Comparative study of pro-Inflammatory and anti-Inflammatory cytokines for understanding the severity in patients infected with SARS-CoV-2 and other Respiratory viruses

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Abstract
A series of hyper immune responses as combined reaction of innate and adaptive immunity may lead to cytokine storm prolonging sickness and causing severities among patients of respiratory infection in general and attack COVID-19 patients in particular. We measured the levels of inflammatory cytokines viz. TNF-alpha, Interleukin-6 and Interferon-gamma. The initiation of immune system against viral infection involves role of cytokines. In Severe Acute Respiratory Distress Syndrome-Coronavirus-2 (SARS-CoV-2) infection, high levels of cytokine were observed which led to severity in immune-compromised patients, elderly and even mildly infected patients. High levels of cytokines have not only been observed in SARS-CoV-2 but are also prevalent in other human coronavirus infections. There are other respiratory diseases occurring in the form of epidemics and outbreaks in all parts of the world, yet understanding of the fact that when system gets infected by more than one respiratory virus, the cytokine expression observed has not been looked upon.

This is irrespective of the fact that antibodies may already be present against respiratory diseases within the system over the course of time. We hereby hypothesise that if cytokine storm due to such condition may lead to activation of Th1/Th2 response, develop severity/complications and ultimately may lead to lung damage. In this study, we measured the cytokine response profiles (TNF-alpha, interleukin-6 and IFN-gamma) in patients with respiratory viral infections and we observed that TNF-α is the main factor contributing to cytokine storm and that hyper innate immune response is brought by mucosal immunity during infection by SARS-CoV-2.

Keywords: Cytokines, SARS-CoV-2, Respiratory viruses, TNF alpha, Cytokine storm.

Introduction
Respiratory viruses including recently appeared Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infect nasopharyngeal route extending its infectivity up to alveolar cells of lungs in case of SARS-CoV-2[5]. The immune response offered to these infections is primarily triggered by mucosal immunity where number of cells viz. macrophages and dendritic cells become antigen presenting cells to activate cytokine secretion and generate specific immunity by B-cell[4,8]. However, before adaptive immune response, which is indirect for being generated through mucosal immune cells, innate immune response of body against infection is pronounced. Cytokine activation especially by formation of APC by macrophages generates tissue inflammation including the cells lining the lungs alveoli[1,12]. This may cause the alveolar cell conduciveness for facilitating the viral infection which reaches to these cells, days after innate immune response activation.

A cohort study of patients of respiratory infection including those of SARS-CoV-2 was undertaken in 88 patients and observations showed raised expression of Tumor Necrosis Factor-Alpha (TNF-alpha) as associated with high fever, cough and breathlessness among patients. To implicate specific cytokine which could be possibly responsible for the cytokine storm, our observation report TNF-alpha as the single cytokine whose pro-inflammatory action could facilitate the virus entry into pulmonary alveolar cells and could cause corresponding severity of disease.

Material and Methods
Blood samples of indoor patients admitted to respiratory ward of Sharda Hospital, Greater Noida, U.P., India were collected. First, their consent for the study was taken and then their clinical symptoms and reason for admission were also recorded. Based on these symptoms, blood was drawn and then brought to the laboratory. The serum was separated and then subjected to estimation of cytokines employing method as per manufacturer's protocol (M/s Elab Sciences, USA). Three cytokine ELISA kits were used i.e. for Interleukin-6, Tumour necrosis factor-alpha and Interferon-gamma detection. Reverse transcriptase-polymerase chain reaction assays were utilized to identify respiratory viruses present in nasal secretions.

Results
Table 1 shows association between raised level of interleukin-6 with the clinical condition of patients. Higher level of IL-6 was associated with less clinical severities as expressed in term of symptoms such as cough, sputum, breathlessness and fever. Graphical representation of the same is shown in fig. 1. Table 2 shows higher level of

Interferon-gamma associated with low clinical severities in terms of cough, sputum, high fever and breathlessness. Graphical representation of the same is shown in fig. 2. Table 3 shows association of TNF-α with clinical severities. All the clinical parameters of respiratory infections such as cough, high fever, sputum and breathlessness were associated with the higher level of TNF-α. Graphical representation of the same is shown in fig. 3.

Table 1
Clinical profile of patients with high and low level of Interleukin-6 (IL-6)

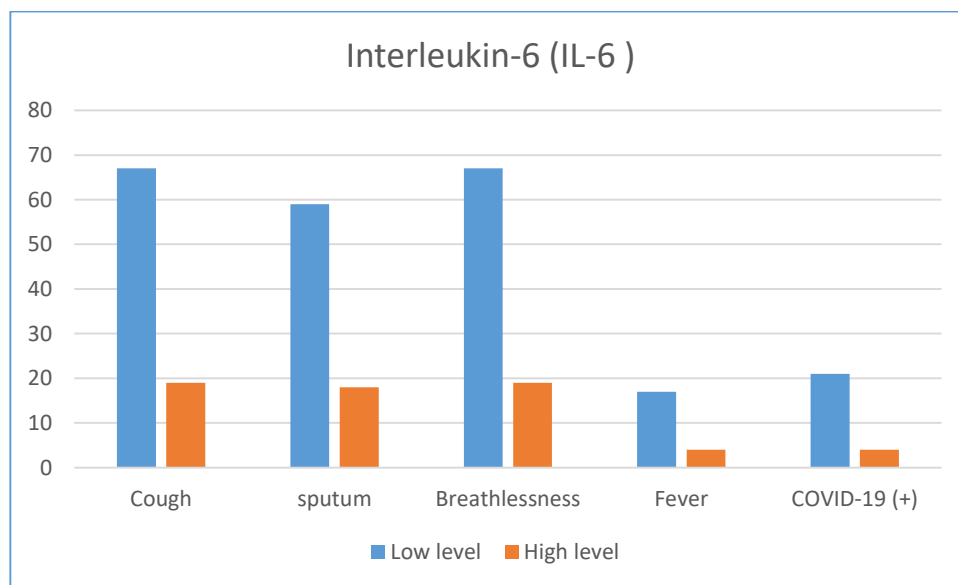| Interleukin-6 levels	Major complaints				COVID-19 (+)
	Cough	**Sputum**	**Breathlessness**	**Fever**	
Low level	67 (98.52%)	59 (86.76%)	67 (98.52%)	17 (25%)	21 (30.88%)
High level	19 (95%)	18 (90%)	19 (95%)	4 (20%)	4 (20%)

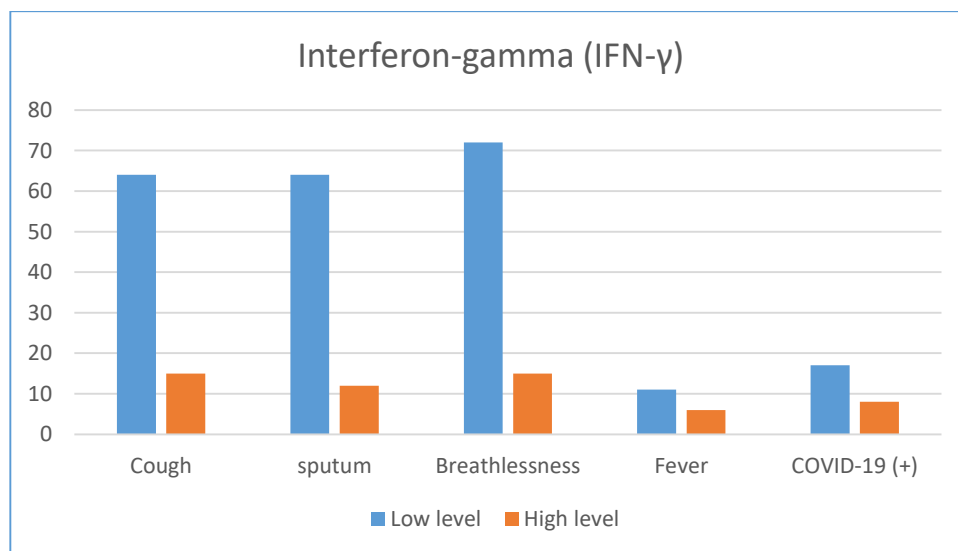**Fig. 1: High and low levels of IL-6 among COVID-19 patients**

Fig. 2: High and low levels of IFN-γ among COVID-19 patients

Table 2
Clinical profile of patients with high and low level of Interferon-gamma (IFN- γ)

IFN-γ	Cough	Sputum	Breathlessness	Fever	COVID-19 (+)
Low level	64 (88.88%)	64 (88.88%)	72 (100%)	11 (15.27%)	17 (23.61%)
High level	15 (93.75)	12 (75%)	15 (93.75%)	6 (37.5%)	8 (50%)

Table 3
Clinical profile of patients with high and low level of Tumor-necrosis factor-alpha (TNF- α)

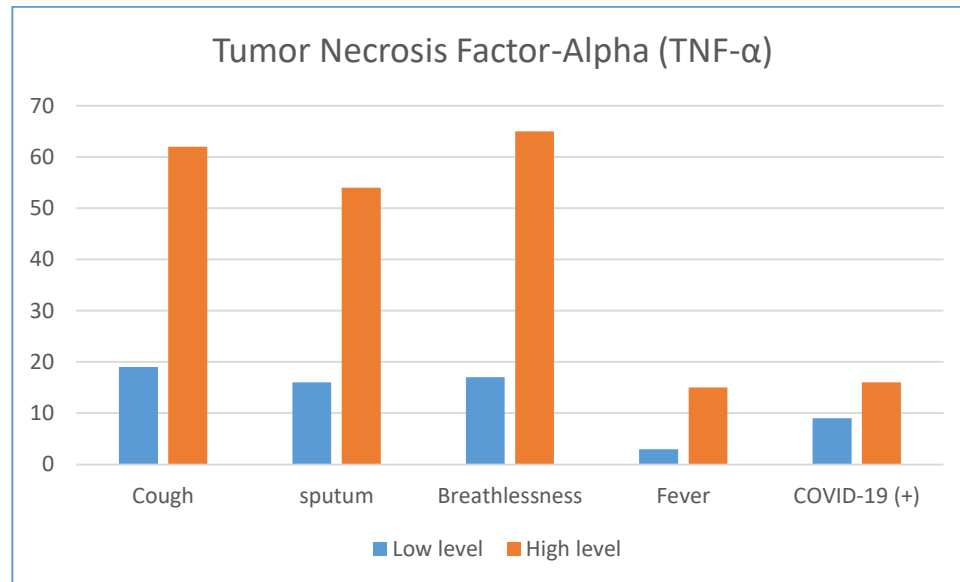| TNF-α	Cough	Sputum	Breathlessness	Fever	COVID-19 (+)
Low level	19 (86.36%)	16 (72.72%)	17 (72.27%)	3 (13.63%)	9 (40.90%)
High level	62 (93.93%)	54 (81.81%)	65 (98.48%)	15 (22.72%	16 (24.24%)

Fig. 3: High and low levels of TNF-α among COVID-19 patients

Discussion

Classically, when human body gets infected or experiences non self-proteins in case of developing cancer, our innate and adaptive immune response gets activated with former preceding later. In case of infections through the routes such as through nasopharyngeal route, the effector response of immunity is initiated through mucosal immunity by means of innate response offered by macrophages, dendritic cells and T helper cells present locally in mucous layer. These cells become Antigen Presenting Cells (APC) and sensitize secretion of number of cytokines viz. Interferon- γ, TNF-α and Interleukin-6 etc.[6] Many a times the over activation of innate immunity by secretion of non-targeted cytokines cause adverse clinical conditions[7,13] which could also lead to cytokine storm causing vascular leakage, cell apoptosis and acute respiratory distress syndrome[2,10], a clinical complicated condition experienced in COVID-19 patients.

We have studied relative roles of these major cytokines and have reported that while IL-6 and IFN-γ do not contribute for severity of symptoms, over secretion of TNF-α was mainly associated with severe clinical condition which could lead to the state what is termed as cytokine syndrome. In present study, we highlight a specific focus that TNF-α is a possible cytokine responsible for cytokine storm among COVID-19 patients. Earlier workers have also reported higher plasma level of TNF-α [9] and have emphasized that TNF-α could sensitize over secretion of other inflammatory cytokine viz. IL-6 and IFN-γ [11]. It has also been reported that anti TNF-α therapy was more effective as compared to steroid supplementation to the patients[3].

Conclusion

Present study implicates single cytokine, TNF-α as the responsible secretion for cytokine storm among COVID-19 patients. The study also reports a novel observation that hyper innate immune response is due to mucosal immunity and similar condition may be experienced in case of all infections affecting mucosal layer.

Acknowledgement

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