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Research Article

Circulating CXCL8 levels in determining the severity of COVID-19 infection among patients aged 50 years and older

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Abstract: **Background:** COVID-19, resulting from the SARS-CoV-2 virus, manifests with a spectrum of severity, spanning from mild respiratory symptoms to severe pneumonia and multi-organ failure. CXCL8 (IL-8) is a chemokine that plays a role in the inflammatory response. It has been shown that levels of CXCL8 are increased in different infections and inflammatory diseases.Aim: This study aims to evaluate the role of circulating CXCL8 levels in determining the severity of COVID-19 infection among patients aged 50 years and older.**Method:** This cross-sectional study was conducted in Kirkuk city at Al-Shifaa Epidemiological Hospital, a COVID-19 epidemic center, from November 1, 2021, to June 30, 2022. A total of 138 patients aged 50 years or older, presenting with signs and symptoms of COVID-19, were included in the study. Additionally, 100 healthy individuals without any COVID-19 symptoms or chronic diseases were recruited as a control group. Nasopharyngeal swabs were collected from each participant and tested for SARS-CoV-2 using real-time PCR. Blood samples were obtained by venipuncture, centrifuged to isolate serum, and stored at -20°C for the determination of CXCL8, CXCL9, and CXCL10 levels using the ELISA technique.**Results:** The study found that 100 out of 138 (72.46%) suspected patients tested positive for SARS-CoV-2 by real-time PCR, compared to only 2% in the healthy control group (P-value = 0.0001). Among the 100 COVID-19 positive patients, 37% had severe infections, and 63% had moderate infections. The results indicated that CXCL8 levels were significantly elevated in patients with severe COVID-19 (39.63 ± 7.3 pg/ml) compared to those with moderate infection (12.31 ± 3.6 pg/ml) and the control group (4.16 ± 2.18 pg/ml) (P-value = 0.001). Moreover, CXCL8 levels increased with age, with the highest levels observed in patients over 69 years, suggesting a correlation between age and CXCL8 levels. A significant sex-based difference in CXCL8 levels was also observed, with males having higher levels than females in both moderate and severe cases (P-values = 0.017 and 0.043, respectively). This suggests that CXCL8 could serve as a potential biomarker for predicting disease severity

Keywords: SARS-CoV-2, CXCL8, Severity, Biomarker, Cytokine.

INTRODUCTION

As of November 21st, 2021, the global tally of confirmed COVID-19 infections is at over 257 million, with a death toll exceeding 5 million. SARS-CoV-2 is the causal agent of the illness. The development of vaccinations targeting SARS-CoV-2 represents a significant advancement in mitigating the impact of COVID-19. Nevertheless, the pandemic is still ongoing, and the continual transmission of the virus allows for the accumulation of changes in its genetic material. These alterations can provide benefits like as improved replication, evasion of the immune system, enhanced ability to spread, or even failure of diagnostic tests to identify the virus [1,2]. Given the rapid development of SARS-CoV-2 variations and the sluggish pace of worldwide immunization, it is imperative to have a comprehensive understanding of this new virus and the associated illness. Activation of the host's innate and adaptive immunity, including the stimulation of T cells, CD4 and CD8+T cells, and the generation of several proinflammatory cytokines, is necessary to regulate viral replication, prevent the spread of the virus, and eliminate infected cells. Nevertheless, the virus can cause tissue damage, leading to an excessive production of proinflammatory cytokines,

macrophages, granulocytes, and other immune cells[3-5]. Multiple clinical trials have suggested the potential of inhibiting cytokine synthesis or reducing their concentration in the bloodstream as viable approaches[2]. Nevertheless, the ongoing epidemic necessitates further randomized controlled research to comprehensively comprehend the etiology of the illness and explore potential therapeutics. Scientists have uncovered shared traits among very sick COVID-19 patients. The following items are included: There is a sudden worsening of symptoms between 7 to 14 days after the disease starts. The blood shows low levels of lymphocytes, increased levels of C reactive protein and pro-inflammatory cytokines such as IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, and IL-17A. Additionally, there are extremely high levels of inflammatory markers, hypercoagulation, and damage to multiple organs. According to clinical data, pneumonia is the most frequent consequence that occurs after SARS-CoV-2 infection. This complication is characterized by fast viral replication, which leads to the infiltration of numerous inflammatory cells and a cytokine storm. It ultimately results in acute lung damage followed by acute respiratory distress syndrome (ARDS) [8,9]. Researchers at several global centers have utilized genetic, biochemical, and radiological tests to assess the clinical features of patients and ascertain the trajectory of illness progression. Nevertheless, these examinations were conducted on a restricted cohort of individuals, and the published findings exhibit disparities among them. The role of IL-6 and IL-8 (CXCL 8), which belong to the cytokine and chemokine family, has been established in SARS and MERS. However, their importance in SARS-CoV-2 (COVID-19) infection is now under research (10,11,12). This study is conducted to evalute the role of circulating CXCL8 levels in the severity of COVID-19 infection

MATERİALS AND METHODS

This Cross-sectional study was carried out in Kirkuk city (Al-Shifaa Epidemiological Hospital /covid-19 epidemic center) from the period starting from 1st of November 2021 to the end of June 2022. The study included 138 patients suffered from signs and symptoms of Covid-19 with age ≥50 years, and from both sexes. The study also included 100 healthy person who haven't and symptoms of Covid and haven't any chronic diseases as a control group.

METHODS

Nasopharyngeal swabs collected from each person in the study, Swabs were inserted in tubes contained Viral Transport Medium (VTM). The VTM tubes then mixed, closed thoroughly, labeled, delivered to the laboratory within 1 hour of collection and stored in deep freeze until PCR extraction and amplification tests done. Three ml of blood were collected by vein puncture using syringe from each persons enrolled in this study. Blood samples were placed into sterile test tubes, centrifuged at 3000 rpm for 15 minutes, the obtained sera were aspirated using automatic micropipette and transferred to Eppendorf tubes and stored in deep freeze at -20°C for determination of CXCL8, CXCL9 and CXCL10 using ELISA technique.

RESULTS

The study. Show that. The study showed that 100 of 138 (72.46%) of suspected patients have positive PCR test for SARS COV-2 by real-time PCR compared with 2% of healthy control group (P. value 0.0001), Table 4.1. all those 100 patients were admitted to the Hospital for further management of the infection as 37% were with severe infection and 63% were with moderate infection, Figure 4.1.

Table 1: Prevalence of SARS COV-2 among suspected patients and control group

	Patients		Control	
SARS COV-2	No.	%	No.	%
PCR Positive	100	72.46	2	2
PCR Negative	38	27.54	98	98
Total	138	100	100	100

P. value: 0.0001

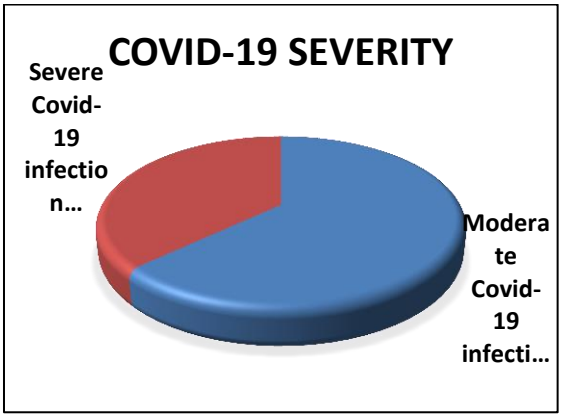

Figure 1: Covid-19 Severity

The study showed that majority of patients were above 60 year, as the risk of covid-19 infection increased with age as elderly was most affected (P:0.001). The study also found that males were more affected by the virus from females, Table 4.2 and 4.3

Table 2: Distribution of covid-19 patients according to age

Age (years)		COVID-19 patients (PCR+ve)		P. value
		Moderate	Severe	
50-59	No.	26	12	0.001
	%	41.27	32.43	
60-69	No.	20	1	
	%	31.75	2.7	
>69	No.	17	24	
	%	26.98	64.86	
Total		63	37	
		100	100	

Table 3: Distribution of covid-19 patients according to sex

Sex		COVID-19 patients (PCR+ve)		P. value
		Moderate	Severe	
Males	No.	34	24	0.28
	%	53.97	64.86	
Females	No.	29	13	
	%	46.03	35.14	
	%	100	100	

The study showed that the highest mean of CXCL8 level was found in patients with severe Covid-19 infection (39.63 pg/ml) and reduced significantly in patients with moderate infection (12.31 pg/ml) and the lowest mean was in the control group (4.16 pg/ml), the difference was highly significant at P. value: 0.001, Table 4

Table 4: Mean of CXCL8 among the studied groups

Studied Groups	CXCL8 (IL-8), Pg/ml		
	No.	Mean	SD
Moderate	63	12.31	3.6
Severe	37	39.63	7.3
Control group	100	4.16	2.18

P. value: 0.001

Table 5 presents the distribution of CXCL8 (IL-8) levels among different age groups for patients with moderate and severe COVID-19. For moderate COVID-19 patients, the mean CXCL8 levels progressively increase with age: patients aged 50-59 years have a mean CXCL8 level of 10.5 ± 3.2 pg/ml, those aged 60-69 years have a slightly higher level of 11.2 ± 3.4 pg/ml, and patients above 69 years show the highest mean level of 13.1 ± 3.7 pg/ml. This trend suggests a potential association between advancing age and increasing CXCL8 levels in moderately affected patients. For severe COVID-19 patients, a similar pattern is observed, with markedly higher CXCL8 levels in older age groups. Patients aged 50-59 years have a mean CXCL8 level of 35.4 ± 6.1 pg/ml, those aged 60-69 years have a level of 36.1 ± 5.8 pg/ml, and the >69 age group exhibits the highest mean level at 42.8 ± 7.9 pg/ml. The significant elevation of CXCL8 levels in severe cases compared to moderate cases across all age groups indicates that CXCL8 may serve as a biomarker for COVID-19 severity, especially in older patients.

Table 5: CXCL8 Levels by Age Group and COVID-19 Severity

Age Group (years)	Moderate COVID-19		Severe COVID-19	
	No.	CXCL8	No.	CXCL8
50-59	26	10.5 ± 3.2	12	35.4±6.1
60-69	20	11.2 ± 3.4	1	36.1±5.8
>69	17	13.1 ± 3.7	24	42.8±7.9
P-avlue	0.01		0.01	

Table 6 illustrates the levels of CXCL8 (IL-8) in male and female patients with moderate and severe COVID-19, alongside their respective p-values. In moderate COVID-19 cases, male patients (n = 34) show a higher mean CXCL8 level of 12.8 ± 3.7 pg/ml compared to female patients (n = 29), who have a mean level of 11.7 ± 3.5 pg/ml. The p-value of 0.017 indicates a statistically significant difference

between the sexes, suggesting that male patients may exhibit slightly higher CXCL8 levels even in moderate cases. For severe COVID-19 cases, the difference in CXCL8 levels between sexes becomes more pronounced. Male patients (n = 24) show a mean CXCL8 level of 40.2 ± 7.1 pg/ml, which is marginally higher than that of female patients (n = 13), who have a mean level of 38.5 ± 7.6 pg/ml. The p-value of 0.043 indicates that this difference is also statistically significant, reinforcing the trend that male patients tend to have higher CXCL8 levels than females in severe COVID-19. These findings suggest a potential sex-based difference in the inflammatory response to SARS-CoV-2 infection, as reflected by CXCL8 levels, which could have implications for understanding sex-specific pathophysiology and management of COVID-19.

Table 6: CXCL8 Levels by Sex and COVID-19 Severity

Sex	Moderate COVID-19		Severe COVID-19	
	No.	CXCL8	No.	CXCL8
Males	34	12.8 ± 3.7	24	40.2±7.1
Females	29	11.7 ± 3.5	13	38.5±7.6
P-avlue	0.017		0.043	

DİSCUSSİON

Based on the study's findings, a significant proportion of patients were aged 60 or above, which is logical considering that the likelihood of acquiring a COVID-19 infection increases with age, and older individuals are the most often impacted. Based on the data shown in Table 4.1, the study also revealed that the virus had a more significant effect on men compared to females. Consistent with our study findings, Najim et al. performed research in Kirkuk city and observed that the majority of patients were older, with men being the most often afflicted group [1]. The study done in Baghdad revealed that a significant majority of COVID-19 patients were males aged 50 years or older [2]. Kumar et al. conducted a study that showed most patients were over the age of 60. In addition, the risk of COVID-19 infection increases with age, and older adults are the more severely affected population [3]. Moreover, a research conducted on 138 patients with novel coronavirus (2019-nCoV)–infected pneumonia ncov found at Zhongnan Hospital of Wuhan University in China. Patients included in the study were aged 56 years (median [range, 42-68 years]) They were also found to be predominantly male (64.3%) [4]. In contrast, males comprised 70.3% of the total among those who died (37 patients). Those aged 50 and over are at higher risk of coronavirus which would appear in a more severe form than those under the age of 50. Health complications and comorbidities are more frequently found in the older age group, which is a likely explanation for this.[5] Additionally, a study published in The Lancet, which examined 191 COVID-19 patients in Wuhan, China during the early epidemic, indicated that men were more likely to experience severe symptoms than women (62% of patients). Additionally, the investigation identified a correlation between an increased likelihood of contracting COVID-19 and advanced age. In Wuhan, China, a study of 52 critically ill patients conducted in the intensive care unit (ICU) revealed that 67% of the patients were male, while 52% were geriatric individuals over the age of 60 [7]. In a recent study conducted at Jin Yintan Hospital in Wuhan, China, 41 patients were analyzed, and it was discovered that 73% of them were male. The investigation also disclosed that no children or adolescents contracted the illness, and the majority of patients were aged 50 to 64. The higher death rate found in this age group can be attributed to age-related comorbidities, which are the main contributing factor. However, clinicians should not necessarily apply age-related patterns observed in the general population to particular cases. Without this information, a patient's classification as high risk or low risk may not accurately reflect their real health state. This can lead to erroneous risk assessment, inadequate allocation of resources, and poor patient management [12]. The study's findings indicate that levels of CXCL8 were consistently increased after SARS-CoV-2 infection. Pius-Sadowska et al. observed that individuals with a severe infection in the COVID-19 model exhibited elevated levels of CXCL8 in their plasma. This discovery aligns with our own [13]. The apparent conflicts between molecular and protein-level processes can be elucidated to some extent. An assessment was conducted on patients who had contracted severe cases of COVID-19 in order to establish their initial cytokine levels. The cells involved in the generation of the chemokines that we studied were human macrophages, T lymphocytes, neutrophils, fibroblasts, osteoblasts, endothelial cells, and epithelial cells. Previously generated chemokines were released into the bloodstream at the occurrence of acute infection symptoms. The results of Abers et al. [14], who identified a similar connection to the one we found, seem to support this notion. Furthermore, our study's findings revealed a direct relationship between the severity of the disease and the levels of CXCL8 plasma. This link was also confirmed by our study's results. Substantially, increased levels of CXCL8 have previously been shown to be a powerful proinflammatory chemokine and critical neutrophil chemoattractant [15]. CXCL8 is a cytokine that presents in elevated levels in serum from patients with inflammatory diseases. It may also be associated with poor clinic outcomes in a number of medical disorders [16]. In addition, patients who

tested positive for SARS-CoV-2 in the plasma had significantly increased levels of selected inflammatory cytokines and chemokine ligands (CXCL10; CCL5). This concentration gain was subsequent to mechanical breathing extending for a longer duration. This sector needs further investigation as the published studies did not show a consensus18, and several authors have reported conflicting results [17]. Expression level of CXCL10 is an important marker for nonviral ARDS apart from viral V-ARDS. The data of this study indicate that CXCL10 is induced strongly in the human lung epithelium, murine infection models and actual SARS-CoV-2 infected humans along with other chemokines such as CXCL9 and CXCL11 following an acute viral response. Which is exactly what is happening in all three of the mentioned situations. CXCL9, CXCL10 and CXLC11 are assigned to the subgroup of CXC–chemokines which contain immunostimulatory properties. Autocrine and paracrine signaling occurs when chemokines interact with its receptor CXCR3 found in variety of cell types including epithelial, endothelial, B/T-lymphocytes, macrophages, natural killer cells and dendritic cells [18-22].

CONCLUSIONS

It was concluded that CXCL8 levels was hilghy related to Covid-19 severity which may be used as biomarker for prediction of the severity of such patients .

Conflict of Interest: The authors declare that they have no conflict of interest

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Ethical approval: The study was approved by the AL-Kitab University, Kirkuk, Iraq

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