

Occurrence of auto antibodies in COVID-19 Infected Patients

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Abstract

The study included 60 patients 40 males and 20 females infected with COVID-19 virus, diagnosis (PCR method) by staff of virology laboratory from Imam Hussein Teaching Hospital in Thi-Qar province, and 30 controls, 15 males and 15 females. This study was approved by the ethics commissions of the participating hospitals, with a waiver of informed consent. The study included measuring the level of interleukin-6, anti-double-strand antibody and Anti-cyclic Citrulline Peptides, for patients during and after infection and comparing between them by using paired t-test and ANOVA to compare between patients and control, as well as between patients according to chronic diseases, the independent t-test also used for compartment of patients and controls categories according to gender. The results showed a significant increase in the level of interleukin-6 among patients during infection and there were non-significant differences according to gender, while the level of anti-double-strand antibody and Anti-cyclic Citrulline Peptides were high after infection, while the results recorded significant differences according to gender in the level of anti-double-strand antibody in the second age group, and Anti-cyclic Citrulline Peptides in the first age group, while the results recorded a significant increase in the level of immune parameters when compared to the control.

Keywords: auto antibodies, COVID-19, Infected Patients

Introduction

The COVID-19 pandemic has already affected several thousand people and has become the biggest health challenge worldwide (Roland *et al.*, 2020). The clinical presentation of varies range from asymptomatic and mildly symptomatic to acute respiratory distress syndrome (ARDS) and death, due to the unknown number of people experiencing shedding symptoms and the lack of symptoms in the community, the total number of infections is uncertain [1] (Wiersinga *et al.*, 2020). So far, an effective treatment is not available. However, the results of preliminary studies and clinical trials improve our understanding of pathogenesis and Covid-19 treatment. Here, we share our personal views on the immune disease of this infection [2] (Parasher, 2021). The virus enters the cell via angiotensin-converting enzyme-2 (ACE-2) and is sensed (mainly) by the toll-like receptor 7 (TLR7), which is found in endosomes. TLR7 activation leads to production of alpha interferon, TNF-alpha, interleukin (IL) -12 and IL-6 [3] (Amraei and Rahimi, 2020). This results in the formation of CD8 + cytotoxic T cells and, through the CD4 + T helper cell, leads to the formation of antigen-specific B cells and the production of antibodies [4] (Gil-Etayo *et al.*, 2021). This adaptive immune response controls the viral infection and determines clinical recovery, during cytokine secretion associated with TLR7 signaling, IL6 may play an important role. Interleukin-6 plays a multidirectional role across the immune system [5] (Kim *et al.*, 2020). It is important in the formation of follicular helper T cells, Th17 subgroup deviation, and the formation of long-lived plasma cells. However, IL-6 can inhibit CD8+ cytotoxic T cells by inhibiting interferon gamma secretion. Moreover, IL-6 by induction of suppression of cytokine signaling (SOCS-3) and increased expression of PD-1 can paralyze the cell-mediated antiviral response during a cytokine storm. Recent studies show that SARS-CoV-2 infected individuals admitted to the hospital with more severe disease have higher rates of clinically relevant auto antibodies (AABs) [6] (Agasing, 2020), with critically ill COVID-19 patients often possessing detectable levels of lupus anticoagulant antibodies, an antibody profile often implicated in Hypercoagulable states [7] (Yhojan *et al.*, 2020). In [8] addition, auto antibodies against type II interferon were found in COVID-19 subjects during acute illness (Rockx *et al.*, 2020); such antibodies may subvert the induction of an effective anti-SARS-CoV-2 immune response, against the backdrop of this evolving autoimmune picture,

an increasing number of reports have documented post-acute sequelae of COVID-19, or 'long COVID, found in those who survive acute illness yet continue to have prolonged symptoms ranging from sustained acute symptomatology, overall decrease in physiological reserve, and onset of new symptoms, including neurocognitive dysfunction [9] (Phipps *et al.*, 2020).

Material and Method

Patient and Sample Collection

The study included 60 patients 40 males and 20 females infected with COVID-19 virus, diagnosis by staff of virology laboratory from Imam Hussein Teaching Hospital in Thi-Qar province, and 30 controls, 15 males and 15 females. This study was approved by the ethics commissions of the participating hospitals, with a waiver of informed consent from the period from November 2020 to March 2021. The level of immune parameters was measured for patients during and after the infection and the results was compared between them and with the control by using third generation ELISA technique.

Statistical Analysis

The all data of the current study was analysis by using Excel Microsoft version 2010, and statistically by using SPSS (Statistical Package of Socio Science) by using one way ANOVA for variation, least significant difference, independent t-test and paired t-test at P. value < 0.05.

The Results

A Compartment of all Total Parameters in Patient during and after Infection

The current results recorded that there were statistically significant difference in levels of anti- DS and anti - ACCP were significant increased after infection, while the concentration of IL-6 was decreased significantly after infection at P. value < 0.05.

Table 3-1: Concentration of anti- DS, anti- ACCP and IL-6

Parameters	Patients = 60	Mean + SD	P. value
Anti- DS	During Infection	13.7 ± 5.4	< 0.01
	After Infection	25.2 ± 7.9	
Anti-ACCP	During Infection	11.1 ± 2.8	< 0.01
	After Infection	17.4 ± 6.2	
IL-6	During Infection	82.8 ± 21.7	< 0.01
	After Infection	45.4 ± 15.2	

Concentration of anti- DS in Patient During and after Infection and Control

The results of current study noted the anti- DS increased in patient after infection in both male and female and a significant difference with patient during infection and control at P. value < 0.05, while the result noted a non-significant differences according gender between male in both categories infection and control.

Table 3-2: Concentration of anti-DS in patient and control

Anti-DS IU/ml	Cases No.	Mean + SD		t test P. value
		Male	Female	
During Infection	40/ 20	14.1 ± 5.8	13.1 ± 4.5	0.493

After Infection	40/ 20	26.8 ± 7.4	22.1 ± 8.2	0.031
Control	15 / 15	11.3 ± 4.0	13.0 ± 5.2	0.340
P. value		< 0.01	< 0.01	
LSD		2.83 / 3.83	4.0 / 4.3	

Concentration of anti-CCP in Patient During and after Infection and Control

The results of current study illustrated the concentration of anti- CCP a significant increase in patient after infection in both male and female and a significant difference with patient during infection and control at P. value < 0.05, while the result noted a non-significant differences according gender between male in both categories infection and control.

Table 3-3: Concentration of anti- CCP in patient and control

Anti- CCP EU/ml	Cases No.	Mean + SD		t test P. value
		Male	Female	
During Infection	40/ 20	11.6 ± 2.8	9.99 ± 2.4	0.031
After Infection	40/ 20	17.1 ± 6.5	18.1 ± 5.3	0.542
Control	15 / 15	10.5 ± 2.5	11.3 ± 2.8	0.381
P. value		< 0.01	< 0.01	
LSD		2.10 / 2.85	2.45 / 2.64	

Concentration of IL-6 in Patient During and after Infection and Control

The results recorded the concentration of IL-6 a significant increase in patient during infection in both male and female and a significant difference with patient after infection and control at P. value < 0.05, while the result also noted a non-significant differences according gender between male in both categories infection and control.

Table 3-4: Concentration of IL-6 in patient and control

Gender IL-6 pg/ml	Cases No.	Mean + SD		t test P. value
		Male	Female	
During Infection	40/ 20	81.4 ± 22.4	85.7 ± 20.7	0.481
After Infection	40/ 20	46.1 ± 14.1	43.9 ± 17.2	0.597
Control	15 / 15	35.4 ± 8.45	33.3 ± 11.5	0.569
P. value		< 0.01	< 0.01	
LSD		7.8 / 10.5	11.0 / 11.9	

Concentration of anti- DS and anti-CCP in Patient with and without Chronic Disease during Infection and Control

The study showed the concentration all patient categories recording a significant increased compartment with control at P. value < 0.05. While the result also noted a non-significant

difference all patient categories compartment with control in concentration of DS and anti-CCP.

Table 3-5: Concentration of anti-DS and anti- CCP in patient and control

Groups During Infection	Cases No.	Mean + SD	
		Anti-DS	Anti-CCP
With Diabetic	15	14.6 ± 5.87	11.1 ± 2.85
With Pressure	14	14.2 ± 6.11	10.6 ± 3.04
Without Disease	31	13.1 ± 4.99	11.3 ± 2.82
Control	30	12.1 ± 4.69	10.9 ± 2.68
P. value		0.40	0.872
LSD		Non-Sig	Non-Sig

Discussion

COVID-19 patients are at a high risk for thrombotic arterial and venous occlusions. Antiphospholipid antibodies (aPL) are pathogenic auto antibodies targeting phospholipids and phospholipid-binding proteins, that can cause so called Antiphospholipid Syndrome, characterized by a rapidly occurring multiorgan thrombotic damage. The aPL are including anticardiolipin (aCL), anti-beta-2 glycoprotein I (aβ2GPI), anti-phosphatidylserine/Prothrombin (aPS/PT) and lupus anticoagulant (LA) [10] (Gao et al., 2021). The shared path genetic mechanisms and clinical-radiological aspects between the hyper-inflammatory diseases and Covid-19 may suggest that SARS-CoV-2 could act as a triggering factor for the development of a rapid autoimmune and/or auto inflammatory dysregulation, leading to the severe interstitial pneumonia, in genetic predisposed individuals.

Anti-Double Strand Antibody

The result recorded significant increased concentration of anti-DS in COVID-19 patient after viral infection compartment with same patients after infection, the results also recorded the anti-SD increased in patients' compartment with control in male gender, the results also showed significant between male and female after infection status, and no significant difference between patients according to chronic disease. The study of [11] Slimani *et al.*, (2021), in Morocco, they study systemic lupus Erythematous and Varicella-like rash following covid-19 in a previously healthy patient, they concluded the patients with covid-19 may be develop to autoimmune disease as systemic lupus and auto-inflammatory diseases as Erythematous and rheumatic arthritis. Viruses considered as one of the triggers for autoimmune and auto-inflammatory diseases, such as SLE, including parvovirus B19, Epstein-Barr virus, cytomegalovirus, herpes virus-6, HTLV-1, hepatitis A and C virus, and rubella virus [12] (Crow *et al.*, 2019), [13] Smatti *et al.*, (2019), showed that the viral-induced autoimmunity can be activated through multiple mechanisms, mainly by molecular mimicry, other mechanisms include epitopes spreading, bystander activation, and immortalization of infected

Anti-cyclic Citrulline Peptides

Anti-cyclic citrulline peptide (anti-CCP) detection considered one of the most marker that have been used for the diagnosis of rheumatoid arthritis, the sensitivity of the test is similar to RF but it has better specificity. The result recorded significant increased concentration of

anti- CCP in COVID-19 patient during viral infection compartment with same patients after infection, the results also recorded the anti- ACCP increased in patients compartment with control in both gender, the results also showed significant between male and female during infection status. The study of [14] Serge *et al.*, (2020), in Burkina Faso, they study patients with rheumatoid arthritis after covid-19 infection, and concluded the RF in patients produced as a result of immune disorder in covid-19 infected patients due to antibody formation specialized and non-specialized for the disease. Also a study of [15] González-Zorrilla *et al.*, (2020), they study immune response COVID-19-associated Hepatic Encephalopathy, and concluded A significant increase ACCP concentration was observed in patients with COVID-19-associated hepatic encephalopathy compared to patients with hepatic encephalopathy. The association between RA and COVID-19 is very complex and can be explained in two different directions. On the one hand, in fact, the possible role of external pathogens that cause acute and chronic arthritis in the form of either the direct colonization of the joints by the pathogen or the autoimmune reaction resulting from the host's response to the infection, so arthritis has been observed in COVID-19 patients due to an immune system disorder [16] (Ruiz, 2020). There are also other auto antibodies were found in COVID-19, such as auto antibodies anti-cyclic peptide containing citrulline (CCP) were found in COVID-19, the existing auto antibodies might be associated with the severity of illness and autoimmune symptoms. The convalescent plasma should be tested for auto antibodies before their plasma donations are accepted. Notably, due to the multiple peptide sequence sharing between SARS-CoV-2 spike glycoprotein and the human proteins have been found, molecular mimicry has been proposed as a cause of the autoimmune phenomena observed in COVID-19 [17] (Angileri *et al.*, 2020). Therefore, one of the potential side effects of giving a mass vaccine could be an emergence of autoimmune diseases especially in individuals who are genetically prone for autoimmunity [18] (Shoenfeld *et al.* , 2020).

Interleukin-6

The results recorded the concentration of was high during covid-19 infection status compartment with same patients after infection status, the results also noted their not difference in IL-6 concentration between gender, and between patients according to chronic disease. A previous study performed by [19] Han *et al.*, (2020), in China, they study cytokines profile as predicts for severity of infection, and noted that the highest level of IL-6 and CRP was in patient group classify as critical group, while the lowest level was in group of moderate disease, their study also indicated that during the field survey, it was found that people with slightly high levels of IL-6 and CRP are the most vulnerable people. Also the study of [20] Chakraborty *et al.*, (2020), in Taiwan, they study IL6 receptor antagonist for the therapy of cytokine storm syndrome in SARS-CoV-2 infected patients, they noted the level of IL-6 was elevated in patients and significantly increased when the disease status progression, they also noted the increased stimulation of production IL-6 lead to random stimulation of cytokines and may lead to an immune storm. The significant increased level of IL-6 level in serum of covid-19 infected patients with severe or critical status. IL-6 considers is a one of the major cytokines, which can trigger inflammatory storm. This may consequence of impair oxygen diffusion in the body. Due to this, respiratory muscles become fatigued and that may finally leads to respiratory failure [21] (Buonaguro *et al.*, 2020).

Conclusion

The current study concluded a significant increase in the level of interleukin-6 in patients during infection, while the level of autoimmune antibodies increased after infection, and the study also showed that chronic diseases had no effect on the level of measured immune parameters.

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