The effect of coronavirus on cytochrome c oxidase (complex IV) and correlation with Severe COVID-19 Infection

Dr. Raid J. M. Al-Timimi [1] Zainab NoamanEyada[2] Dr. Haitham AL-Kubaisy[3] [4] Dr. Yasir .M. Abdulateef[4]

1 *Assistant Professor of Medical Biochemistry.* Al-Nahrain University /College of Medicine / Department of Chemistry and Biochemistry

2 *Doctorate student of Medical Chemistry.* Al-Nahrain University /College of Medicine / Department of Chemistry and Biochemistry.

Zainab611992@gmail.com

3Professor of Internal Medicine and Infectious illnesses, University of Anbar /College of Medicine / Department of Medicine.

4Assistant Professor of Microbiology and Immunity. University of Anbar /College of Medicine / Department of Microbiology.

*Corresponding author : Raid.J.M Al-Timimi

Email : rjtimimi68@yahoo.com

Abstract

Objective: Iraq's COVID-19 has very high morbidity and fatality rates. among the leading causes of death was mitochondrial cell damage.

Aim: to study the effect of cell mitochondrial damage through cytochrome c oxidase on COVID-19 patient morbidity and mortality.

Methods: This study was carried out at Al-Nahrain University/College of Medical in Bangkok, Iraq. The study included 100 individuals with COVID-19 immunoglobulins IgG, IgM, and cytochrome c oxidase enzyme and acute inflammatory biomarkers, procalcitonin (PCT), and Interleukin-6. Clinical analysis results were used in statistical analyses..

Results: The cytochrome c oxidase enzyme increased vertically as COVID-19 patient severity increased, and there was a significant correlation between the enzyme and acute inflammatory indicators. Cytochrome c oxidase enzyme and COVID-19 immunoglobulins are related.

Keywords: cytochrome c oxidase, COVID-19, deterioration , Interlukin-6,procalcetonin severity,

Introduction

Covid-19 is a disease that is spread by the SARS-CoV-2 virus. An RNA virus causes it. Most people who get the virus will have different respiratory problems. The virus can spread through a person's air droplets when they cough, sneeze or talk. [(1)]. Effect of COVID-19 on mitochondria are the cytoplasmic ground substance we find. Aside from the structures, we already know those granules, which Benda (1902) called threadlike granules or mitochondria because they tend to form threads. [(2).] When the virus gets into the cell, it hurts the cell. It happens when different biochemical processes work on different parts of a cell. This led to the loss of ATP and damage to the mitochondria, It resulted in the failure of oxidative phosphorylation, the development of a high-conductance channel in the mitochondrial membrane, and the progressive loss of ATP [(3)] The mitochondria of eukaryotes, bacteria, and archaea include a huge transmembrane protein complex called complex IV, also referred to as cytochrome c oxidase [(4)]. The last enzyme in a cell's respiratory chain is found in the electron transport chain. It is found in the membrane. It moves one electron from each cytochrome c molecule, four protons, and one oxygen molecule. Water is now made up of two

molecules. It binds four protons from the inner aqueous phase and transports four additional protons across the membrane. Proton electrochemical potential transmembrane variation, which is used by ATP synthase to create ATP, is increased as a result.

It moves four protons, one oxygen molecule, and one electron from each of the four cytochrome c molecules. Water is now made up of two molecules. It transfers four more protons across the membrane in addition to the four bound protons from the inner aqueous phase. As a consequence, there is an increase in the transmembrane variation of proton electrochemical potential, which is utilised by ATP synthase to produce ATP [3,4]. Effect covid in biological markers: acute inflammatory markers like C-reactive protein (CRP), Ferritin, and LDH[5]. These results point to high levels of the cytochrome c oxidase enzyme in mitochondria that cause damage to the mitochondria and imply a potentially significant role for a cytokine storm in the pathogenesis of COVID-19. D-dimer indicates distal tissue necrosis to the affected artery[6], the subsequent bacterial infection is indicated by procalcitonin (PCT) [7]. Interleukin-6 (IL6) exhibits an overreactive immune response and COVID-19 infection [8]. Potential ,IgG appears at the end of the second week, while COVID-19 immunoglobulin, which appears near the end of the first, exhibits antibodies against covid-19 acute immunoglobulin. [9].

Patients Materials and Methods:

Study design:

This case-control research was carried out by the Department of Chemistry and Biochemistry at Al-Nahrain University's School of Medicine. The Ethics Committee approved the study protocol for Al-Nahrain University's Faculty of Medicine. Dar Al Salam Field Hospital 1 and Lagash Land Laboratory obtained data on 100 Iraqi patients with varying COVID-19 severity using PCR. All patients gave consent, were admitted to the hospital, and had their blood collected prior to taking any medicine. The research was carried out between September 2021 and April 2022. All participants are above the age of 18 and under 90.

The control group consists of (100 samples) of sex- and age-matched volunteers who seem to be in good health and who are receiving the Covid 19 vaccine from the main healthcare facility. (AL-Kadhmiyae, AL-Zahra Clinic)

Case group: Consists of 100 samples with COVID-19 infection that has been positively identified by PCR on a nasopharyngeal swab. According to Iraqi guidelines and duration of disease. The severity of the disease is divided into three groups:

1. stage 1 : n=40 COVID-19 patients with durations ranging from 1 to 6 days.
2. stage 2: n=37 individuals with COVID-19 with durations ranging from 7 to 14 days.
3. stage 3: n=23 COVID-19 patients with a period of up to 14 days

Exclusion criteria:

Patients with COVID-19 who were previously ill with cancer, liver damage, or kidney failure.

Blood sample collection and preservation:

A total of around 5ml of venous blood was donated by each participant. The serum was isolated from the blood by centrifuging it for 10 minutes at 3000 rpm after allowing the blood to clot for 15 minutes at room temperature. measurement of serum level The human cytochrome c oxidase kit was run using the Elisa Human Reader, which was purchased from the Elabscience Corporation, USA. Other tests that were performed were those for C-reactive protein (CRP), ferritin, total lactate dehydrogenase (LDH), D-dimer, IL6, PCT, and COVID-19 IgG and IgM.

Statistical Analysis:

SPSS software version 25.0 was used to carry out all statistical calculations (SPSS, Chicago). Continuous data was subjected to a normality test (Shapiro-Wilk test). After either an analysis of variance (ANOVA) or a Student t-test (between two groups) was done to compare normally distributed data, the least significant difference (LSD) was used to compare the two groups (between more than two groups). For data that wasn't evenly spread out, the Mann-Whitney U test (between two groups) or the Kruskal-Wallis test were used (between more than two groups). The Chi-square test was used to look at categorical variables that were shown as numbers and percentages. If the p-value was less than 0.05, there was a statistically significant difference between the two groups.

RESULTS :

The level of all cytochrome c oxidase markers is shown in Table (1)

Variable	Healthy (n=100)	COVID-19			p-value
		stage1 (n=40)	stage2 (n= 37)	stage 3 (n=23)	
Cytochrome C, ng/mL					<0.001
Mean±SD	1.75±0.28a	2.24±0.8b	3.37±1.5c	5.42±0.76c	
Median	1.77	2.0	2.41	5.37	
Range	1.32-2.28	1.67-5.71	2.01-6.05	4.00-6.45	

Table (2) and figure (1)show the correlation of Age & cytochrome C oxidase with age and covid-19 duration

Variables		Age	Covid-19 duration
Cytochrome C	r	0.122	0.632
	p	0.225	<0.001
Age	r		0.255
	p		0.010

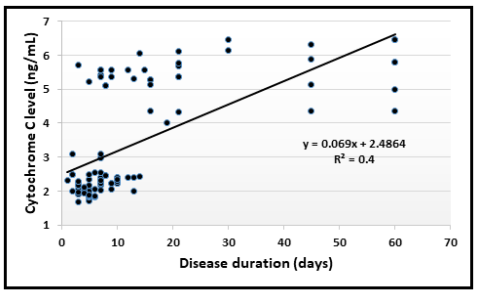
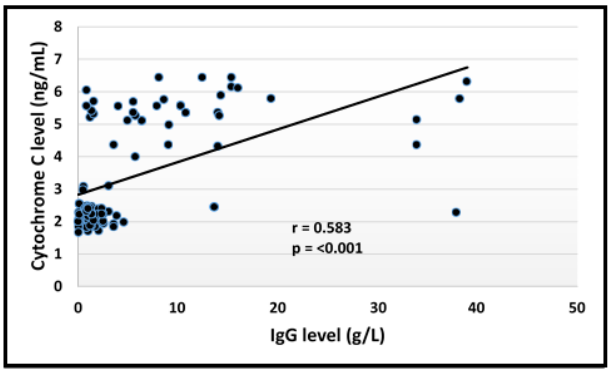


Table (3) denotes that cytochrome C level and IgG and IgM levels

Variables		IgG	IgM
Cytochrome C	r	0.583	0.404
	p	<0.001	<0.001
IgG	r		0.538
	p		<0.001

Figure (2) corelation of cytochrome-C-oxidase and IgG

Figure (3) corelation of cytochrome-C-oxidase



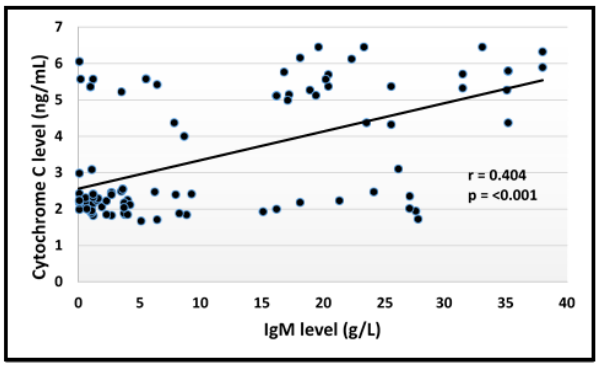


Table (4) Correlation of cytochrome-c oxidase with inflammatory markers

		Interlukin-6	Procalcetonin	lactatdehydrogenase	c-reactive protein	D-dimer	Ferritin
Cytochrome C	r	0.712	-0.122	0.592	0.556	0.428	0.553
	p	<0.001	0.225	<0.001	<0.001	<0.001	<0.001

As demonstrated in Table 3-7 and Figures 4 and 5, cytochrome C levels have a positive connection with Il-6 levels (r = 0.712; p = 0.001), LDH levels (r = 0.592; p = 0.001), CRP levels (r = 0.566; p = 0.001), D-dimer levels (r = 0.428; p = 0.001), and ferritin levels (r = 0.553; p = 0.001).

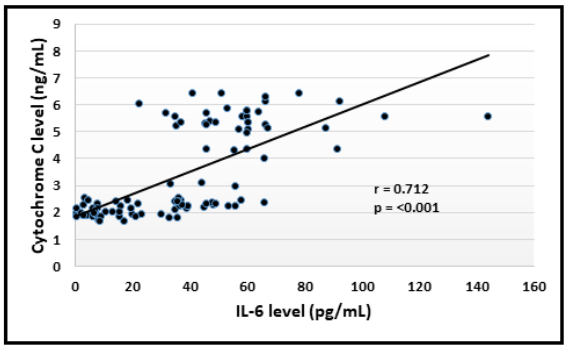


Figure (4) corelation of cytochrome-C-oxidase and IL6

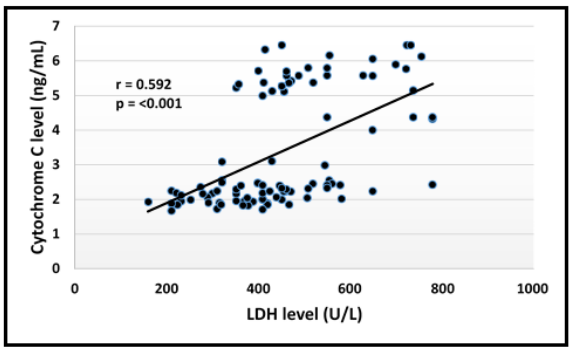


Figure (5) corelation of cytochrome-C-oxidase and LDH

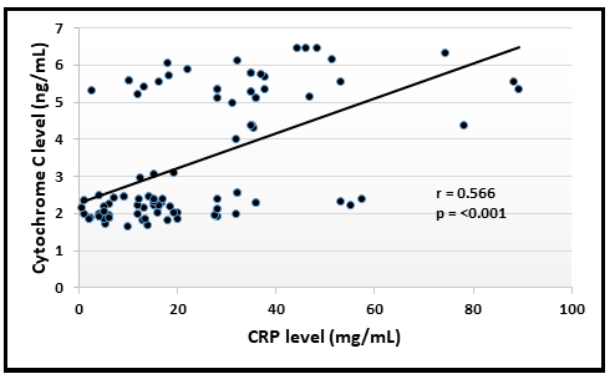


Figure (6) corelation of cytochrome-C-oxidase and CRP

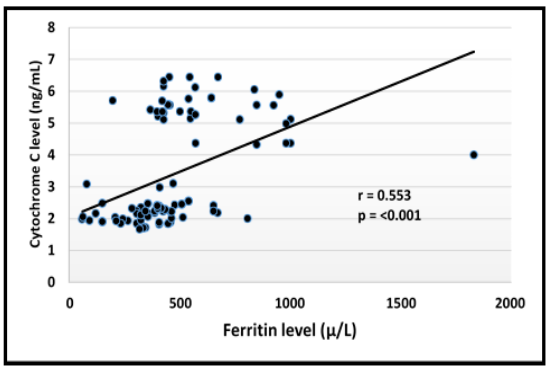


Figure (7) corelation of cytochrome-C-oxidase and Fe

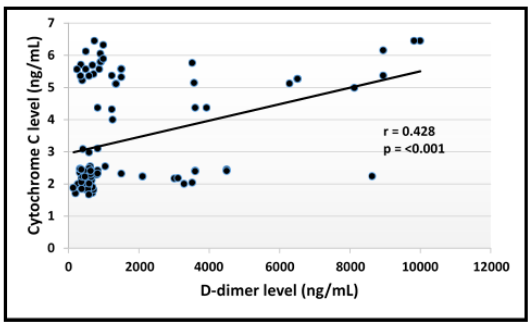


Figure (8) corelation of cytochrome-C-oxidase and D-Dimer

Discussions

This paper was done to study the effect of cell mitochondrial damage through cytochrome c oxidase on COVID-19 patient morbidity and mortality. Table 1 shows cytochrome c oxidase is highly significant in control groups compared to three groups of covid-19 patients. Cytochrome c oxidase is the enzyme that has a good marker for mitochondrial damage; it is elevated with the severity of COVID-19, which means the virus causes damage to mitochondrial and causes depletion of ATP that needs the body to perform many different functions and explains why the disease severity of the patients.

Table 2 & figure 1 show increases with duration despite the virus life span ending due to mitochondrial damage in the tissues like lungs..etc., where the virus causes mitochondrial damage, which leads to tissue damage and then continuity of tissue inflammation and disease prolonged. Another study shows that cytochrome c oxidase is associated with the severity of pulmonary arterial hypertension [15,16] due to pulmonary tissue mitochondrial damage, hypoxia, and pulmonary hypertension. Table 3 Figures 2 and 3 demonstrate a strong positive association between cytochrome C level and IgG, IgM (r =0.583, p = 0.001), respectively, with IGM beginning to grow at the end of the first week and dramatically increasing by the third week, indicating a favorable immunological response. Also, the level of these two immunological markers was greater in group 3 patients than in groups 2 and 1 patients; if the patient did not curr in the third week, the severity of the illness worsened. Early findings suggested that IgM antibodies against SARS-Cov-2 could appear before IgG antibodies, and that detecting both IgM and IgG might assist clinicians establish whether a person has SARS-Cov-2. As demonstrated in table 4 and pictures, cytochrome c oxidase is associated with inflammatory indicators (IL6, PCT, LDH, CRP, D-Dimer, ferritin). (4), (5), (6), (7) and (8) this prove of our explanation up.

In Table 4 and Figures 3. Moreover, cytochrome C oxidase had a positive connection with IL-6 level (r = 0.712; p = 0.001), As an inflammatory biomarker, serum may be used to detect interleukin-6 and other pro-inflammatory cytokines. It may be used to treat COVID-19 in a number of ways, including risk assessment, illness tracking, prognosis determination, drug selection, and predicting how a patient will react to therapy. [13] As a result, IL6 levels are higher in patients with stage 2 severe infectious illness. This indicates that the link with Apoptosis is important. In table 4 and figure 5, I discovered that patients with COVID-19 who had high blood LDH levels were an excellent indication of the severity and mortality rate of infectious illnesses (r = 0.592; p = 0.001). [14] so it is highly significant with Cytochrome c oxidase. In table 4 and figure 6, CRP level (r = 0.566; p = <0.001) with cytochrome c oxidase For those infected by SARS-CoV-2, Multiple studies show that plasma C-reactive protein (CRP) levels are related to how nasty a dengue infection is, and people with higher plasma CRP levels at the first stage of dengue are more likely to get Apoptosis of the cell, which is a type of cell death. [15] . Table 4 and Figure 8 show that there is a link between the level of D-dimers and cytochrome c oxidase (r=0.428; p= 0.001). Checking a person's D-dimer level is one way to find

out if they have thrombosis. Researchers have found that the early stages of COVID-19 illness are linked to high levels of D-dimer and fibrinogen. A bad prognosis is linked to a three-to fourfold rise in D-dimer levels. [16] . Figure 7 shows the levels of ferritin ($r = 0.553$; $p = 0.001$) because Ferritin is a key part of immune dysregulation, especially in people with very high levels of Ferritin. It does this by directly shutting down the immune system and making inflammation worse, which can lead to cytokine storms. [17]

Aknowlefgement: Greet thanks to the Al-Nahrain University's Department of Chemistry and Biochemistry in the Faculty of Medicine for their aid and support.

Authors' declaration:

There are no conflicts of interest. We affirm that all of the figures and tables in the text are ours. Moreover, the figures and photographs, which are not mine or ours, have been granted permission for re-publication and are connected to the book.

- The authors sign off on the acceptability of ethical considerations.

- Ethical Clearance: The project was authorized by the University of al-local Nahrain's ethical council.

Authors' contributions statement: This work was carried out in collaboration between all authors. A A diagnosis the cases then

Recommendations: Further research in this subject is needed to predict patient severity pathways and how to avoid and treat this problem.

Fund: No fund regarding this paper.

Reference:

1- Vuorinen, V., Aarnio, M., Alava, M., Alopaeus, V., Atanasova, N., Auvinen, M., ... &Österberg, M. (2020). Modeling aerosol transport and virus exposure with numerical simulations in relation to SARS-CoV-2 transmission by inhalation indoors. Safety Science, 130, 104866.

2- Kluge, M. A., Fetterman, J. L., & Vita, J. A. (2013). Mitochondria and endothelial function. Circulation Research, 112(8), 1171-1188.

3- Ferrier, D. R. (2014). Biochemistry. Lippincott Williams & Wilkins

4- Miranda-Astudillo, H., Colina-Tenorio, L., Jiménez-Suárez, A., Vázquez-Acevedo, M., Salin, B., Giraud, M. F., ... & González-Halphen, D. (2018). Oxidative phosphorylation supercomplexes and respiratory reconstitution of the colorless alga Polytomella sp. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1859(6), 434-444.

5- Hasty, F., García, G., Dávila, H., Wittels, S. H., Hendricks, S., & Chong, S. (2021). Heart rate variability is a possible predictive marker for the acute inflammatory response in COVID-19 patients. Military Medicine, 186(1-2), e34-e38.

6- Wichmann, D., Sperhake, J. P., Lütgehetmann, M., Steurer, S., Edler, C., Heinemann, A., ... & Kluge, S. (2020). Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Annals of internal medicine, 173(4), 268-277.

7- Kooistra, E. J., van Berkel, M., van Kempen, N. F., van Latum, C. R., Bruse, N., Frenzel, T., ... &Pickkers, P. (2021). Dexamethasone and tocilizumab treatment considerably reduces the value

of C-reactive protein and procalcitonin to detect secondary bacterial infections in COVID-19 patients. Critical Care, 25(1), 1-12.

8- Chen, L. Y., Biggs, C. M., Jamal, S., Stukas, S., Wellington, C. L., &Sekhon, M. S. (2021). Soluble interleukin-6 receptor in the COVID-19 cytokine storm syndrome. Cell Reports Medicine, 2(5), 100269.

9- Ma, H., Zeng, W., He, H., Zhao, D., Jiang, D., Zhou, P., ... &Jin, T. (2020). Serum IgA, IgM, and IgG responses in COVID-19. Cellular & molecular immunology, 17(7), 773-775.

10- Mahir Ali Jasim1, Hazim Ghazzay, Haitham Noaman, Mothana Khalil, Samir Johna (2021). The outcome of telemedicine services for COVID-19 patients in "Al-Anbar" province west of Iraq. Journal of Emergency Medicine, Trauma and Acute Care. 2021(3): 2-4.

11- Van Elslande, J., Houben, E., Depypere, M., Brackenier, A., Desmet, S., André, E., ... & Vermeersch, P. (2020). Diagnostic performance of seven rapid IgG/IgM antibody tests and the Euroimmun IgA/IgG ELISA in COVID-19 patients. Clinical Microbiology and Infection, 26(8),1082-1087

12- Abdulsalam Al-Ani, Hazim Ismael Ghazzay, Ameel F. Al Shawi, Haitham Noaman Eyada Al-koubaisy, Maadh Aldouri , Fatimah Al-Ani (2022). Association of chronic diseases with mortality among hospitalized patients with COVID-19 treated with convalescent plasma: Evidence from a single center – Iraq. JEMTAC: 2022(2); 1-7.

13- Castelnovo, L., Tamburello, A., Lurati, A., Zaccara, E., Marrazza, M. G., Olivetti, M., ... & Mazzone, A. (2021). Anti-IL6 treatment of serious COVID-19 disease: A monocentric retrospective experience. Medicine, 100(1).

14- Wu, M. Y., Yao, L., Wang, Y., Zhu, X. Y., Wang, X. F., Tang, P. J., & Chen, C. (2020). Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel coronavirus (COVID-19) pneumonia. Respiratory Research, 21(1), 1-6.

15- Chen, W., Zheng, K. I., Liu, S., Yan, Z., Xu, C., & Qiao, Z. (2020). Plasma CRP level is positively associated with the severity of COVID-19. Annals of clinical microbiology and antimicrobials, 19(1), 1-7.

16- Rostami, M., & Mansouritorghabeh, H. (2020). D-dimer level in COVID-19 infection: a systematic review. Expert review of hematology, 13(11), 1265-1275.

17- Vargas-Vargas, M., & Cortés-Rojo, C. (2020). Ferritin levels and COVID-19. Revista Panamericana de Salud Pública, 44, e72.