Serum Antibodies Against Neutrophil Extracellular Traps (NETs) and Their Relation to Severe COVID-19 infection

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Abstract

Background: Acute and chronic SARS-CoV-2 patients experience a wide range of clinical manifestations, ranging from mild asymptomatic symptoms (colds) to severe , often fatal

Aim: The current study aimed to evaluate some immunological variables in patients with COVID-19 for chronic and acute cases

Materials and methods: the study included collecting (120) blood samples from individuals suspected of having respiratory distress syndrome, of both sexes, with ages ranging from 29 years to 60 years, for the period from 15/12/2021 to 20/7/2022 from Nineveh Private Hospital and Ibn Sina Governmental Hospital. The samples registered in the study were divided after confirming the diagnosis of COVID-19 infection into two groups, the first group included (25) patients suffering from Chronic Respiratory Distress Syndrome and lying in the intensive care unit and needing ventilatory support, the second group included (25) patients who They suffer from moderate symptoms such as coughing, fever, runny nose with upper respiratory tract infections, pneumonia, and do not need oxygen from an external source. The control sample included (25) blood samples from healthy individuals with ages ranging from 24-52 years and from both Both sexes do not suffer from any transmissible diseases, viral or bacterial infections, or autoimmune diseases. Through the study, some immunological variables were evaluated for the study sample, which included the estimating the phagocytic activity, and at the level of specialized humoral immunity, the level of COVID-19 IgM antibodies was estimated. NETs-IgM, NETs-IgG.

Results: At the level of humoral immunity, the results showed a significant increase in the levels of COVID-19 IgM and COVID-19 IgG antibodies in patients with chronic cases of COVID-19, compared to both acute patients and the control sample at a significant level of $P \leq 0.05$, and a significant increase in the levels of NETs-IgM antibodies in patients with chronic conditions compared to acute cases and the control sample. As for NETs-IgG antibodies, the study recorded a significant increase in its levels in patients with chronic and acute conditions compared to the control sample at a significant level of $P \leq 0.05$.

Keywords: *COVID-19; IgG; IgM; Severe; NET.*

INTRODUCTION

COVID-19 is an epidemiological challenge caused by thenovel coronavirus SARS-CoV-2 (1) which is currently putting severe pressure on many health systems around the world. The virus belongs to the family of coronavirus viruses that include SARS-CoV-1 and Middle East respiratory syndrome (MERS-CoV). Viruses Corona is a special receptor for lung cells (2). SARS-CoV-2 is known to use the same receptor 1-SARS-CoV to enter the host cell, angiotensin-converting enzyme (ACE-2). (3) . Acute and chronic SARS-CoV-2 patients experience a wide range of clinical manifestations, ranging from mild asymptomatic symptoms (colds) to severe, often fatal. The latter form usually appears with interstitial pneumonia and is moderate to severe in oxygen saturation and therefore hypoxia. Many patients develop respiratory failure, called acute respiratory distress syndrome (ARDS) (4), requiring immediate admission to the intensive care unit. Unlike the usual acute respiratory distress syndrome, patients with chronic conditions show general and progressivedamage to lung tissue and often require a continuous positive airway (pressure) (5). As a result, patients, especially in chronic conditions, suffer from Respiratory failure, which is called interstitial pneumonia (6). Thegreatest risk is deep lung infections. The epithelial cells that make up the respiratory and lung pathways are where the body's cells first come into contact with the FAwhen it invades the cells of lung tissue, these cells respond by releasing high concentrations of reactive chemicals, called cytokines, and triggering the immune response. Not only can inflammatory cytokines, including IL-2, IL8 and IL-1ß TNF-α and IFN-α play a role in killing a thousandaerosat, but also cause significant destruction of lung tissue (7). Cytokines mainly stimulate immune cells such as macrophages, neutrophils, and lymphocytes to the site of injury – the lungs and respiratory passages (8). There are specific immune cells called macrophages within the lung tissue (especially around the air sacs called alveoli) that provide the next level of resistance to theRussian virus. These cells develop anti-neurotic immune compounds, but can stimulate the formationof serious lung tissue damage (9). A group of severeinflammatory reactions due to neutrophil recruitment and infiltration in different organs with the formation of neutrophil extracellular traps (NETs), as well as different immune responses by immune system cells in the epicenter of infection involving various complications from COVID-19 infection (10,11). Because the reaction of immune responses has an effect equivalent to the virulence of this virus, it was necessary to evaluate the immune responses of COVID-19 patients in patients with chronic and acute conditions to find out the most important factors that stimulate the high immune reaction, so the study aimed to evaluate levels of COVID-19 type COVID-19 IgM and IgG and COVID19 IgG antibodies to COVID-19 patients in chronic and acute conditions and comparing them with the control sample.

Materials and Methods

A number (120) blood samples were collected for individuals suspected of having respiratory distress syndrome of both sexes and from 29 years to 60 years for the period from 15/12/2021 to 20/7/2022 from Nineveh National Hospital and Ibn Sina Governmental Hospital. The samples recorded in the study were divided after confirming the diagnosis of COVID-19 into two groups. , The first group included (25) patients with chronic respiratory distress syndrome and are lying in the intensive care unit and need ventilator support, the second group included (25) patients with moderate symptoms such as cough, fever, and runny nose with upper respiratory tract infections pneumonia, and do not need oxygen from an external source, and the control sample included (25) blood samples for healthy individuals and ages ranging from 24years to 52 years and of both sexes do not suffer from any communicable diseases or viral orbacterial infections or autoimmunediseases. Venous blood samples were drawn by sterile medical syringes of (5) ml and Baccording to the quantity required for eachexperiment and the blood sample for each patient was divided into: Put (3ml) of the blood model in a clean, sterile and anticoagulant free gel tube test, leave the blood for half an hour until coagulatione, and separate the serum after the centrifugation process at (3000 cycles / min) for a period of (15) minutes, and keep the serum samples at a temperature of (-20) ° C until the serological tests Ferritinand CRP, COVID IgG, COVID IgM, NET-IgG , NET-IgM. EDTA tubes were used to collect venous blood in size (2ml) for a period not exceeding two hours, to perform blood tests, including complete blood image (CBC) and phagocytic activity.

Results:

Table 1 refers to a set of statistical indicators represented by (sample size, arithmetic mean, standard deviation, lowest value, and highest value) of the variable of absolute numbers of phagocytes for the study groups.

Table 1: Mean of absolute numbers of phagocytes among the studied grups

Case Summaries						
TYPE		N	Mean	Std. Deviation	Min	Max
Control	Phagocytic cells Cell/µl	25	31.7857	3.84665	25	38
Non -sever cases of COVID-19		25	24.25	4.98912	12	3
Sever cases of Covid -19		25	25.125	7.45542	12	37

The results of the Duncan test for comparison pairs are shown as shown in Table 2. There are significant differences in the absolute numbers of phagocytes between Sever cases of COVID-19 and the control sample, as well as significant differences between Non-sever cases of COVID-19 and the control sample). Figure 1 Phagocyte containing dark formazan granules compared to the control sample

Table 2: Duncan test for two pairs of comparisons and test the significant level at the variable (absolute numbers of phagocytes)

Phagocytic cells			
Duncan			
TYPE	N	Subset for alpha = 0.05	
		1	2
Control	25		31.7857
Non -sever cases of COVID-19	25	24.2500	
Sever cases of Covid -19	25	25.1250	
P-value		.650	1.000

*P⩽ 0.05

Images 1: A phagocyte containing dark formaz granules compared to the control sample

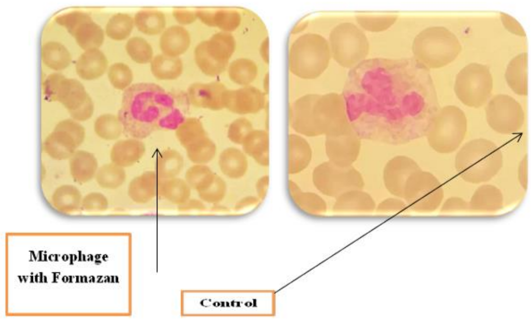

Table 3 refers to a set of statistical indicators represented by (sample size, arithmetic mean, standard deviation, lowest value, and highest value) of the antibody variable IgM -NET for the study groups.

Table 3: Mean of IgM-NET antibodies level among the studied groups

Case Summaries						
TYPE		N	Mean	Std. Deviation	Min	Max
Control	IgM -NET µg/mL	25	.3258	.22792	.12	1.06
Non -sever cases of COVID-19		25	.8681	.65256	.28	2.44
Sever cases of Covid -19		25	.7214	.40244	.33	1.46

The results of the Duncan test for comparison pairs, as shown in Table 4, show that there were no significant differences in the concentration of IgM -NET antibodies between Sever cases of COVID-19 andNon-sever cases of COVID-19, in terms of the p-value value which reached (0.398), which is greater than (0.05). There were significant differences in the concentration of NET-IgM antibodies between Sever cases of Covid-19 and the control sample (as well as significant differences between Non-sever cases of COVID-19 and the control sample).

Table 4: Duncan test for two pairs of comparisons and test the level of significance at the variable (NET-IgM)

NET-IgM			
Duncan			
TYPE	N	Subset for alpha = 0.05	
		1	2
Control	25	.3258	
Sever cases of Covid -19	25		.7214
Non –sever cases of COVID-19	25		.8681
P-value		1.000	.398

*P⩽ 0.05

Table 5 refers to a set of statistical indicators represented by (sample size, arithmetic mean, standard deviation, lowest value, and highest value) of the IgG -NET antibody variable for the study groups.

Table 5: Mean of IgG –NET antibodies level among the studied groups

Case Summaries							
TYPE		N	Mean	Std. Deviation	Min	Max
Control	IgG -NET U/L	25	.25	.17894	.1	.83
Non -sever cases of COVID-19		25	.4503	.16454	.19	.83
Sever cases of Covid -19		25	.4881	.17971	.21	.83

The results of the Duncan test for comparison pairs, as shown in Table 6, show that there are no significant differences in the concentration of IgG -NET antibodies between Sever cases of COVID-19 andNon-sever cases of COVID-19, in terms of the p-value value which reached (0.525), which is greater than (0.05). There were significant differences in the concentration of NET-IgG antibodies between Sever cases of Covid-19 and the control sample, as well as significant differences between Non-sever cases of COVID-19 and the control sample.

Table 6: Duncan test for two pairs of comparisons and test the level of significance at the variable (NET-IgG)

NET –IgG			
Duncan			
TYPE	N	Subset for alpha = 0.05	
		1	2
Control	25	.2500	
Non –sever cases of COVID-19	25		.4503
Sever cases of Covid -19	25		.4881
P-value		1.000	.525

*$P \leqslant 0.05$

Table 7: refers to a set of statistical indicators represented by (sample size, arithmetic mean, standard deviation, a lowest value, and the highest value) of the IgM antibody variable against the Corona virus for the study groups.

Table 7: Mean of total IgM antibodies level among the studied groups

Case Summaries						
TYPE		N	Mean	Std. Deviation	Min	Max
Control	COVID-19 IgM	25	.2809	.15189	.08	.65
Non -sever cases of COVID-19		25	2.2617	5.10804	.15	24.66
Sever cases of Covid -19		25	6.7300	4.47369	1.17	15.90

There were no significant differences in the concentration of COVID-19 IgM antibodies between the control sample and Non-sever cases of COVID-19, in terms of the p-value value, which amounted to (0.174), which is greater than (0.05). There were significant differences in the concentration of COVID-19 IgM antibodies between Sever cases of COVID-19 and the control sample, as well as significant differences between Sever cases of Covid-19 and Non-sever cases of COVID-19.

Table 8: Duncan test for a pair of comparisons and test the level of the variable (IgM antibodies against Corona virus)

COVID-19 IgM			
Duncan			
TYPE	N	Subset for alpha = 0.05	
		1	2
Control	25	.2809	
Non –sever cases of COVID-19	25	2.2617	
Sever cases of Covid -19	25		6.7300
P-value		.174	1.000

$^*P \leqslant 0.05$

Table 9: refers to a set of statistical indicators represented by (sample size, arithmetic mean, standard deviation, lowest value, and highest value) of the IgG antibody variable against the Corona virus for the study groups.

Table 9: Mean of total IgG antibodies level among the studied groups

Case Summaries						
TYPE		N	Mean	Std. Deviation	Min	Max
Control	COVID-19 IgG µg/mL	25	0.4208	0.28103	.04	.84
Non -sever cases of COVID-19		25	3.2004	2.72566	.10	9.42
Sever cases of Covid -19		25	19.5344	13.30628	2.90	45.34

The results of the Duncan test for comparison pairs, as shown in Table 10, show that there were no significant differences in the concentration of COVID-19 IgG antibodies between the control sample and Non-sever cases of COVID-19, in terms of the p-value value which amounted to (0.280), which is greater than (0.05). There were significant differences in the concentration of COVID-19 IgG antibodies between Sever cases of COVID-19 and the control sample, as well as significant differences between Sever cases of Covid-19 and Non-sever cases of COVID-19.

Table 10: Duncan test for two pairs of comparisons and test the level of the variable (IgG antibodies against Corona virus)

COVID-19 IgG			
Duncan			
TYPE	N	Subset for alpha = 0.05	
		1	2
Control	25	0.4208	
Non –sever cases of COVID-19	25	3.2004	
Sever cases of Covid -19	25		19.5344
P-value		.280	1.000

$^*P \leqslant 0.05$

Discussion

Patients with COVID-19 showed a significant reduction in the number of lymphocytes and an elevated number of neutrophils which are responsible for phagocytic activity in the blood compared to the control sample (1)

COVID-19 patients in acute and chronic cases have a weak innate immune response and are therefore more susceptible to secondary bacterial infections and opportunistic lung infections. The collaboration between antigen-specific antibodies and phagocytes (infiltrating and single-cell-derived macrophages and partially alveolar macrophages) has shown an important role in the elimination of COVID-19 in infected mouse and human models. Our findings provide a better understanding of the mechanisms controlled by host defenses in COVID-19 infection. Ideally, this information could contribute to the development of new protocols or treatments For COVID-19 patients (2,3). The process of cytophagytosis by neutrophil cells is more effective in the presence of opsonins such as IgG and IgG. IgM IgG or IgM bound to the microbial surface is recognized by C1q which activates the classical complementary pathway. PMNs express IgG receptors (FcγRI, FcγRII, FcγRIII and opsonic C3b supplementary molecules) and iC3b (CR1, CR3 , CR4 (12). Observed improvement in COVID-19 patients treated with IV immunobulin or by reduced supplement activation suggests that the low neutrophil-mediated bacterial killing seen in COVID 19 patients may result from reduced levels of neutrophils seen in COVID 19 patients. Kimmunoglobulin in these people. Neutrophils are also believed to be effective against COVID-19. Autopsies also showed that lung tissue had been penetrated by neutrophils through capillaries (13) During COVID-19 infection, NETs may be formed directly by neutrophils (14) may also be indirectly stimulated or shaped by activated platelets and autocoagulant antibodies. As a result, the body then exhibits a compensatory response to hyperimmunity in pursuit of virus removal. This is characterized by the presence of a peripheral cell chamber in which simple B cells take a path outside the follicle to become antibody-producing cells, thus bypassing natural checkpoints against autoimmunity (15), While this strategy may quickly produce a large amount of neutralizing antibodies toviruses, it also paves the way for the production of de novo for various pathogenic autoantibodies. NETs appear to lead to the production of autoantibodies in systemic autoimmune diseases such as lupus, rheumatoid arthritis, and antineutrophilic cytoplasmic antibodies associated with vasculitis and for example, it has been suggested that increased NET formation, the presence of NET antibodies, and impaired NET removal, are all associated with disease activity and organ damage in lupus (16). Our group found something similar in individuals with primary antiphospholipid syndrome (APS) (17). We found that high levels of anti-NET IgG and IgM are present in COVID-19 patients in intensive care. These NET antibodies not only weakened the endogenous ability of DNases Serological on the survey of NETs, but also associated with the weakened respiratory condition and the severity of the disease in general. While the ongoing vaccination campaign is working to reduce the incidence of COVID-19 and deaths, millions of survivors of COVID-19 infection continue to experience long-term symptoms of the disease. The diverse and functional autoantibodies produced during COVID-19 infection are certainly a reasonable contributor to post-COVID-19 syndrome. Interestingly, one recent study noted that out of 9 COVID-19 survivors , 5 of them developed chronic "long-term" symptoms, and all had autoantibodies (13,14). A previous study also showed that an anti-IgG to NET remains inside the body for up to 4 years among some patients with antiphospholipid syndrome (APS) (17). The data presented here suggest the presence of another functional antibody in COVID-19 , and that the potential long-term persistence of these antibodies requires further investigation.

Conclusions

The most important conclusions of the study wereas follows:

1. Significant decrease in the number of phagocytes and the effect of phagocytosis in chronic and acute cases of COVID-19 patients compared to the control sample.

2. Significantly higher levels of COVID-19 IgM antibodies and COVID-19 IgG in chronic COVID-19 patients compared to both acute patients and control sample.

3. A significant increase in the levels of antibodies to NETs-IgM cell neutrophil traps in patients with chronic conditions compared to acute cases and control sample, as for NETs-IgG antibodies, the study recorded a significant increase in its levels in patients with chronic and acute cases inthe control sample.

Recommendation

1. Follow-up of the effect of variation in immunological measures after recovery from infection with the Corona virus, especially in patients with chronic conditions.

2. Conducting a study to estimate the effectiveness of inflammatory factors, especially complement, by performing Haemolytic Complement (CH50) Activity of Serum tests.

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