University of Diyala

College of medicine

Department of microbiology

Review article in

Role of cytokines in COVID-19

A project submitted to the council of College of Medicine / University of Diyala in Partial fulfillment of the Requirements for the Degree of bachelor in medicine and general surgery

Student name:

Jassim Muhammed

Supervised by:

Dr. Hiba Hadi

2022

بسم الله الرحمن الرحيم

(يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ
اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انشُزُوا فَانشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ).

صدق الله العلي العظيم

من سورة المجادلة الآية 11

Dedication

I dedicate this small project to the most precious and my first teachers my parents may Allah bless them.

Acknowledgments

I owe a great many thanks to the great people who helped and supported me to complete this project**.**

My deepest thanks to (**Dr. Hiba Hadi**) my teacher and supervisor for guiding me in the article by correcting the mistakes and giving his valuable opinions in many aspect in both scientific facts and literature ones. I hope all the best and successes to her in her career and future.

Introduction

In December 2019, a new corona virus appeared, the SARS-CoV-2 which belongs to *the Coronaviridae* family, (from the latin corona = crown, which is due to their morphology as spherical virions with a core shell and surface projections resembling a solar corona). These viruses are enveloped, with a positive sense single-stranded RNA genome (26–32 kb). The virus has spread so rapidly worldwide, and the growing cases were so alarming that the WHO Emergency Committee declared it a global health emergency. It was not the first-time countries have been affected by this family of viruses, as in the past two decades, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), caused two large-scale aggressive pandemics known for their silent spreading (1).

It is showing symptoms slowly over an incubation period of about 2 weeks, necessary for the virus to replicate in the upper and lower respiratory tract and forming lesions. The disease outcome depends on several factors, including the host immune status. Indeed, most people infected with the SARS-CoV-2 will experience mild to moderate respiratory illnesses and recover without requiring special treatment. However, older people, and especially the ones with medical history like cardiovascular disease, diabetes, chronic respiratory disease and cancer, are more likely to develop serious illness (2). It was reported that up to 17% of patients infected with the virus, develop an acute respiratory distress syndrome (ARDS) which was associated with organ injury like acute renal injury, acute respiratory injury and septic shock, which can eventually lead to organ failure (3).

It has been well described that ACE-2 (angiotensin-converting enzyme - 2) receptor acts as a site for cellular binding for SARS-CoV-2.

However, its affinity for the ACE-2 receptor is 10–20 folds higher than other coronaviruses, amounting to its higher transmissibility. ACE-2 receptor expression is found in pulmonary and extrapulmonary tissues including type 2 alveolar epithelial cells in the lungs, bronchus, nasal mucosa, heart, esophagus, kidney, stomach, bladder, and ileum making them susceptible entry sites. With the help of TMPRSS2 (transmembrane serine protease 2), the virus is endocytosed by proteolytic cleavage of ACE-2, which is followed by its cytosol replication and cell-to-cell transmission (4). The common clinical manifestations of the disease are reported as respiratory illness and high-grade fever. A few studies also reported other systemic manifestations such as neurologic symptoms and renal complications. More reports on rare clinical conditions such as stroke induced deaths and large vessel occlusions in the younger population are also reported (5).

SARS-CoV-2 elicits an innate immune response and causes an immediate rise in the neutrophils and other immune cells along with a marked reduction in the T cells (CD4+ and CD8+). However, the reduction of T cells along with the enhanced production of IL-6 and IL-8 has been reported as a remarkable characteristic of SARS-CoV-2 infection (6). The systemic inflammatory response along with other co-morbid factors may lead to other diverse complications, such as cardiac failure, renal dysfunction, hepatic damage, and multiple organ disruption.

In this review, we will discuss the role of cytokines and the cytokine storm in COVID-19 infection.

Literature review

Cytokines are a group of polypeptide signaling molecules responsible for regulating a large number of biological processes *via* cell surface receptors. Key cytokines include those involved in adaptive immunity (e.g., IL-2 and IL-4), proinflammatory cytokines and interleukins (ILs) (e.g., interferon (IFN)-I, -II, and -III; IL-1, IL-6, and IL-17; and TNF-α); and anti-inflammatory cytokines (e.g., IL-10). In response to stress-generating internal processes (e.g., cancer or microbial infection), host cells secrete cytokines with a highly important role in cell metabolism reprogramming as a defensive response (7). In the short time since the emergence of COVID-19, numerous studies have described abnormal levels of the following cytokines and chemokines in the patients: IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, M-CSF, G-CSF, GM-CSF, IP-10, IFN-γ, MCP-1, MIP 1-α, hepatocyte growth factor (HGF), TNF-α, and vascular endothelial growth factor (VEGF). The key point in SARS-CoV-2 infection could be the depletion of antiviral defenses related to innate immune response as well as an elevated production of inflammatory cytokines (8).

Interleukin 1 (IL-1) actively participates in the inflammatory response to infection, and its main sources are activated monocytes and macrophages SARS-CoV-2 appears to act on the activation and maturation of IL-1β, which in turn activates other proinflammatory cytokines, such as IL-6 and TNF-α. Hence, IL-1β forms part of the cytokine storm produced by coronavirus infections. Yang et al. detected elevated levels of the antagonistic receptor of IL-1 (IL-1Ra) in 14 severe cases of COVID-19, and this marker has been associated with increased (9).

IL-2 plays a key role in the proliferation of T cells and in the generation of effector and memory T cells. It is involved in adaptive

immunity and increases glucose metabolism to promote the proliferation and activation of T, B, and NK cells. The absence of this interleukin has been associated with a poor control of effector cells and the consequent development of autoimmunity. Elevation of this interleukin and its association with disease severity have also been reported in patients with other types of coronavirus (11).

Interleukin 6 (IL-6), also called "hepatocyte stimulating factor", "hybridoma growth factor" or "B cell stimulating factor", is a glycoprotein expressed in many cell types such as T and B lymphocytes, monocytes/macrophages, dendritic cells, fibroblasts, and endothelial cells. Its plasma concentration rises during septic shock and various attacks such as trauma and burns. As a pleiotropic cytokine, IL-6 stimulates the growth and differentiation of B lymphocytes and increases the generation of platelets. It also activates the hepatocytes and induces the secretion of inflammation proteins such as reactive protein C (CRP) and fibrinogen. Therefore, IL-6 is involved in the regulation of the immune system, hematopoiesis, inflammation and is a key player in the Cytokine Release Syndrome. The plasma level of IL-6 in COVID-19 patients surpasses the superior limit of normal value in both groups for which the oxygen saturation (SpO2); $SpO2 \geq 90\%$ and the $SpO2 < 90\%$. In many studies, the higher level of IL-6 in serum was positively correlated with the COVID-19 severity (12).

Tumor necrosis factor (TNF-α), the most studied cytokine in the TNF superfamily, is another key factor in the COVID-19 CRS pathophysiology. It is produced by several cell types, including macrophages, mast cells, T cells, epithelial cells, and smooth muscle cells in the airways. Its synthesis is mainly stimulated by PAMPs and IL-1, via NF-κB activation, and also IL-17. In healthy subjects, inhaling TNF-α

triggers bronchial hyper-responsiveness and inflammation of the airways induced by increased neutrophils recruitment. This cytokine can also cause T cells apoptosis (13). TNF-α promotes the production of other cytokines such as the IL-1β and IL-6. Many studies have found that severe COVID-19 cases display increased plasma levels of TNF-α (14).

Interleukin 1β (IL-1β) is one of the most important inflammatory pleiotropic cytokines that plays an important role in inflammatory diseases and seems to be involved in COVID-19 CRS**.** The principal sources of this cytokine are macrophages, monocytes, dendritic cells, B lymphocytes, neutrophils and synovial fibroblasts. The IL-1β effects are quite similar to those of TNF-α, and promote the production of several hematopoietic factors, in particular IL-6 (15). In addition to its immune functions, IL-1β induces the synthesis of type 2 cyclooxygenase (COX-2), phospholipase A (PLA) and inducible nitric oxide synthase (iNOS). These enzymes are responsible for the formation of nitric oxide (NO), prostaglandin E2 (PGE2) and the platelet activating factor (PAF), involved in fever, pain, vasodilation, hypotension and inflammation components. Moreover, IL-1β increases the expression of chemokines and adhesion molecules, especially in mesenchymal and endothelial cells; thus, promoting the infiltration of immunocompetent cells into injured tissue (16).

M-CSF, also known as colony-stimulating factor-1, is a primary growth factor that belongs to the family of colony-stimulating factors. It regulates the growth, proliferation, and differentiation of hematopoietic cells, including monoblasts, promonocytes, monocytes, macrophages, and osteoclasts. It is secreted by various cell types, including monocytes, fibroblasts, osteoblasts, stromal cells, endothelial cells, and tumor cells. The actions of M-CSF are mediated by a type III tyrosine-kinase receptor. It requires the synergic action of IL-1 and IL-3 during the early

differentiation of myeloid line cells, while at subsequent stages it can directly control the proliferation and differentiation of mononuclear cells of the phagocytic system. M-CSF expression increases during infectious processes, augmenting the production of myeloid cells (17). Liu et al. found significantly elevated levels of this factor in patients with COVID-19 and associated the hyperexpression of this and other cytokines with lung damage, which may assist in predicting disease severity (18).

Granulocyte colony-stimulating factor (G-CSF) is essential for the proliferation and maturation of polymorphonuclear granulocyte cells (PMNs), preparing the organism for defense against invasion by certain pathogens. The immunological functions of PMNs in infections include chemotaxis, phagocytosis, and the release of lysosomal enzymes and other signaling molecules. Thus, G-CSF has hematopoietic growth factor properties and simultaneously functions as a mediator of anti-infectious and anti-inflammatory responses. G-CSF levels were elevated in patients with COVID-19 and even higher in those requiring ICU admission. This could lead the multiorgan failure related to severe cases (19).

Interferon gama (IFN-γ)is a type-II IFN produced by a wide variety of lymphocyte cells, including CD4+ and CD8+ T cells, Treg cells, FoxP3+ CD8- T cells, B cells, and NK cells. Monocytes, macrophages, dendritic cells, and neutrophil granulocytes can also produce this cytokine. Although numerous cells can be the source of IFN-γ, it is mainly produced by T and NK cells. MSCs can also secrete low IFN-γ levels to regulate hematopoiesis (20). *Huang et al.* found that serum IFN-γ levels were higher in patients with COVID-19 than in healthy individuals and proposed that the elevation of this and other cytokines might result from the activation of Th1 and Th2 cells (11).

Cytokine storm

Although the concept of an uncontrolled, cytokine-mediated response was already viable in the 1980s, first described in relation to malaria and sepsis, and subsequently in 2000s in the context of pancreatitis, variola virus and influenza virus H5N1, the first occurrence of the term 'cytokine storm' (CS) dates back to 1993 when it was reported in the context of graft-versus-host disease (GVHD). CS can be directly induced by a broad range of infections and by certain drugs. In the latter scenario, it is described as 'infusion reaction' or 'cytokine release syndrome' (21).

Mechanistically, a stressed or infected cell, through receptor-ligand interactions, activates large numbers of white blood cells, including B cells, T cells, natural killer cells, macrophages, dendritic cells and monocytes. This results in a release of inflammatory cytokines, which activate more white blood cells in a positive feedback loop. CS starts locally post-primary infection and spreads throughout the body via systemic circulation. The classical signs of inflammation calour (heat), dolour (pain), rubor (redness), tumour (swelling or oedema) and loss of function—are observed. Initially, the localized response is meant to eliminate the trigger and involves protective mechanisms, i.e. increase in blood flow, facilitation of leucocyte extravasation and delivery of plasma proteins to the site of injury, increase in body temperature (advantageous in case of bacterial infections) and pain triggering (warns the host of the occurring challenge) (22).

There are many therapeutic strategies to decrease the impact of cytokine storm. Tocilizumab (TCZ) is a recombinant humanized anti-human IL-6 receptor monoclonal antibody, preventing IL-6 binding to its receptor to exert the immunosuppression promoted by IL- 6. Michot et al. (23) reported that 42-year-old male suffering from respiratory failure

due to SARS-CoV-2 infection. After 4 days of TCZ treatment, the CRP decreased from 225 to 33 mg/L and ultimately clinically fully recovered. Similarly, some case reports showed TCZ is an efficacy and safety approach in COVID-19, even patients with other diseases combined, such as multiple myeloma, end-stage renal disease, and sickle cell disease (23).

Glucocorticoid therapy is used widely among critically ill patients with other coronavirus infections (e.g., SARS, MERS). Corticosteroids have been administered to ICU patients infected with SARS-CoV-2. Glucocorticoids exhibit pharmacologic effects at any therapeutically relevant dose through classic genomic mechanisms. Some immunosuppressive effects are based on transactivation, and glucocorticoid induces gene transcription and protein synthesis of NF-κB inhibitors and lipocortin-1. Through inhibition of NF-κB signaling, glucocorticoids induce inhibition of synthesis of downstream proteins such as IL-1, IL-6, granulocyte-macrophage colony-stimulating factor, and inducible cyclooxygenase-2 (24).

Cytokine adsorption involves using a method, such as extracorporeal membrane oxygenation (ECMO), to filter harmful substances directly. An extracorporeal cytokine hemoadsorption device called Cytosorb R has been reported to capture and reduce inflammatory mediators. Bruenger and colleagues reported that the plasma level of IL-6 and procalcitonin decreased in one patient with severe ARDS after Treatment with ECMO using a hemoadsorption device (25).

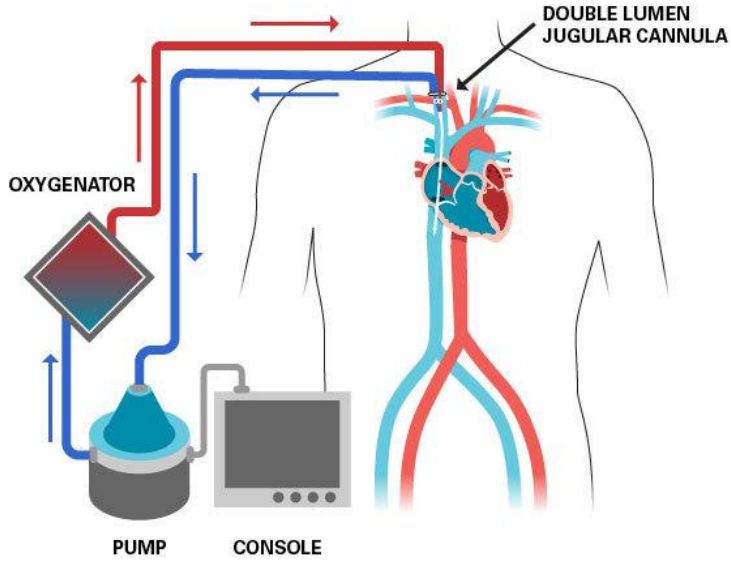

Figure 1. ECMO

Discussion

Th1 activation is a key event in the activation of specific immunity. However, unlike SARS patients, patients with COVID-19 also have elevated levels of Th2 cell secreted cytokines (such as IL-10), which inhibit the inflammatory response. The serum levels of IL-6 in patients with COVID-19 are positively correlated with the severity of the disease (27).

IL- 1 levels are related to the virulence of the process, and significantly higher serum levels have been observed in SARS-CoV-2 cases with severe symptoms than in mild cases or in those infected with the 2003 SARS-CoV or 2012 MERS coronavirus (10). IL-2 participates in the prevention of autoimmune diseases and is essential to control immune responses and maintain self-tolerance so it absence is due to exaggerated immune response (11).

It is likely that elevated IL-6 level reflects the cytokine-mediated hyperinflammatory state as evidenced by the similarly predictive values for CRP level. Further, even though IL-6 and CRP levels are significantly

elevated in patients requiring ventilation, they are relatively low compared with the levels observed in patients with septic shock (26). IL-6 is essential for the generation of T helper 1. The excessive IL-6 may explain the overactivated Th1 cells observed in COVID-19 patient (28)

TNF- alpha is increased in COVID-19 infection. Such increase was also associated with disease severity and inversely correlated with the reduction of T lymphocytes. It has been reported that the prevalence of severe and complicated cases of COVID-19 was lower in patients with anti-TNF-α therapy compared to patients taking steroids (14).

The ability of CD4+ T cells to produce interferon (IFN) was also significantly boosted in very ill patients. In extremely severe individuals, CD4+ T cell hyperfunction will trigger macrophage activation syndrome, which will result in cytokine storm. (29). The over-production of inflammatory IFN-g and chemokines might be resulting from the lack of IL-10- mediated down-regulation of the immune responses to SARS-CoV infection (11).

Conclusion

Cytokines has very important role in the normal physiology of the human body and can be of significant importance in diagnosis of diseases. Based on what we reviewed, we found an elevation in the serum levels of many cytokines in COVID-19 patients such as IL-1, IL-6, TNF, IFN, and we found decrease in one cytokine (IL-2).

Recommendation

We recommend to conduct more studies on the role of cytokines and biomarkers in COVID-19 specially in Diyala province to determine which cytokine could be of diagnostic and prognostic value in future.

References

1. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. Journal of pharmaceutical analysis. 2020 Apr 1;10(2):102-8.
2. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The lancet. 2020 Feb 15;395(10223):514-23.
3. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The lancet. 2020 Feb 15;395(10223):507-13.
4. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Frontiers of medicine. 2020 Apr;14(2):185-92.
5. Roe, K. Explanation for COVID-19 infection neurological damage and reactivations. *Transbound. Emerg. Dis.* 2020, 67, 1414–1415.
6. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, Ogishi M, Sabli IK, Hodeib S, Korol C, Rosain J. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4570.
7. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2014 Nov 1;1843(11):2563-82.
8. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine & growth factor reviews. 2020 Aug 1;54:62-75.

9. Yang Y, Shen C, Li J, Yuan J, Yang M, Wang F, Li G, Li Y, Xing L, Peng L, Wei J. Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. MedRxiv. 2020 Jan 1.
10. Qin C, Ziwei MP, Tao SY, Ke PC, Shang MM. Dysregulation of immune response in patients with COVID-19 in Wuhan, China; Clinical Infectious Diseases; Oxford Academic. Clinical Infectious Diseases. 2020.
11. Huang KJ, Su IJ, Theron M, Wu YC, Lai SK, Liu CC, Lei HY. An interferon-γ-related cytokine storm in SARS patients. Journal of medical virology. 2005 Feb;75(2):185-94.
12. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, Klein M, Weinberger T. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. Journal of Allergy and Clinical Immunology. 2020 Jul 1;146(1):128-36.
13. Makwana R, Gozzard N, Spina D, Page C. TNF-α-induces airway hyperresponsiveness to cholinergic stimulation in guinea pig airways. British journal of pharmacology. 2012 Mar;165(6):1978-91.
14. E. Brenner, R. Ungaro, J. Colommbel, M. Kappelman, Current Summary Data, SECURE-IBD Database, 2020, 2020, https://covidibd.org/current-data/ (accessed June 20, 2019).
15. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annual review of immunology. 2009 Apr 23;27:519-50.
16. Darif D, Hammi I, Kihel A, Saik IE, Guessous F, Akarid K. The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong?. Microbial Pathogenesis. 2021 Apr 1;153:104799.
17. Chiba Y, Mizoguchi I, Hasegawa H, Ohashi M, Orii N, Nagai T, Sugahara M, Miyamoto Y, Xu M, Owaki T, Yoshimoto T. Regulation of myelopoiesis by proinflammatory cytokines in infectious diseases. Cellular and molecular life sciences. 2018 Apr;75(8):1363-76.
18. Y. Liu, C. Zhang, F. Huang, Y. Yang, F. Wang, J. Yuan, Z. Zhang, Y. Qin, X. Li, D. Zhao, S. Li, S. Tan, Z. Wang, J. Li, C. Shen, J. Li, L. Peng, W. Wu, M. Cao, L. Xing, Z. Xu, L. Chen, C. Zhou, W.J. Liu, L. Liu, C. Jiang, 2019-novel Coronavirus (2019-nCoV) Infections Trigger an Exaggerated Cytokine Response Aggravating Lung Injury, 2020. , http://www.chinaxiv.org/abs/202002.00018.
19. Del Rio C, Malani PN. 2019 novel coronavirus—important information for clinicians. Jama. 2020 Mar 17;323(11):1039-40.

20. Tang M, Tian L, Luo G, Yu X. Interferon-gamma-mediated osteoimmunology. Frontiers in immunology. 2018 Jun 29;9:1508.
21. Yuen KY, Wong SS. Human infection by avian influenza A H5N1. Hong Kong Medical Journal. 2005.
22. Fara A, Mitrev Z, Rosalia RA, Assas BM. Cytokine storm and COVID-19: a chronicle of pro-inflammatory cytokines. Open biology. 2020 Sep 23;10(9):200160.
23. Michot JM, Albiges L, Chaput N, Saada V, Pommeret F, Griscelli F, et al. Tocilizumab, an anti-IL6 receptor antibody, to treat Covid-19- related respiratory failure: a case report. Ann Oncol. 2020.
24. Firestein GS, Budd RC, Gabriel SE, McInnes IB, O'Dell JR. Kelley's Textbook of Rheumatology E-Book. Elsevier Health Sciences; 2012 Aug 31.
25. Bruenger F, Kizner L, Weile J, Morshuis M, Gummert JF. First successful combination of ECMO with cytokine removal therapy in cardiogenic septic shock: a case report, 2015.
26. Surbatovic M, Popovic N, Vojvodic D, Milosevic I, Acimovic G, Stojicic M, Veljovic M, Jevdjic J, Djordjevic D, Radakovic S. Cytokine profile in severe Gram-positive and Gram-negative abdominal sepsis. Scientific Reports. 2015 Jun
27. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet respiratory medicine. 2020 Apr 1;8(4):420-2.
28. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, Song S, Ma Z, Mo P, Zhang Y. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. The Journal of infectious diseases. 2020 May 11;221(11):1762-9.
29. Lee Y, Kirubarajan A, Patro N, Soon MS, Doumouras AG, Hong D. Impact of hospital lockdown secondary to COVID-19 and past pandemics on surgical practice: A living rapid systematic review. The American Journal of Surgery. 2021 Jul 1;222(1):67-85.