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The correlation between IFN-γ and the severity of COVID-19 infected patients

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***Abstract*---**This study was carried out at Wasit Province / Iraq in cooperation with alzahraa hospital and Fairouz Hospital/ unit of microbiology. The aim of this study was to evaluate the serum levels of interferon (IFN-γ), and to evaluate the protective Role of this cytokine, in patients infected with (SARS-CoV-2). Samples were collected from many places at Wasit province. It included 66 blood samples from Iraqi patients with covid-19, and 22 healthy subjects as a control. The study was conducted during the period from October 2021 - January 2022, The age range in both groups was (14–72) years. The patients were classified into mild group (22 patients),moderate group(22 patients) and severe group (22 patients). Serum samples were collected from all participants and tested for the cytokine levels by ELISA (enzyme-linked immunosorbent assay) method. The mean serum levels of (IFN-γ), in the (COVID-19), patients were significantly higher than those observed in the control group Also, there was no difference in (IFN-γ) levels between severe and moderates groups and this levels is slightly lower than mild group . There were significant differences in the means of the three selected variables between samples with (COVID-19), and those without the infection. The calculated P-value was less than (0.001) for the three investigated variables. According to these findings suggest the relationship between (IFN-γ), and severity of (covid-19).

***Keywords*---**SARS-CoV-2, COVID-19, respiratory coronavirus, pneumonia, IFN-γ.

Introduction

Several case with pneumonia from un-identified origins were emerged in Wuhan, China in 2019. The patients were attributed to the novel beta coronavirus "SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (1). Severe acute

respiratory syndrome coronavirus 2 was the virus caused the severe respiratory diseases and referred as corona-virus diseases-19 (COVID19), and had been declared pandemic by the World Health Organization (WHO) in March 2020 (2,3,4). The existence of a previously unknown CoV-2 strain in five patients with pneumonia hospitalized from December 18 to December 29, 2019, was revealed by genomic sequencing of viral isolates from five patients with pneumonia (5). COVID-19 presently affects most countries worldwide, There have been more than 6.1 million confirmed cases of the Corona virus disease (COVID-19) in the world, as of the 1st of June 2020. About 16–21% of people with the virus in China have become severely ill with a 2–3% mortality rate. With the most recent estimated viral reproduction number, in a completely non-immune population, the average number of other people that an infected individual will transmit the virus to stands at about 3.77 (6). Given the origin of the initial COVID-19 case, the virus was most likely passed from animal to human (7). Coronaviruses (CoV) are members of the Coronaviridae family and belong to the genus Coronavirus. CoVs are pleomorphic RNA viruses with distinctive crown-shaped peplomers ranging in size from 80 to 160 nM and a genomic size of 27–32 kb (8). As a result, enclosed (CoV) are among the biggest known RNA viruses. Coronaviruses may infect a wide range of hosts, including humans and other animals cause upper respiratory tract infections in humans and are also hazardous diseases in domesticated (9). In COVID-19 patients, enormous numbers of research were published, from an experiment and epidemiologic study, tried to understanding immunological pathway of COVID19 and potential treatments. CD8+, CD4+, and CD3+ lymphocyte counts were typically decreased acording to diseases stage " (10). Beside it, cytokines storm was also presented in severe patient due to elevation in interleukin like IL-6, TNF-α, IL-10 and IL-8 (11,12). So, difference among hosts immune response played main roles in disease severity

Materials and Methods

Samples collection

This study was carried out in alzahraa hospital and Fairouz Hospital in the city of Wasit province. This study included the collection of 88 blood samples, which were obtained from various areas in Wasit province during the period from the October 2021 - January 2022, The blood samples were collected from (covid-19) patients who attended alzahraa and Fairouz Hospital and some private laboratories. The present work has been accepted via the local ethics committee at scientific research by ethical approval of health, higher education and scientific research ministry in Iraq, all patients' take part in the study were already informed about the aim of the study, agreed, and signed consent. Before blood sampling, personal information for each patient was obtained, including name, age, gender, smoking, family history, disease duration and the type of therapy regime. all blood samples were placed in a cool-Box under aseptic condition, Serum was isolated by centrifugation at 3000 rpm for 10 min and the serum was divided into aliquots in eppendorf tubes and this tube was stored in the freezer at (-80°C), Serum levels (IFN-γ),(IgM) and(IgG) in the samples were measured by Enzyme Linked Immunosorbent Assay (ELISA) using The Kits From(CUSABIO, CHINA) at wavelength of 450 nm .

Study Groups

The total number of participants in the study was eighty eight people between men and women, study groups included the following:

Group 1: sixty six people with covid-19 They were diagnosed by (R T-PCR), their samples were collected from Fairouz Hospital / and al Zahraa Teaching Hospital /wasit and some private laboratories . Their clinical information were obtained from their files.
Group 2: twenty two people apparently healthy people were classified as control group.

Statistical analysis

Data were entered, coded, and analyzed in SPSS (statistical package for social sciences) software program version 26.Data analysis were done using different tests. Frequency and percentages were used for the description of categorical variables. The mean and standard deviation were used to describe the continuous variables. Both Chi-square and Fisher's exact test were used for the assessment of the association between categorical variables. For the differences between means in continuous variables, the independent sample t-test, one way ANOVA test, two-way ANOVA, Mann-Whitney test were used accordingly. Spearman correlation coefficient was used to assess the presence of correlation in non-normally distributed variables. A P-value equal to or less than 0.05 was considered significant. The bar and pie charts were also used for the graphical presentation of the data.

Results and Discussion

A total of 88 people were recruited for this study to assess the effect of (IFN-γ) on the severity of (COVID 19), infection. Of those participants, there were 22 persons with negative (RT-PCR) test (mean no COVID-19). Other 66 patients with positive PCR were divided into 3 equal groups (22 patients) who were classified as severe, moderate, and mild cases. The mean and standard deviation (SD) of the 88 persons who participated in the study were 49.55±17.11 years old (minimum age =14, and the maximum =72 years old).

Other sociodemographic features presented by the participants were presented in (table 1). More than half of them (53.4%) were females and the majority (83%) were living in urban places. The prominent blood group among the sample was A+ (33%) followed by O+ (29.5%). Among the selected 66 (COVID-19) patients, about 42 (63.6%) were on steroid treatment while only 18 (27.3%) were on antiviral treatment (Remdesivir). There are 5 (7.6%) of patients who suffered from secondary bacterial infection. Only 4 (6.1%) of those COVID-19 patients ended with death (table 2). There was no significant difference between the patients and control groups concerning age and sex distribution. socio-demographic features are presented in (Table 1) and Frequency distribution of (COVID-19) disease history of the patients are presented in (Table 2).

Table(1): Frequency distribution of the socio-demographic features of 88 participants

Variables	Categories	Frequency	Percent
Gender	Female	47	53.4%
	Male	41	46.6%
Place of living	Rural	15	17.0%
	Urban	73	83.0%
Smoking status	Non-smoker	70	79.5%
	Smoker	18	20.5%
Job-status	Governmental	23	26.1%
	Self-employer	23	26.1%
	Housewife	37	42.0%
	Retired	4	4.5%
	Student	1	1.1%
COVID-19 vaccination	Non-vaccinated	76	86.4%
	Vaccinated	12	13.6%
Blood group	AB+	10	11.4%
	AB-	2	2.3%
	A+	29	33.0%
	A-	1	1.1%
	B+	18	20.5%
	B-	2	2.3%
	O+	26	29.5%

Table (2): Frequency distribution of COVID-19 disease history among 66 infected patients

Variables	Categories	Frequency	Percent
COVID-19 disease severity	Severe	22	33.3%
	Moderate	22	33.3%
	Mild	22	33.3%
Steroid drugs	No steroid	24	36.4%
	Steroid	42	63.6%
Antiviral drugs	No-remdesivir	48	72.7%
	Remdesivir	18	27.3%
Secondary bacterial growth	No bacterial growth	61	92.4%
	Bacterial growth	5	7.6%
Disease outcome	Cure	62	93.9%
	Death	4	6.1%

The results of the laboratory investigation made for the whole sample including interferon-gamma, IgM antibody, and IgG antibody values were described in (table 3) according to their health status. The mean interferon-γ value for those who don't have (COVID-19) was 30.38 Pg/ml while for those with mild (COVID-19) was 355.03 Pg/ml. The mean IgG titer was found to be 0.724 U/ml for the (non-COVID-19) sample but it reaches 3.646 U/ml in those with severe (COVID-19). Regarding the mean IgM titer, it appears to be 0.825 U/ml and 5.123 U/ml in the same previous order.

Table (3): Descriptive statistics of interferon-γ, IgG, and IgM antibodies among all health status categories of the study sample (88)

Disease status	Interferon-γ (Pg/ml)		IgG (U/ml)		IgM (U/ml)	
	Mean (Standard deviation)	(Min-Max.)	Mean (Standard deviation)	(Min.-Max.)	Mean (Standard deviation)	(Min.-Max.)
Severe COVID-19	229.88(56.310)	102-309	3.646(1.068)	1.588-5.501	5.123(1.194)	3.261-6.640
Moderate COVID-19	266.03(29.208)	223-316	3.244(1.147)	0.680-4.607	3.921(1.589)	1.457-6.406
Mild COVID-19	355.95(21.346)	323-398	2.985(1.190)	1.325-4.990	3.079(0.972)	1.021-4.457
No COVID-19	30.38(7.303)	16-40	0.724(0.191)	0.413-1.109	0.825(0.085)	0.686-0.972
Total sample	220.75(124.260)	16-398	2.650(1.500)	0.413-5.501	3.237(1.918)	0.686-6.640

There were significant differences in the means of the three selected variables between samples with (COVID-19), and those without the infection. The calculated *P*-value was less than 0.001 for the three investigated variables as shown in (table 4).

Table (4): Mean differences of Interferon-γ, IgG, and IgM between COVID-19 control(non-COVID-19) participants

Variables	COVID-19	Not COVID-19	*P*-value (Mann-Whitney Test)
Interferon-Y	283.95 (65.576)	30.16 (7.009)	<0.001
IgG	3.292 (1.1520)	0.724 (0.191)	<0.001
IgM	4.041 (1.515)	0.825 (0.085)	<0.001

In (table 5), the mean Interferon-γ was also different between the (COVID-19) patient categories. The *P*-value was <0.001. Patients who suffer from severe (COVID-19) had the lowest mean (229.88±56.310) while those with a mild form of the disease had 355.95±21.346. this result agrees with a study done by(13). in Brazil who observed the higher IFN-γ levels were detected in early (COVID-19), infection than healthy populations these levels were not sustained after ten days of symptoms. In those with sustained IFN-γ levels, the mortality increased. Also agree with study by (14).in Iran who found the IFN-γ levels were significantly higher in mild group than in control group and the IFN-γ was increased significantly in severe and mild groups compared to healthy control group. on other hand present study disagrees with(15).in Irbil Iraq,they observed the IFN-γ concentrations in the control, moderate (COVID-19), severe cases of (COVID-19), and recovered from (COVID-19) groups, did not vary significantly among groups.

Table (5): Mean differences and descriptive statistics of Interferon-γ among (66) COVID-19 patients

COVID-19	Mean	Standard Deviation	95% Confidence Interval for Mean		*P*-value (One way ANOVA test)
			Lower Bound	Upper Bound	
Severe	229.88	56.310	204.91	254.84	<0.001
Moderate	266.03	29.208	253.08	278.98	
Mild	355.95	21.346	346.49	365.41	
Total	283.95	65.576	267.83	300.07	

there is a significant (*P*-value <0.001) positive strong correlation (r=0.806) between the level of Interferon-γ and the (COVID-19), classes (severe, moderate, and mild COVID-19). In current study, the results show high levels of Interferon-γ in mild group compare with control group thats refere to IFN-γ related with viral load and this agrees with a study by (16).who shows the IFN-γ was highly correlated with viral load, suggesting that the virus can boost the secretion of these cytokines.

the level of Interferon-γ has a significant (*P*-value <0.001) negative median correlate (r= -0.484) with IgM. While it has no significant correlation (*P*-value=0.687) with IgG titer (r=0.051) among patients with the different classifications of (COVID-19). (Table 6) shows that there is no significant difference (*P*-value=0.159) between the COVID-19 classes regarding the IgG value. The means were 3.646, 3.244, and 2.985 for patients with severe, moderate, and mild (COVID-19), respectively this results agreed with(17).who observed The positive rates of IgG detection were not significantly different among the mild, severe and critical groups. Also, study agreed with study by(18).

Table (6): Mean differences and descriptive statistics of IgG among (66) COVID-19 patients

COVID-19	Mean	Standard Deviation	95% Confidence Interval for Mean		*P*-value (One way ANOVA test)
			Lower Bound	Upper Bound	
Severe	3.646	1.068	3.172	4.120	0.159
Moderate	3.244	1.147	2.736	3.753	
Mild	2.985	1.190	2.457	3.513	
Total	3.29	1.152	3.008	3.575	

There was a significant difference between (COVID-19), classes concerning IgM value (<0.001) as shown in (table 7). , quantitative analyses of antibody levels over the disease course revealed that SARS-CoV-2-specific IgM levels were higher in patients in the severe group, as compared with the other groups, which might be because of high disease activity and/or a compromised immune response in these patients.The present study agrees with a study done by(19).who show the severe cases of (COVID-19) tended to have a more vigorous response in IgM antibodies to (COVID-19) illness. also The increased IgM level in the deceased case group might be related to the higher disease severity in these patients and indicate a poor prognosis. In summary, we observed that the IgM antibody response to (SARS-CoV-2), occurred earlier and peaked earlier than the IgG antibody response; the IgM antibody response began to decline at week 3 of the illness, while the IgG antibody response persisted and was maintained in patients with (COVID-19).

Table (7): Mean differences and descriptive statistics of IgM among (66) COVID-19 patients

COVID-19	Mean	Standard Deviation	95% Confidence Interval for Mean		*P*-value (One way ANOVA test)
			Lower Bound	Upper Bound	
Severe	5.123	1.194	4.594	5.652	<0.001
Moderate	3.921	1.589	3.216	4.626	
Mild	3.079	0.972	2.648	3.510	

Total	4.041	1.515	3.669	4.414	

Table (8): Differences in mean interferon-y, IgG, and IgM according to disease outcome

Variables	Disease outcome	N	Mean	Std. Deviation	P- value (independent sample t-test)
Interferon-γ	Recovery	18	243.28	52.594	0.014
	Death	4	169.58	24.392	
IgG	Recovery	18	3.818	1.069	0.110
	Death	4	2.870	0.725	
IgM	Recovery	18	5.034	1.166	0.471
	Death	4	5.525	1.416	

Table (8)There are no significant differences between patients who died and those who recovered from (COVID-19), in IgG levels p-value 0.110 also in IgM levels p-value 0.471 where as there are significant differences between patients who died and those who recovered from (COVID-19), in Interferon-γ levels p-value 0.014 this results show the recovery patients mean levels 243.28 whereas the death patients levels 169.58 and these refere to decrease of IFN gamma levels in death patients than the recoverd patients groups, The baseline levels of IFN-γ were negatively associated with the increase of fibrosis and mortality in (COVID-19) . These data suggest that early intervention of anti-viral infection using IFN-γ could be substantial in the inhibition of fibrosis and death for better functional recovery these results agree with results by (20).indicate that decreased circulating IFN-γ is a risk factor of lung fibrosis and death in (COVID-19).

Conclusions

According to the study's findings, the following conclusions have been made at: The postulated mechanism for Coronavirus illness in 2019 is Cytokine dysregulation (COVID-19), Patients with COVID-19 had considerably greater mean blood levels of(IFN-γ), than those in the control group. Additionally, there was no distinction in IFN- levels between the severe and moderate groups, and these levels are marginally lower than those of the mild group. Patients in the severe group had greater IgM levels than those in other groups, whereas there was no discernible difference in IgG levels across the mild, moderate, and severe groups. Patients who have low or high grade(IFN-γ), circulation are more likely to develop fibrosis or hyperinflammation, which both increase mortality rates.

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