ORIGINAL ARTICLE

Complement Component C3 and C4 in Patients COVID-19 Induced Cytokine Released Storm

HUSAM OUDAH ALJWAID[1*], THAMER J GHALI[1], ALI A. AL-HISNAWI [2,3], AND KASIM SAKRAN ABASS[4]
[1]*Department of Medical Laboratory Technique, College of Medical Technology, National University of Science and Technology, Thi-Qar, Iraq*
[2]*Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq.*
[3]*Researchs Unit of Al- Razzaza Lake and West of Euphrates River, College of Science, University of Kerbala, Kerbala, Iraq.*
[4]*Department of Pharmacology and Toxicology, College of Pharmacy, University of Kirkuk, Kirkuk, Iraq*
**Corresponding author email: haljwad@yahoo.com*

ABSTRACT

Aim: To evolution the complement component C3 and C4 in patients COVID-19 induced cytokine released storm.
Methods: The case control study was conducted in Imam AL-Hussain Hospital in Thi-Qar Governorate from February 2020 to September 2020. In our study was forty of them with cytokine storm-induce SARS-COVID 19 depended on clinical feature and lab testing. Hence, fifty persons normally as a control group. The age group between 25 to up to 80 age and both gender included in the current study.
Results: The level of IL6 in patients was 107.41±18.18 Pg/ml, this value showed to be in cytokine storm phenomenon while the control group was 17.41±5.45 Pg/ml. The differences were highly significant different (*P*= 0.00). Complement component of C3 and C4 levels of infected patient were (2.47±0.399) and (0.655±0.133), respectively showed highly elevated than the normal range .
Conclusion: However, ferritin was out of the rang (>2000 ng/mL) and C-reactive protein (CRP) was highly elevated (134.05±22.67mg/L) showed highly elevated than the normal range. The mean of was ranged interleukin 6 >107.41, ferritin >2000 ng/mL, while, C.R. Protein 134.05mg/L suggested patient with cytokine release storm. However, several anti-cytokine approaches have proven effective in reversing cytokine storm syndromes.
Keywords: Complement; Thi-Qar; C3; C4; COVID-19; Cytokine

INTRODUCTION

Cytokines storm is a physiological reaction in which the immune system causes an uncontrolled and excessive release of a large amount of pro-inflammatory cytokines. The host immune response to the SARS-CoV-2 virus is hyperactive resulting in an excessive inflammatory reaction. Several studies recorded cytokine profiles from patients infected with this virus suggested that the "cytokine storm" imbedded directly with prognosis of severe of lung injury and multi-organ failure[1,2]. Most patients with severe COVID-19 displayed significantly increased serum levels of pro-inflammatory cytokines (e.g. IL-6, IL-1β, IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP-10, MCP-1, CCL3, and TNFα)[3]. IL-6 plays a key role in the pathogenesis of the cytokine storm owing to its pleiotropic properties. Several studies showed that the serum levels of IL-6 are increased in COVID-19 patients and that its circulating levels are positively related to disease severity[4,5]. IL-6 level was >100pg/mL in three persons. IL-6 >100pg/mL might represent the emergence of "inflammatory storm[6]. Elevated ferritin levels due to secondary haemophagocytic lymphohistiocytosis (sHLH) and cytokine storm syndrome have been reported in severe COVID-19 patients[7]. CRP levels are increased in COVID-19 patients and it has been shown that survivors had median CRP values of approximately 40 mg/L, while non-survivors had median values of 125mg/L, indicating a strong correlation with disease severity and prognosis[8]. The deregulated activation of multiple innate immune pathways, including the complement system, the cytokine circuitry, and several pro-coagulant and thrombogenic pathways, is believed to fuel a hyper-inflammatory state that drives ARDS and may lead to multiple organ injury in COVID-19[9,10]. C3 activation is positioned upstream of these proinflammatory innate immune circuits that contribute to thromboinflammation and organ damage in COVID-19[11]. Cytokine release through excessive complement 1211ignaling on pro-inflammatory macrophages and other leucocytes is thought to contribute to the cytokine storm associated with sepsis and MOF. Furthermore, blockade of complement anaphylatoxin C5a in experimental sepsis virtually prevents the appearance of MOF and improves the outcome[12].

Recent research has shown that SARS-CoV-2 S protein is heavily glycosylated with residues that are rich in L-fucose or D-mannose[13,14]. Hypothetically, the virus could activate the complement pathway through interaction with a lectin, expressed at the alveolar epithelium (15,16) and also in the circulation[17,18]. Viral particles entering the circulation would come into contact with MBL as well as the ficolins and CL-11. It is therefore plausible that interaction of SARS-CoV-2 with these lectins triggers the inflammatory and coagulation cascades in the lung and circulation. They reported finding strong immune-histochemical staining for MBL, MASP-2, C4, C3 and C5b-9 in the lung[19]. As well as, suggested that type I and type II alveolar epithelial cells were main tissue targets for complement deposition. Moreover, the presence of MBL and MASP-2 infers a role for the lectin pathway in this process though it does not establish causality. Serum C5a levels were elevated in their patients with severe lung disease, providing indication of systemic activation of complement or leakage of the activated fragment from the diseased lung. Simultaneously (20), reported on a group of five COVID-19 patients who

died with respiratory failure and possibly coagulopathy. Here, the predominant lesion in the lung was micro-vascular thrombosis associated with MASP-2, C4 and C5b-9 deposition with colocalisation for SARS-CoV-2 S protein, but with relative sparing of alveolar cells

The present study was conducted to understood the action of complement component C3 and C4 in cytokine released storm induced COVID-19.

MATERIALS AND METHODS

Patients data diagnosed with COVID-19 pneumonia whose blood serum were collected and tested of IL-6, Ferritin, C-reactive protein (CRP) and complement C3,C4 in Imam AL-Hussain Hospital in Thi-Qar Governorate in Iraq. The diagnosis of severe or critical patients were depending on clinical sign and symptom, respiratory distress, RR ≥30 times / minute, requiring treatment in ICU, oxygen saturation ≤93%; patient under mechanical Ventilation. Shock occurs and other organ failure may be occure , CT. Scan imaging >50% lung progress and RT. PCR in addition Lab test of ferritin >2000 ng/mL. CRP > 125 Mg/L. IL6 > 100 Pg/ml suggested patient severing from cytokine storm phenomenon.

Cytokines storm-related biomarkers: Complement C2, C4, IL-6, Ferritin, CRP, CBC, LDH, D. dimer, troponin, liver function test and renal function test were conducted and completed in the Iraq laboratory. Interleukin-6 was detected by ELISA technique according to the manufacturer's instruction. Ferritin was detected by Roche electrochemiluminescence method. C3, C4 and CRP were detected by immunoturbidimetry method.

Statistical analysis: The data were analyzed using description statistic (mean and standard deviation) independent sample *t* test the level significant was set at $P < 0.05$ SPSS (Statistical Packing for Social Sciences) version 20 .

RESULTS

Patient included in this study: Ninety persons including in our study. Forty of them with cytokine storm-induce SARS-COVID 19 depended on clinical features and lab testing . Fifty persons normally as a control group. The age group between 25 to up to 80 age and both gender included in the current study.

Mean ± SD serum levels of IL6 in controls and patients group: The level of IL6 in patients was 107.41±18.18 Pg/ml, this value showed indicated to cytokine storm phenomenon which revealed higher significant differences to the control group 17.41±5.45 Pg/ml (*P*=0.00) Table 1.

The mean ± SD serum Complement C3 and Complement C4: Complement component of C3 and C4 levels of patients were (2.47±0.399) and (0.655±0.133) respectively, showed highly elevated than the normal range. Ferritin was out of the rang (>2000 ng/mL) while CRP was highly elevated (134.05±22.67mg/L) than the normal range . Ferritin and CRP values in the current study similar to that happen in cytokines storm Table 2.

Table 1. Mean ± SD serum levels of IL6 in patients and control groups

	No	IL6 Range (1.5 - 48) Pg/ml	*P value*
Patients	40	107.41 ± 18.18	0.00
Control	50	17.41 ± 5.45	
Total	90		

Table 2. The mean ± SD serum C3 , C4 , Ferritin and CR. Protein in controls and patients group.

	NO	Complement C3	Complement C4	Ferritin	CRP
Value	40	2.47±0.399	0.655±0.133	>2000 ng/mL	134.05±22.67
Normal range		(0.9-1.8)g/L		Adult males (20-390) Adult female (10-150)	<10 Mg/L

DISCUSSION

Interleukin 6 and cytokine storm : IL6 level in the current study was highly elevated in patients group, this funding was in agreement with[21] who reported that serum samples of patients with cytokine released storm have elevated levels of IL-6. Furthermore, support to the present results, (22) suggested that IL-6 >100pg/mL might represent the emergence of "inflammatory storm" induced by SARS COVID-19. IL-6 is an important member of the cytokine network and plays a central role in acute inflammation[23]. IL-6 can promote T-cell population expansion and activation and B-cell differentiation, regulate the acute phase response, and affect the hormone-like properties of vascular disease, lipid metabolism, insulin resistance, mitochondrial activity, neuroendocrine system and neuropsychological behavior[24]. An excessive generation of IL-6 during infections and tissue injury is believed to be responsible for cytokine release syndrome[25]. IL-6 dysregulation leads to the activation of complement and coagulation, inducing vascular leakage[26,27]. In addition, IL-6 peak levels have been associated with pulmonary disease progression in COVID-19 (28). Importantly, on day 2, our patients showed elevated IL-6 levels. In a recent retrospective cohort study of 201 patients in Wuhan, China[29]. The acute phase response of cytokine storm is relatively over-exaggerated. Since high serum levels of cytokines are inversely related to the total lymphocyte count, low levels of cytotoxic T cells may contribute to reduced viral clearance[30].

Ferritin, CRP and cytokine storm : Serum ferritin and CRP were highly elevated in COVID-19 patients, the serum inflammatory biomarkers may have a role in assessing disease progression, since a poor prognosis in COVID-19 appears to be correlated with abnormal serum markers and clinical attributes of cytokine storm[31]. Ferritin is a key mediator of immune dysregulation, especially under extreme hyperferritinemia, via direct immune-suppressive and pro-inflammatory effects, contributing to the cytokine storm[32]. CRP is an acute phase reactant produced by the

liver largely in response to IL-6, and CRP levels serve as a reliable surrogate for IL-6 bioactivity[33,34]. CRP levels are increased in COVID-19 patients and it has been shown that survivors had median CRP values of approximately 40mg/L, while non-survivors had median values of 125mg/L, indicating a strong correlation with disease severity and prognosis[35]. Other predictors of poor outcome include the serum levels of ferritin and lactate dehydrogenase (LDH). Elevated ferritin levels due to secondary haemophagocytic lymphohistiocytosis (sHLH) and cytokine storm syndrome was reported in severe COVID-19 patients. Based on body temperature, organomegaly, blood cell cytopenia, triglycerides, fibrinogen, AST and ferritin levels, a predictive has been proposed to estimate the risk of developing secondary haemophagocytic lymphohistiocytosis[36]. In some series, peak CRP levels and fold change in CRP have identified patients at risk for severe CRS.

Complement C3 and C4 and cytokine storm: The levels of Complement C3 and C4 levels were significantly higher in patients group than the control group and this finding was in agreement with Risitano (2020), who observed that the patients with the severe COVID-19 showed widespread complement activation, characterized by the C3a generation and C3-fragment deposition. C3 activation is positioned upstream of these pro-inflammatory innate immune circuits that contribute to thromboinflammation and organ damage in COVID-19[37]. Recent studies conducted by[38] showed that the strong staining for immune-histochemistry analysis of lung tissue from patients who died of COVID-19 the complement components mannose-binding lectin C4, C3, in alveolar epithelial cells. COVID-19 related inflammatory responses could also be induced by the dysregulation of the complement system, a critical component of the host innate immunity. Although it is aimed to prevent viral replication, excessive activation of complement components such as C3, C3a, C5, C5a, and mannose binding lectin associated serine protease (MASP2), possibly by viral proteins, has been associated with increased inflammation both in SARS-CoV and SARS-CoV-2 infections[38].

CONCLUSIONS

In conclusion, this is the first paper that provided original data concerning an complement component C3 and C4 in patients COVID-19 encouraged cytokine released storm. The inhibit complement activation to prevent complement-mediated inflammatory reactions that may due to tissue destructive inflammation in COVID-19 patients. Furthermore the C3 inhibitors recommended to decrease complement activation, complement-mediated micro-vascular injury and coagulopathy. Interleukin-6 receptor antagonist to cilizumab useful in decrease effected of cytokine storm Finally, in spite of this paper, further studies are required under different laboratories and different complement components compounds in order to improve and to increase our knowledge about this very interesting infection with COVID-19.

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