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The association between Tumor Necrosis Factor-alpha level (TNF-α) and moderate COVID-19 patients in Egypt

Sabah Farouk Alabd and Ahmed Abdelhalim Y. Mahmoud

Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt

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Abstract:

Background: Infection with viral agents causes upregulation of cytokines such as Tumor Necrosis Factor-alpha (TNF-α), which is considered an important mediator of inflammation. TNF-α has been associated with a poor prognosis in patients with the severe acute respiratory syndrome (SARS). **Patients and methods:** This study included 66 mild COVID-19 patients with confirmed COVID-19 infection and 22 healthy people as a control group, these study subjects were randomly selected irrespective of the age group and both genders were included, 1 ml blood sample was collected for performing serum TNF-α levels test, Reagents of EIAab is located at East Lake Hi-Tech Development Zone, Wuhan China. Human tumor necrosis factor ELISA kit TNF-α serum levels immunoassay test catalog number E0133h. **Results:** This study reveals that serum TNF-α levels for mild COVID-19 patients and healthy control people were non-significant with a p-value of 0.1191 between the two groups. **Conclusion:** the serum TNF-α level is not a significant biomarker for diagnosis or prognosis of mild COVID-19 patients (Outpatients and patients under home observation), while other studies reported patients with COVID-19 demonstrated significantly elevated levels of TNF- α upon admission to hospitals.

Keywords: COVID-19, Tumor necrosis factor-alpha, TNF-α, Outpatients, cytokine storm.

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1. Introduction:

Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China. On 11 March 2020, the World Health Organization (WHO) declared COVID-19 to be a pandemic [(1)], patients with COVID-19, caused by SARS-CoV-2, can have mild symptoms, or experience a severe and/or life-threatening infection [(2)]. Comorbidities, such as lung disease, diabetes, and obesity, increase the risk of adverse COVID-19 outcomes [(3)].

Severe disease is associated with a "cytokine storm", a delayed onset burst of pro-inflammatory cytokines in circulation. The cytokines associated with fatalities are TNF, IL-6, IL-8, IFNγ, and possibly others [(4)].

Previous studies have shown some pro-inflammatory cytokines to play a crucial role throughout acute lung injury, for instance, acute pancreatitis and sepsis [(5,6)]. They also demonstrated that infection with viral agents causes upregulation of cytokines such as Tumor

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Necrosis Factor-alpha (TNF-α), which is considered an important mediator of inflammation [7,8].

The direct cause of death from acute COVID-19 involves cytokine storm damage to the lungs and multiple organs of the body: heart, kidney, and liver, leading to multiple organ exhaustion [9].

Numerous pathologies are associated with elevated TNF levels, from autoimmune disorders to sepsis and cancer. In the respiratory system, TNF causes bronchial hyperreactivity, narrowing of the airways, damage to the respiratory epithelium, stimulation of collagen synthesis, and fibrosis [10,11], Tumor necrosis factor-α (TNF-α) is one of the overproduced pro-inflammatory cytokines. Tumor necrosis factor-α has been associated with a poor prognosis in patients with the severe acute respiratory syndrome (SARS). An increase of cytokines could be seen in various inflammatory conditions, including cytokine release syndrome (CRS). TNF-α serum levels elevate in COVID-19 patients, especially those with severe symptoms [12,13].

In this study, the levels of TNF-α, were measured through ELISA in sera from healthy volunteers as a control group, and patients with moderate COVID-19, Outpatients, and patients under home observation are included in this study, while hospitalized patients are not included, this study aims to investigate the association between TNF-α serum levels and mild COVID-19 infection.

2. Patients and methods

2.1. **Study population Patients**

This study included 66 non-hospitalized patients with confirmed COVID-19 infection, and 22 healthy people as a control group, these study subjects were randomly selected irrespective of the age group and both genders were included. It was performed following the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All the studied population was informed about the purpose of sample collection and patients were free to refuse sample collection.

2.2. **Data collection**

In this cross-sectional study, we obtained data regarding 66 non-hospitalized patients with confirmed COVID-19 via real-time reverse transcription-polymerase chain reaction (PCR), and 22 healthy people as a control group, they came to Alyameny laboratory in Alexandria, Egypt for biomarkers and complete blood count investigations**,** we reviewed the medical records and compiled data between August 12 and December 30, 2020.

2.3. Collection and processing of blood samples:

1 ml blood sample was collected for performing serum TNF-α levels test (Wuhan EIAab science Co.is located at East Lake Hi-Tech Development Zone, Wuhan China) on 66 Positive COVID-19 patients and 22 healthy peoples for individuals matching in age and gender.

2.4. Assay procedure as manufactory instructions:

Wuhan EIAab science Co for reagents is located at East Lake Hi-Tech Development Zone, Wuhan China. Human tumor necrosis factor ELISA kit TNF-α serum levels immunoassay test catalog number E0133h and samples allowed to be at room temperature before testing, (serum was separated from a blood sample by centrifugation).

2.5. Statistical analysis

Data were analyzed using SPSS statistical software, version 20.0(SPSS, Chicago, Illinois, USA). All continuous data are presented as means and standard deviations, while categorical data are presented as numbers and percentages. A Mann-Whitney *U* test or **Student *t*-test was used to compare categorical variables. Multivariate regression analysis was performed to analyze relationships between mild COVID-19 infected patient's serum TNF-α levels, and healthy people's TNF-α serum levels this model was generated using independent variables achieving a p-value. Then, the best-fit model was generated without interaction variables. For all calculations, a p-value of less than 0.05 was considered statistically significant.

3. Results:

Table (1): shows the serum TNF-α level detected by the ELISA technique for mild COVID-19 Patients and a healthy control group, The present study included patients aged from14 years to 75 years mean age was 44.5 ±30.5 who were confirmed to have Covid-19 based on real-time reverse transcription-polymerase chain reaction, male gender was more frequent (n=36, 54.5%) than female gender (n=30, 45.5%). This study reveals that serum TNF-α levels for mild COVID-19 patients and healthy control people were a non-significant p-value of 0.1191 between the two groups.

Table 1: Mean, and minimum-maximum values of serum TNF-α levels in mild COVID-19 patients and healthy controls, and the results of statistical assessments. Values are mean ± SD.

*Mann-Whitney *U* test, **Student *t* test.

	Patients (n=66)	Controls (n= 22	p-value
TNF-α	10.45±4.65 (5.8-15.1)	7.89± 1.36 (6.53-9.25)	0.1191

P-Value 0.1191

4. Discussion:

TNF- α is a pleiotropic inflammatory mediator produced by several cells of the immune system to regulate a wide variety of immune responses [(14)], TNF- α is made of 157 nonglycosylated amino acids following proteolytic cleavage of the membrane-bound form, which is made of 233 amino acids [(15)], Of significant importance is the relation between TNF- α and the current COVID-19 situation [(16)]. TNF- α plays a central role in mediating the cytokine response required to initiate and potentiate the inflammatory response in the lungs [(17)], which leads to increased accumulation of pulmonary inflammatory fluid and vascular permeability [(18)].

The expression of TNF- α is increased during lung injury, where it provokes a variety of biological responses to modulate the anomaly by inducing the expression of numerous other inflammatory mediators, which is a key step consisting of stimulating multiple genes involved in protective processes [(19)]. It was noticed that patients with COVID-19 demonstrated significantly elevated levels of TNF- α upon admission to hospitals [(20)], this study was conducted on Outpatients and patients under home observation who have mild COVID-19 infection, while hospitalized patients are not included.

This study was conducted on 66 mild infected COVID-19 patients (out hospitalized and home observation Patients) who were confirmed to have Covid-19 based on real-time reverse transcription-polymerase chain reaction, and 22 healthy people as a control group, this study reveals the serum TNF-α levels for mild COVID-19 patients and healthy control people were non-significant p-value 0.1191 between the two groups, while different other biomarkers have different sensitivity with mild COVID-19 patients,

thrombocytopenia was not significant also with mild COVID-19 infection[21], but CRP has a sensitivity 90.2%[22], while other biomarkers have different sensitivity in mild COVID-19 infection as ferritin which has a sensitivity of 71.4% [23], LDH has a sensitivity 67.7%[24], and D-dimer has a sensitivity 36.4%[25].

However, **Song *et al*** found no significant differences in the level of IL-6 and tumor necrosis factor (TNF) between severe and non-severe patients with COVID-19[26], Another study performed by **Han et al**. demonstrated that levels of IL-6, IL-2, IL-10, IFN-γ, and TNF-α were higher in COVID-19 patients than in healthy subjects [27].

Zheng et al found no statistical differences in TNF-α and IL-6 plasma levels among the three groups of their study, which was also divided according to severity. They also reported decreased cases in IFN-γ serum levels in severe cases [28].

Mortaz et al detected an association between mortality of COVID-19 patients in the ICU and elevated serum levels of soluble TNF-α receptors.

Merza et al. [81], reported that the mean TNF-_ levels were not significantly higher in patients with severe COVID-19 compared with non-severe COVID-19 patients [30].

A previous meta-analysis by **Mulchandani et al**. [82], however, reported that the mean TNF-α levels were significantly higher in patients with severe COVID-19 compared with nonsevere COVID-19 patients [31].

Huang et al., a study on 41 COVID-19 cases (13 ICU cases) reported an increase in serum level of Tumour necrosis factor-alpha (TNF-alpha) in 13 ICU cases only [32]

In severe COVID-19 infection, TNF-α is produced in excess, causing an increase in the systemic inflammatory response that results in tissue damage. One of the functions of TNF-α is to increase the recruitment of phagocytic cells and increase the autolysis reaction of cells [33]. TNF-α is associated with acute lung injury and predicts prognosis [32].

Conflict of interest

There are no conflicts of interest.

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