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Exploration of Cytokines Storm by Detect Cytokines Level among Sera of in Patients with COVID 19 at Thi-Qar Provence – Iraq

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ABSTRACT

(SARS-CoV 2) is a complex viral infection disease with a strong patho immune- physiological reaction contribution to its pathogenesis. Genetic with environment factors account for the majority of population susceptibility to this disease. This study is carried out to evaluate some immunogenic and molecular parameters such cytokines in some Iraqi patients with (SARS-CoV2) and detect the association of immune status and severity of disease. The study included 100 patients with COVID 19. And 30 apparently healthy individuals (as control).When some cytokines (IL-1 alpha, GM-CSF, IL-8, IL-6 and IL-2 receptor) have been measured in the sera of the studied groups. All these cytokines have revealed a highly significant elevation when detected among SARS-CoV2 in comparison with healthy control group, except GM-CSF has showed a significant elevation in SARS-CoV2 patients only. This results goes to establish growth (cytokines storm) which its drive to understand the develop molecular immune pattern as key step is to speedily ramp up research related to the production of an effective vaccine for the SARS-CoV2 respiratory syndrome.

Keywords: SARS-CoV2 respiratory syndrome, COVID19. Cytokines, Cytokines Storm.

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INTRODUCTION

COVID-19 is an infectious disease caused by the most recently discovered coronavirus (SARS-CoV-2). This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019(1–3).Coronaviruses (CoV2) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV) (4).The new coronavirus is spread from one person to another primarily through respiratory droplets generated when an infected person coughs or sneezes. According to recent reports, it may be possible that people infected with the novel coronavirus spread the virus before showing significant symptoms.(5,6) Some patients with confirmed infections with the novel coronavirus have showed little to no symptoms while others have become severely ill and died. Symptoms of infection include: Fever, cough and shortness of breath. At this time, it is believed that symptoms may appear in as few as 2 days or as long as 14 days after exposure. Scientists are working hard to understand this new virus and produce a vaccine. Antibiotics do not work against viruses; they only work on bacterial infections. Therefore, antibiotics should not be used as a means of prevention (6).

Cytokines are protein messengers that convey information between and within cells via specific cell surface receptor molecules. The release of specific cytokines into the systemic circulation has been observed in a variety of inflammatory disease including RA. Their concentration levels usually reflect disease severity and prognosis (7-9) However, since most cytokines are expressed transiently and can be induced or inhibited by other cytokines, it has been suggested that a cytokine network may exist in which cytokines regulate each (10, 11) . A complex cytokine network is involved in normal immune function, and this network is comprised of positive and negative feedback loops that enhance or suppress the response. Recent data indicate that many immune mediated diseases especially rheumatic diseases involve the abnormal regulation of cytokines. This can be manifested either by defective production of suppressive factors or by overproduction of proinflammatory cytokines (12) (Figure 1).

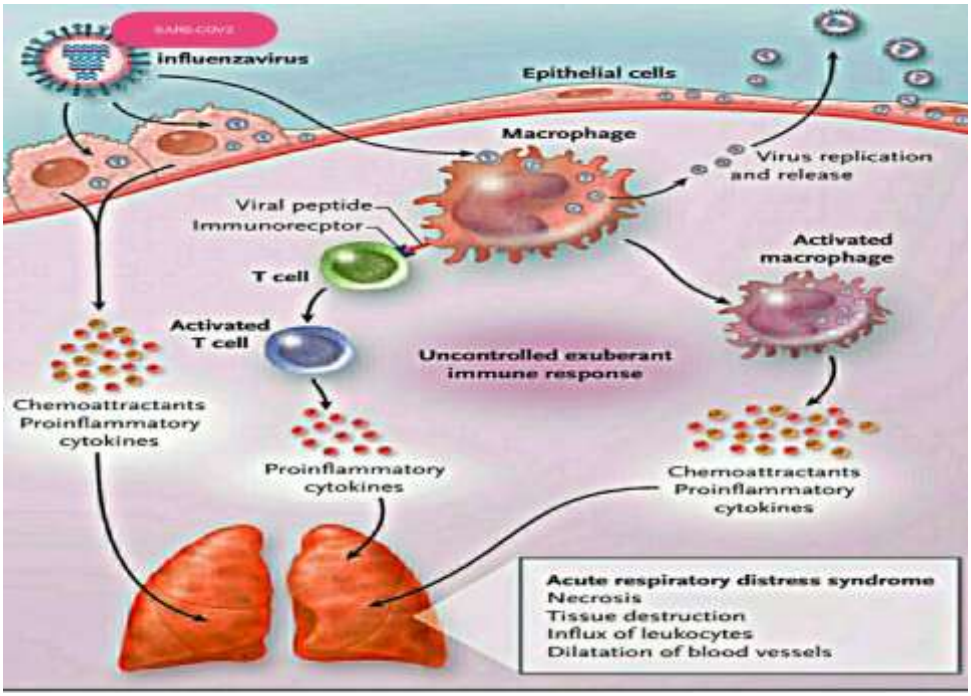

Figure 1: Proposed Mechanism of the Cytokine Storm Evoked by SARS- COV2 virus. The key element in generating the storm is an uncontrolled exuberant immune response to the virus, in which there is an outpouring of pro inflammatory cytokines and chemo attractants. An animated version of this figure is available at www.nejm.org

Cytokines are also divided into pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12 and IL-18) and anti-inflammatory cytokines (IL-4, IL-10, and TGF-β). In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation, and thus can lead to different clinical effects. Anti-inflammatory cytokines or cytokine antagonists counteract the effects of pro-inflammatory cytokines and therefore the relative concentration of a cytokine to its inhibitor or antagonist will determine its final effect.(15-13)
Interleukins (IL-s), This term was given in 1979 to a wide range of proteins, secreted by some leukocytes, acting upon other leukocytes; hence play a significant role in the development of an acute or chronic inflammatory response .(18-16 ,7)
Fourteen cytokines or chemokines were analyzed on 88 RT-PCR-confirmed severe acute respiratory syndrome (SARS) patients. IFN-g, IL-18, TGF-b, IL-6, IP-10, MCP-1, MIG, and IL-8, but not of TNF-a, IL-2, IL-4, IL-10, IL-13, or TNFRI, were highly elevated in the acute phase sera of Taiwan SARS patients. IFN-g was significantly higher in the Ab (þ) group than in the Ab (þ) group. IFN-g, IL-18, MCP-1, MIG, and IP-10 were already elevated at early days post fever onset. Furthermore, levels of IL-18, IP-10, MIG, and MCP-1 were significantly higher in the death group than in the survival group(18-20). For the survival group, IFN-g and MCP-1 were inversely associated with circulating lymphocytes count and monocytes count, but positively associated with circulating neutrophils count. It is concluded that an interferon-g-related cytokine storm was induced post SARS coronavirus infection, and this cytokine storm might be involved in the immunopathological damage in SARS patients that is mean a approach to describe cytokines storm in SARS -COV2 (21-24).

METHODS

Subjects study groups have been investigated, which include:
Patients group a total of 100 Iraqi SARS-CoV-2 patients, aged from 20-65years. Those patients were attending the consultant clinic for pulmonary disease and Intensive care unit in Hussain Teaching Hospital from March 2020 to March 2020. The committee of internal and pulmonary performed the clinical examination under the supervision of Dr. Khudhair Al-Asadi Healthy Control Group. Thirty apparently healthy person included in this study as a healthy control group, who have no history or clinical evidence of SARS-CoV-2 or any other chronic disease, and no obvious abnormalities, were selected from Blood Bank Donors (Figure 2).

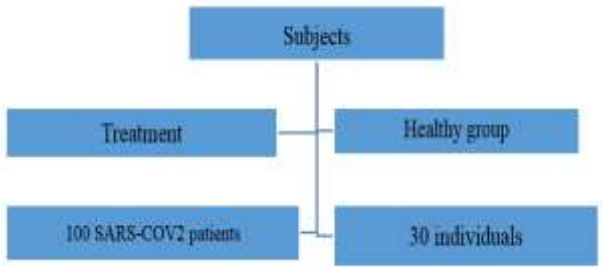

Figure 2: General design of the study

Enzyme Immunoassay for Estimation of serum Cytokines:
A- Interleukin-1α assay. Principle. The immunotech IL-1α enzyme immunoassay is intended for quantification of

human IL-α in plasma, serum or culture supernatant. It is a two immunological steps sandwich type assay, according to (4,25) also see (12, 17, 26).

In the first step the IL-1α captured by a monoclonal antibody bound to the wells of microtiter plate. In the second step, a monoclonal antibody linked to acetyl cholinesterase (ACE) conjugate is added. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromomgenic substrate. The intensity of the coloration is proportional to the IL-1α concentration in the sample or standard (4).

Principle. ELISA test is intended to be used for quantitative determination of native and recombinant human IL-8 or IL-6 in solution such as cell culture, serum or plasma. Interleukin kit is a solid sandwich enzymes linked immunosorbent assay (ELISA) (41, 42). A monoclonal antibody specific for IL-8 or IL-6 is coated on to wells of micro titer plate. Sample or standards are pipetted in to these wells, followed by the addition of biotinylated second antibody (conjugate). During the first incubation, the human IL-8 or IL-6 considered as an antigen binds stimultaneously capture to the antibody on one site, and to the solution phase biotinylated antibody on a second site. After the removal of excess second antibody, Streptovidin–peroxidase is added. This binds to the biotinylated antibody, after a second incubation and washing to remove all of the unbound enzyme. A substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of IL-8 or IL-6 present in the original specimens (43).

Principle. It is a two immunological steps sandwich type assay. In the first step the GM -CSF or IL-2R was captured by a monoclonal antibody bound to the well of microtiter plate. In the second step a biotinylated monoclonal antibody is added together with Streptavidine-peroxidase conjugate. The biotinylated antibody binds to the solid phase antibody – antigen complex, in turn, bind the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic (Substrate). The intensity of the coloration is proportional to the Granulocyte Monocyte – Colony Stimulating Factor (GM – CSF) or IL-2R concentration in the sample or standard (34, 35, 39).

Table 1: The Mean of some cytokines level among the sera of the studied groups (SARS-CoV-2 patients and apparently healthy control).

Cytokines	Number	Mean	Std. Deviation	t-test P-value	Significant
IL-2R (pg/ml) Healthy group patients	30 100	16.76 210.67	5.73 189.21	.0000	HS
GM-CSF (pg/ml) Healthy group patients RA patients	30 100	17.31 30.52	4.36 31.23	.0230	S
IL-8(pg/ml) Healthy group patients	30 100	11.33 134.40	2.22 164.25	.0000	HS
IL-1Alpha(pg/ml) Healthy group patients	30 100	10.15 40.38	2.49 36.94	.0000	HS
IL-6 (pg/ml) Healthy group patients	30 100	15.42 110.967	6.479 31.165	.0000	HS

Note: - IL-2 R: Interleukin -2 receptor, GM-CSF: Granulocyte/ Monocyte -colony stimulating factor, IL-8: Interleukin-8, IL-1 alpha: Interleukin-1 alpha, IL-6: Interleukin-6, pg: Pico gram. Cytokines participate in all phase of immune response, they affect proliferation, differentiation and migration of various cells in immune system and regulate both humoral and cellular immune response. The previous studies mentioned that inflammatory cytokines, such as interleukin 1 (IL-1α) have been implicated as important mediators in inflammatory diseases for instance, in patients with RA beside other cytokines can be elevated productions in SARS-CoV-2 sera such as IL-2R (23.25.28.37). So the concentration of some cytokines (IL-2Res, GM-CSF, IL-8, IL-1α and IL-6) has been estimated in the sera of the studied groups. It is clear that the concentration of IL-8, Il-1α and IL-6 highly elevated in the sera of SARS-CoV-2 patients in comparison with healthy control group with significant differences (P<0.001).

On the other hand, there is significant differences in the concentration of GM-CSF in the sera of treatment in comparison with healthy group (P<0.005), as shown in table (1).

Patients with SARS-CoV-2 showed a highly significant elevation of IL-2 receptor in sera compared with healthy control.

RESULTS

This result seems to agree with previous studies which had shown concentration of IL-2 receptor that significantly higher in SARS-CoV-2 patients compared with healthy control. These findings indicated that serum levels may reflect the degree of immune activation with affected joints (Keystone et al., 1988; Crilly et al., 1993). Other workers had found that the levels are higher in synovial fluid than in serum samples of patients with SARS-CoV-2 (24, 25, 40, 42).

The connection between cytokines among SARS-CoV-2 patients is obtainable in table (2). It has been found that there have been no significant difference between all cytokines among patients.

Table 2: The correlation between cytokines among RA patients

ROW	Il-8 pg/ml	GM_CSF pg/ml	IL-2R pg/ml	IL-6 pg/ml
IL-1 Alpha(pg/ml)				
Pearson Correlation	-0.101	0.084	-0.054	0.249
P-value	0.318	0.406	0.591	0.185
Significant	NS	NS	NS	NS
IL-8(pg/ml)				
Pearson Correlation		0.075	-0.054	0.093
P-value		0.458	0.591	0.626
Significant		NS	NS	NS
GM-CSF (pg/ml)				
Pearson Correlation			-0.059	-0.115
P-value			0.560	0.544
Significant			NS	NS
IL-2R (pg/ml)				
Pearson Correlation				-0.115
P-value				0.544
Significant				NS

Concentrations of IL-2 receptor also increase in the chronic autoimmune rheumatic diseases and are useful in assessing and monitoring the response to treatment in diverse range of disorders associated with immune activation and immune deficiency (Rubin, 1990). The IL-2 receptor concentrations are elevated and shed by activated T-cells which are found in rheumatoid patients(4,12,27). It has been suggested to be a potentially useful adjunct in monitoring disease activity (22.23.24). Other studies indicated that serum IL-2R levels were highly correlated with disease activity, indicating that measurement of IL-2R may be a useful clinical marker in the future (17.27.28.37). Moreover, Suenaga et al. (1998) suggest that IL-2 receptor measurements could be helpful for the early diagnosis of SARS-CoV-2 in patients with joint pain, but without symptoms of bone or joint destruction(4, 20, 28, 29).

In our study, we observed significant difference of GM-CSF levels in SARS-CoV-2 cases when was compared with controls. Similarly, Fiehn et al. (1992) demonstrated statistically significant difference of GM-CSF in SARS-CoV-2 populations when was compared with that in healthy controls. GM-CSF has been postulated to be an important element in disease (39). This cytokine is potent macrophage inducer and increases expression of HLA-DR molecules more than IFN-γ and regulates neutrophile function (39, 40, 41, 42)

Chemokines including IL-8, have potent chemotactic activity for immune system cells. This cytokines do not only participate in the inflammation phase of SARS-CoV-2 but also they participate in the vascular proliferate phase of this disease (19, 20, 24, 30). The current study showed that the serum level of IL-8 in SARS-CoV-2 patients is significantly highly than those healthy controls. These data confirmed the previous study which denoted serum IL-8 level revealed highly significantly differences in comparison with healthy control groups ((13,14,20,31)). Great variation was seen in cytokine concentration between different SARS-CoV-2 patients, although interestingly, we observed that not all cytokines were elevated in the same sample. This variation among different cytokines in the sera of SARS-CoV-2 patients reflects the intricate cytokine network and its regulatory function (39, 42).

DISCUSSION AND CONCLUSION

Moreover, among cytokines, which play a key role in SARS-CoV-2 pathogenesis is IL-1 that represents a central mediator of joint destruction. So continuous elevation of IL-1 may expose the patient to the disease worse prognosis, which is associated with chronic active respiratory syndrome. IL-1α and IL-1β are produced as precursor peptides, which are proteolytically processed and released in response to cell injury and thus induce apoptosis (32-35)

In present study, the level of serum IL-1α was different significantly among SARS-CoV-2 cases and controls. Elevated levels of IL-1α will continue to be a diagnostic target for inflammatory diseases like SARS-CoV-2. IL-1α is not commonly found in the circulation or in body fluids except during severe disease, in which case the cytokine may be released from dying cells. Consequently, it is less easy to get an impression of the role of IL-1α in the pathogenesis of inflammatory disease (6, 8, 11, 16, 32).

Eventually, estimation of IL-6 represents one of the inflammatory cytokines that drive inflammation in SARS-CoV-2 and cause joint damage (Shankar and Handan, 2004). The results of IL-6 concentration was highly significant in SARS-CoV-2 patients in comparison with healthy group. These findings are concurrence with the study done by El-Safari, (2008) who has recorded on increased concentration of IL-6 among SARS-CoV-2 patients. This cytokine is identified as a factor that induces the final maturation of B cells into plasma cells and involved in diverse biologic process, such as the activation of T cells, the induction of the acute phase response (14, 17, 24, 29, 34, 36-38).

There is Some of strategies applied in countries and a part of the cytokine storm literature since the pandemic of SARS and MERS a group immunologist, perhaps related this term mindful of the recent Desert Storm war, coined'cytokine storm' to describe their observations in graft-versus-host disease (GVHD). As we have reviewed, the notion that excessive release of pro-inflammatory cytokines causes GVHD pathology already existed. For instance, recently, a polyclonal anti-tumour necrosis factor (anti-TNF) antibody was shown to reduce mortality in a mouse model of this condition. The term next appeared in COVID 19 as a description of the Severe influenza remains unusual in its virulence for humans. Complications or ultimately death arising from these infections are often associated with hyper induction of pro inflammatory cytokine production, which is also known as'cytokine storm'. For this disease, it has been proposed that immunomodulatory therapy may improve the outcome, with or without the combination of antiviral agents. Applied plans on how various effectors of the immune system initiate the cytokine storm and exacerbate pathological damage in hosts. also identify some of the current immunomodulatory strategies for the treatment of cytokine storms in severe respiratory syndromes, including corticosteroids, peroxisome proliferator-activated receptor agonists, sphingosine-1-phosphate receptor 1 agonists, cyclooxygenase-2inhibitors, antioxidants, anti-tumour-necrosis factor therapy, intravenous immunoglobulin therapy, statins, arbidol, herbs, and other potential therapeutic strategies..

CONFLICT OF INTEREST

None

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