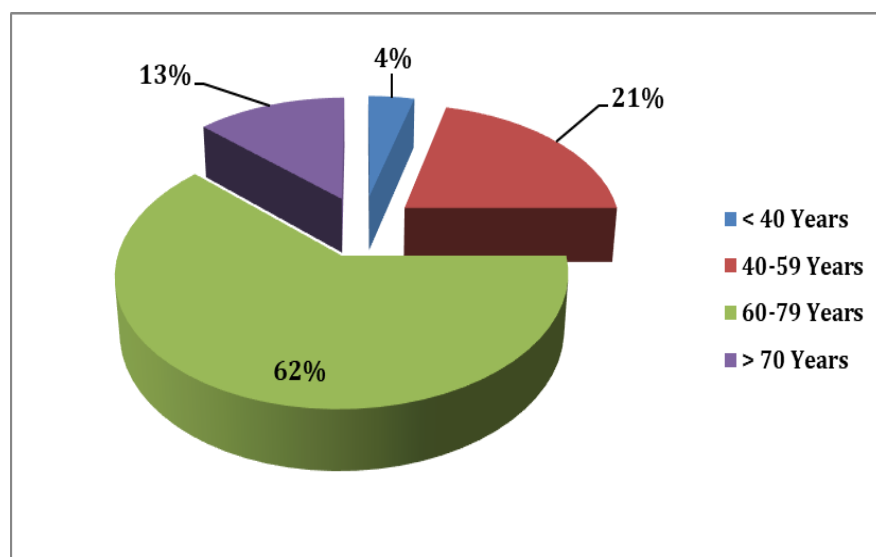


Figure 2: Distribution of Patients Samples According to Age Groups

Table 1: Pearson Correlation between All Parameters in the Study

Pearson Correlation Between All Parameters in The Study											
	Parameter	Ferritin	Dimer	LDH	CRP	RBS	WBC	RBC	HGB	PLT	Gene Load
Ferritin	Pearson Correlation	1	0.763**	0.176	0.389**	0.157	0.349**	0.446**	-0.251-*	-0.073-	0.619**
	Sig. (2-tailed)		0.000	0.080	0.000	0.120	0.000	0.000	0.012	0.469	0.000
Dimer	Pearson Correlation		1	0.334**	0.738**	0.263**	0.399**	0.638**	-0.256-*	-0.304-*	0.648**
	Sig. (2-tailed)			0.001	0.000	0.008	0.000	0.000	0.010	0.002	0.000
LDH	Pearson Correlation			1	0.382**	0.491**	0.264**	0.211*	-0.038-	-0.619-*	0.251
	Sig. (2-tailed)				0.000	0.000	0.008	0.035	0.708	0.000	0.129
CRP	Pearson Correlation				1	0.446**	0.430**	0.671**	-0.118-	-0.454-*	0.669**
	Sig. (2-tailed)					0.000	0.000	0.000	0.243	0.000	0.000
RBS	Pearson Correlation					1	0.266**	0.309**	-0.142-	-0.357-*	0.573**
	Sig. (2-tailed)						0.007	0.002	0.159	0.000	0.000
WBC	Pearson Correlation						1	0.358**	0.130	-0.216-*	0.701**
	Sig. (2-tailed)							0.000	0.196	0.031	0.000
RBC	Pearson Correlation							1	0.131	-0.233-*	0.476**
	Sig. (2-tailed)								0.195	0.019	0.003
HGB	Pearson Correlation								1	-0.006-	-0.320-
	Sig. (2-tailed)									0.954	0.050
PLT	Pearson Correlation									1	-0.387-*
	Sig. (2-tailed)										0.016

Correlation is Significant at the 0.01 level (2-tailed).*

Correlation is Significant at the 0.05 level (2-tailed).*

Discussion

Our study showed that ferritin levels are elevated for COVID-19 patients and increase with COVID-19 severity ($p < 0.001$). We found that ferritin levels are not affected by the patient's gender and age. In the results of the D-dimer test, we found that it was elevated in patients with critical and severe COVID-19 ($p < 0.001$), and that gender and age no effect. The LDH levels of COVID-19 patients in critical and severe cases increased ($p < 0.001$), with an increase in males compared to women (0.064), and a rise in the age group >70 years (0.026). RBS levels of covid-19 patients in critical and severe cases increased (< 0.001), in the age groups >70 years, we found no effect of gender. Increasing CRP levels for COVID-19 patients in critical and severe cases ($p < 0.001$), and we found that gender and age do not significantly affect. The levels of WBC increased in critical and severe cases of COVID-19 patients, especially in the age groups 40-59 years, ($p < 0.001$), we didn't find any effect of gender. In the results of our study, we found that RBC levels were higher in severe cases compared to the control group ($p < 0.001$), and we did not find an effect of gender, and age. The results of our study showed a lower hemoglobin level in severe cases compared to the control group (< 0.001). Age had an effect on hemoglobin levels in age groups (< 40 years, 40-59 years) compared with >70 (0.016). We did not find any effect on gender. The results of our study showed a decrease in the platelet level in critical cases of COVID-19 patients ($p < 0.001$), and high platelet levels especially in females (0.025), and we did not find any effect on age groups. The results of our study showed high NRF2 gene expression levels in severe state COVID-19 patients (0.002), and we found no effect between NRF2 gene expression rate and gender or age groups. Our study's findings revealed a moderately positive association between ferritin levels and NRF2 gene expression levels (0.619). While the results of the relationship between the levels of D-dimer and platelets were inversely weak.

Conclusion

We found a change in biochemical and immunological parameters of Covid-19 patients, some influenced by gender and age. The severity of the disease increases in the elderly with chronic disease. NRF2 gene expression levels were high in covid-19 patients in severe cases. NRF2 gene expression levels are affected by some parameters.

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Ethical approval

Ethical use of data consent was obtained from all the study sharers.

Conflicts of interest: No conflicts of interest to report.

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