The Effect of Caspase-8 as an Apoptotic Marker in Relation to COVID-19 Patients Severity

Zainab Noaman Eyada[1]*, Raid J. M. Al-Timimi[2], Haitham AL-Kubaisy[3], Yasir M. Abdulateef[4]

[1] Doctorate student of Medical Chemistry. Al-Nahrain University / College of Medicine / Department of Chemistry and Biochemistry/Iraq
E-mail: zainab611992@gmail.com

[2] Assistant Professor of Medical Biochemistry. Al-Nahrain University /College of Medicine / Department of Chemistry and Biochemistry/Iraq

[3] Professor of Internal Medicine and Infectious diseases, University of Anbar /College of Medicine / Department of Medicine/Iraq

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

Abstract

Objective: In Iraq, the COVID-19 pandemic was catastrophic, leading to mortality and morbidity. One of the leading causes of mortality was cell apoptosis that leads to organ failure. Aim: To diagnose the cell apoptosis of COVID-19 patients at an early stage so we can predict earlier organ failure. Methods: The research was conducted in the medicinal chemistry department of Al-Nahrain University/College of Medicine in Baghdad, Iraq. One hundred patients of different stages proved COVID-19 and compared with 100 persons in the control group, measure caspase-8 enzyme and acute inflammatory markers, procalcitonin (PCT), and IL-6 with COVID-19 immunoglobulins IgG, IgM in the blood of two groups. We're done—statical studies with clinical analysis results. Results: The marker of cell apoptosis was caspase-8 enzymes, which showed vertical elevation with the increase in severity of COVID-19 patients. There was a strong relationship between the caspase-8 enzyme and acute inflammatory markers. Moreover, there is a relationship between the caspase-8 enzyme and COVID-19 immunoglobulins. Conclusion: The caspase-8 enzyme is a strong marker of severity in COVID-19 patients and indicates a life-threatening condition.

Keywords: caspase-8, COVID-19, severity, deterioration

1. Introduction

The effect of COVID-19 on the cell is not well-apparent. The pathophysiology of the disease was proposed as a dysregulation of the immune response, which is hard to be accepted as a general scheme of the disease in all patients (1). The need to explain why the immune response was so abnormal brought attention to understanding the effect of the virus on a cellular level. All viral effects on the cell could range from Cell lysis, cell fusion, neoplasia or Apoptosis (2).

When the virus enters the cell, it causes cell defects. Various biochemical processes damage the numerous cellular components, resulting in oxidative phosphorylation failure, mitochondrial damage, the creation of high conductance channels in the mitochondrial membrane and gradual ATP depletion(3). Therefore, it leads to the release of cytochrome oxidase and other proteins and causes apoptosis.

Apoptosis is a process that is important for a variety of biological processes, including immunological function, normal cell turnover, embryonic development, metamorphosis and hormone-dependent atrophy. (4).

Among the cysteine proteases that are involved in the processing of cytokines and apoptosis is Caspase-8, which like all other caspases is created as a single polypeptide chain zymogen procaspase that is inactive. Caspase-8 is then recruited into a multimeric complex where it either activates on its own or is triggered by other caspases through trans-cleavage (5). Caspase-8 is then recruited when ligand interaction induces the trimerization of death receptors, which leads to the recruitment of the Fas-associated death domain (FADD). Active caspase-8 can release cytochrome c from mitochondria by either directly cleaving and activating downstream caspases or by cleaving the BH3 Bcl2-interacting protein.(6)

COVID-19 showed acute inflammatory markers, including C-reactive protein, Fe and lactate dehydrogenase(7). These data imply that a cytokine storm may play a significant role in the pathogenesis of COVID-19 and refer to the elevated caspase-8 enzyme in inter-cells that lead to its damage. D-dimer indicates distal tissue necrosis to the affected artery,(8) while procalcitonin (PCT) indicates secondary bacterial infection(9). Interleukin-6 6 (IL6) indicates hyperimmune reaction, a genet COVID-19 infection(10). Finally, COVID-19 immunoglobulin indicates antibodies against COVID-19 acute immunoglobulin, IgM, which forms at the end of the first week, while IgG forms at the end of the second

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week(11).

2. Materials and Methods

This study is a case-control research done at Al-Nahrain University's College of Medicine, which has a department of chemistry and biochemistry. The research proposal was authorized by the Al-Nahrain University College of Medicine's Ethical Committee. One hundred Iraqi patients with variable severity of COVID-19 were documented by PCR and collected in Lagash land laboratory and Dar Al Salam Field Hospital. All the patients had blood taken after their agreement, after being hospitalized and before receiving any medication. The study was done during the period from September 2021 to April 2022. All participants are > 18 and < 90 years of age.
Control group: Includes 100 samples of apparently healthy-aged and sex-matched volunteers coming for COVID-19 vaccinations collected from the primary health care centre (AL-Zahraa clinic, AL-kadhmiyae)
Case group: Includes 100 samples with confirmed COVID-19 infection, diagnosed by the PCR of the nasopharyngeal swab.
According to Iraqi guidelines and based on the duration of the disease, the severity of the disease is divided into three groups:

1. Sub Group I: n=40 in duration from 1 to 6 days for COVID-19 patients
2. Sub Group Ⅱ: n=37 in duration from 7 to 14 days for COVID-19 patients
3. Sub Group III: n=23 in duration up to 14 days for COVID-19 patients
4. Exclusion criteria:

COVID-19 patients having other diseases before contracting the virus: liver disease, renal failure and cancer.

Blood sample collection and storage

About 5 ml of sample blood was obtained from the participants. The blood was clotting at room temperature for 15 minutes; serum was isolated after clothing by centrifugation at 3000 rpm for 10 minutes. The serum Human caspase-8 kit was measured using Elisa Human Reader, purchased from Elabscience Company, USA. Other tests include C reactive protein (CRP, interleukin-6, total lactate dehydrogenase (LDH), procalcitonin, Ferritin, COVID-19 IgG and IgM tests.

Statistical Analysis: SPSS software version 25.0 was used to conduct statistical analysis (SPSS, Chicago). A normality test was conducted on continuously collected data (Shapiro-Wilk test). The data were shown as having a normal distribution and were analyzed using either a Student test (between two groups) or Analysis of Variance (ANOVA), followed by Least Significant Difference (LSD) (between more than two groups). The Mann-Whitney U test (between two groups) or the Kruskal-Wallis test were used to look at data that was not spread out in a normal way (between more than two groups). Moreover, the Chi-square test was used to look at the numbers and percentages that were used to describe categorical variables.
A statistically significant difference was to be shown by a p-value of less than 0.05.

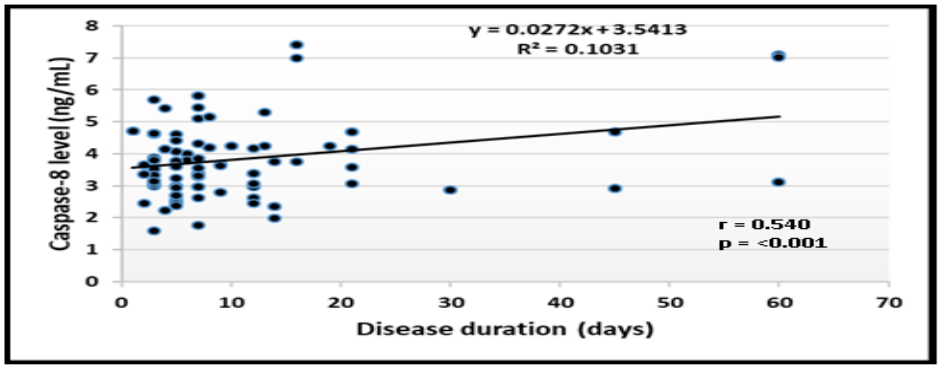
3. Results

The level of all caspase-8 markers is shown in Table 3-1.

Variable	Controls (n=100)	COVID-19 patients			p-value
		Group 1 (n=40)	Group 2 (n= 37)	Group 3 (n=23)	
Caspase 8, ng/mL Mean±SD Median Range	1.97±0.56a 1.77 1.23-3.05	3.08±0.74b 2.98 1.6-4.7	4.28±0.81c 4.2 2.96-5.81	5.76±1.79c 7.0 3.06-7.87	<0.001

Table 3-2 and figure 3-1 show the correlation of caspase-8 with age and disease duration within patient groups.

Variables		Age	Disease duration
Caspase 8	r	0.007	0.540
	p	0.945	<0.001
Age	r		0.255
	p		0.010



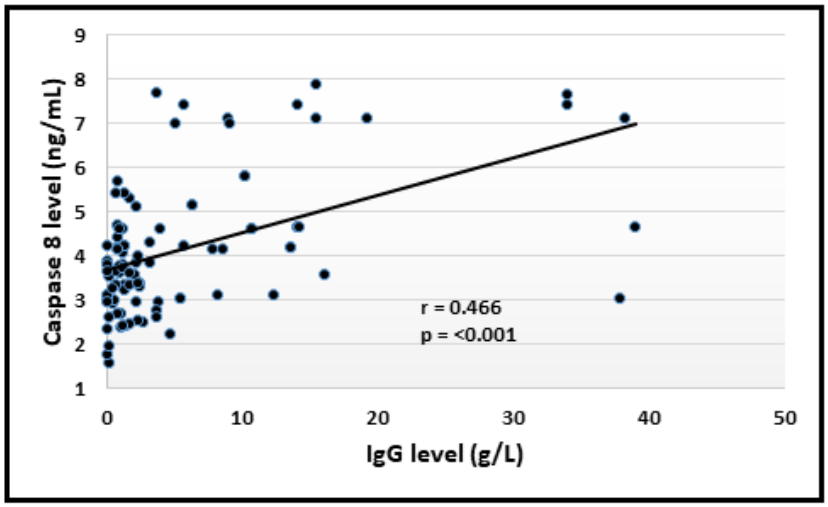
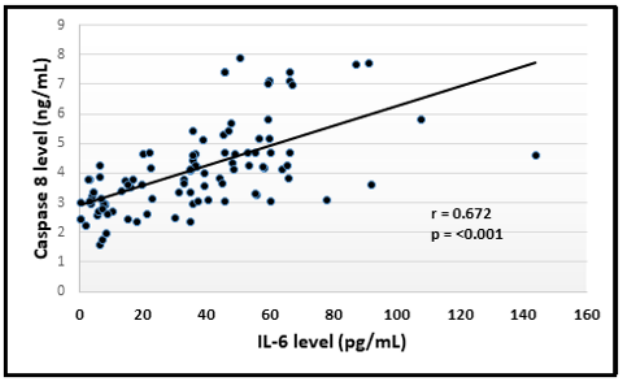


Table 3-3 and Figure 3-2 denote that caspase-8 level and IgG and IgM levels			
Variables		IgG	IgM
Caspase 8	*r*	0.466	0.184
	p	<0.001	0.067
IgG	*r*		0.538
	p		<0.001

Table 3-4. The correlation of apoptotic markers with inflammatory markers.							
		IL-6	PCT	LDH	CRP	D-dimer	Ferritin
Caspase 8	r	0.672	-0.100	0.516	0.470	0.561	0.526
	p	<0.001	0.320	<0.001	<0.001	<0.001	<0.001



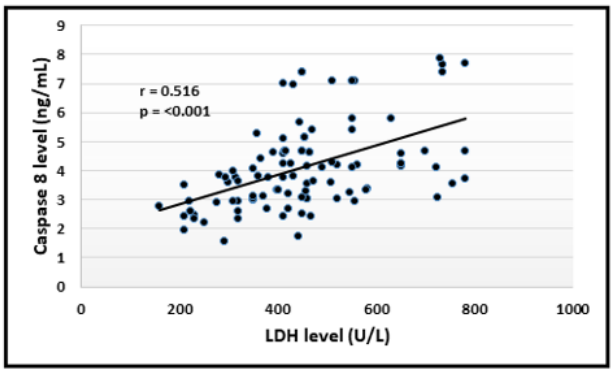
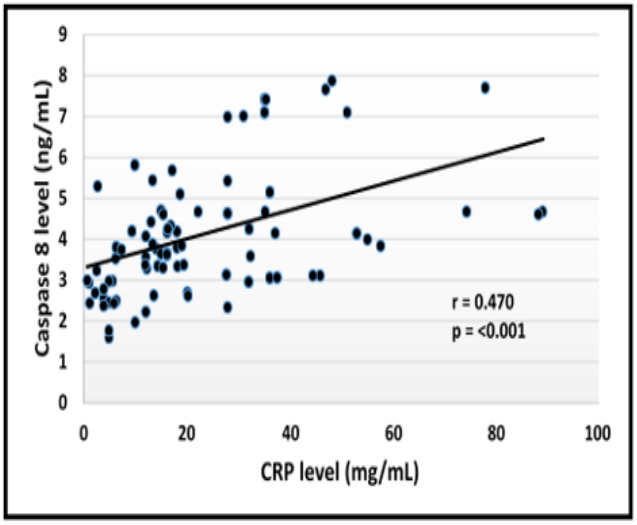


Figure 3-3 Correlation between IL6 & Caspase-8 Figure 3-4 Correlation between LDH & Caspase-8



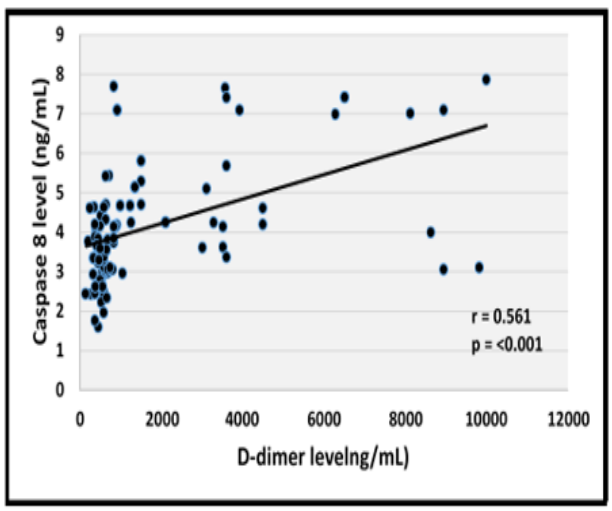


Figure 3-5 Correlation between CRP & Caspase-8 Figure 3-6 Correlation between D-Dimer & Caspase-8

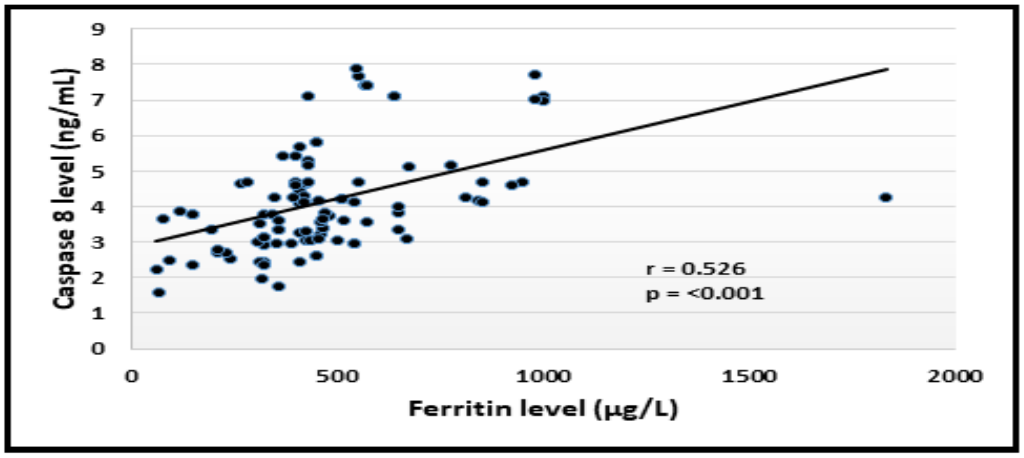


Figure 3-7 Correlation between Ferritin & Caspase-8

4. Discussions

The level of all apoptotic markers was significantly high (p<0.001) in the patients compared to that of the control. It is worth noting that the level of apoptotic markers was significantly higher in group 2 than in group 1, as shown in Table 3-1. Signal molecules tell the cell it's time to "commit suicide" and start the apoptosis process. So, it means that the patients in duration groups 2 and 3 in duration from the 7th day to the 14th show elevated caspase-8 in the patients and consciously elevated this enzyme during the period of the disease. This type of cell death is called "programmed cell death" because it is caused by a chemical problem(12). Correlation between the stage of disease and apoptosis in Table 3-2 and Figure 3-1 shows a clear positive correlation between disease duration and caspase-8 levels (r = 0.540; p = 0.001), which means that when the duration of the disease increases, caspase-8 is also increased. Table 3-3 and Figure 3-2 denote that caspase-8 level was positively correlated with IgG level (r = 0.466; p = <0.001) as IgG begins to increase in the second week and reaches the peak in the third week. Similarly, the IgM increases at the end of the first week and significantly increases with 3rd week, which means a good immune response. Thus, the caspase-8 correlation with IgG is more significant than that with IgM(13). In Table 3-4 and Figures 3-3, caspase-8 was positively correlated with IL-6 level (r = 0.672; p = <0.001), Interleukin-6 and other pro-inflammatory cytokines that can be measured in the serum as inflammatory biomarkers. It may be used in different ways to treat COVID-19, such as to measure risk, track disease progression, figure out prognosis, choose therapy and predict how a patient will react to treatment(14,15). Moreover, IL6 is elevated in week 2 of severe infectious disease. This means its correlation with apoptosis is significant. With the correlation of caspase-8 with LDH level (r = 0.516; p = <0.001), it was discovered that high serum LDH levels were a standalone signal for determining the severity and mortality of infectious disease in COVID-19-positive individuals. (16) So, it is highly significant with caspase-8. CRP level (r = 0.470; p = <0.001) with Caspase-8 For those infected by SARS-CoV-2, According to multiple studies, plasma C-reactive protein (CRP) levels are positively correlated with severe dengue infection and individuals who have higher plasma CRP levels at the first stage of dengue are more likely to develop apoptosis of cell(17). D-dimer level and correlation with caspase-8 is (r = 0.561; p = <0.001), D-dimer level is one way that thrombosis can be found in a patient. The early stages of COVID-19 disease studies have also shown that the early stages of COVID-19 disease are characterized by an increase in D-dimer and fibrinogen levels; a 3–4-fold increase in D-dimer levels is associated with a bad prognosis(18) and Ferritin level (r = 0.526; p = <0.001) as indicated as Ferritin is a key factor in immune dysregulation, especially in cases of extreme hyperferritinemia. It does this by directly suppressing the immune system and making inflammation worse, which contributes to cytokine storms (19).

5. Conclusion

If caspase-8 is elevated in the second week of a COVID-19-positive patient, we expect that the disease of this patient will indicate a finding of apoptosis thereby reflecting ARDS.

6. Recommendations

Further study in this field could be done for expecting patient severity pathways and to find out ways to prevent and treat this complication.

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