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Original Article

Serum tumor necrosis factor-alpha status in hospitalized patients with coronavirus disease-2019 (COVID-19)

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Abstract

Background: Tumor necrosis factor alpha (TNF-α) produces an inflammatory process and plays a critical role against infection and in the control of viral infection. The present study was conducted to determine the status of serum TNF-α in hospitalized patients with coronavirus disease-2019 (COVID-19).

Methods: In this cross-sectional study the serum TNF-α level, sex, and age, were determined in patients with COVID-19. The association between variables was determined using the student t-test, analysis of variance (ANOVA) test, multiple logistic regression analysis, and the statistical package for the Social Sciences (SPSS)-18 ($p < 0.05$).

Results: A total of 91 (women 41.75%, and men 58.24%) patients with a mean serum TNF-α level of 9.9 picograms per milliliter (pg/mL) were considered. In all (100%) patients, the TNF-α serum level was more than the normal limit (P=0.95). 95.60% of patients suffered severe COVID-19, with a TNF-a serum level of 10.20 pg/mL (P=0.87). Mean TNF-α serum levels in women and men were 11.37 pg/mL and 8.8 pg/mL, respectively (P= 0.17). In the age group of > 70 years (11.30 pg/mL), serum TNF-α concentration was higher than the other age groups (p>0.05).

Conclusion: A significant proportion of women and men patients with COVID-19 in the middle and old age had a high concentration of serum TNF-α which may indicate the severity of the disease. Serum TNF-α level is different in women and men of different ages, so it can contribute to treatment strategies.

Keywords: Coronavirus disease (COVID-19), Hospitalized patients, Tumor necrosis factor-alpha **(**TNF-α).

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Tumor necrosis factor alpha (TNF-α) causes a chronic inflammatory process in the body against the infection occurred by intracellular pathogens (1). Depletion of TNF-α with anti-TNF drugs can facilitate latent infection or reactivate viral infection (2-4). In patients with oronavirus disease (COVID-19), the serum TNF-α level increases along with several markers of inflammation such as C-reactive protein (CRP), ferritin, erythrocyte sedimentation rate (ESR), procalcitonin and serum amyloid A protein, as well as many cytokines including Interleukin (IL) -2R, IL-4, IL-6, IL-8, IL-10, and Interferon gamma (IFN-γ) (5-7). Sex differences affect immune responses triggered by TNF-α, which mediate immune responses between cells. Specifically, if women show more type 2 cytokine production, men appear to show more type 1 cytokine production, including TNF-α. Any difference in cytokine production between men and women can lead to plaque formation, leading to an increased risk of cardiovascular events. There are also gender differences in mortality and vulnerability to COVID-19, as disease severity and death are greater in men (3).

It has been reported that sex differences in immune responses may involve the production of cytokines (3). On the other hand, higher superoxide dismutase (SOD) activity in people over 70 years of age increases inflammatory activity. Higher SOD activity is associated with reduced disease-related mortality in women but not in older men, further suggesting gender differences in the regulation of oxidative stress and immune activity with aging (4). Impairment in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) clearances in COVID-19 patients results in severe disease with macrophage activation syndrome and cytokine storm (8-10). At this time, antiviral treatment alone is not sufficient but requires additional anti-inflammatory treatment as well (11-13). This issue is important for recognizing patients who have high levels of inflammatory markers including TNF-α and so may be at higher risk of cytokine storm syndrome. We, therefore, performed the present study to determine the status of serum TNF-α in hospitalized patients with COVID-19.

Methods

The population of this cross-sectional study was consecutively selected among patients who were hospitalized in Ayatollah Rouhani Hospital, Babol, Iran, between May to June 2020 (Ethical code: IR.MUBABOL.REC.1399.322). The diagnosis of COVID-19 infection was confirmed by real-time-polymerase chain reaction (PCR). All patients received standard-of-care treatment during hospitalization. Serum TNF-α level was assessed according to manufacturer's instruction by the direct enzyme-linked immunosorbent assay (ELISA) (Diaclone kit, France and ELISA device brand of Addcare ELISA 200, China). All laboratory tests were done in the Ayatollah Rouhani Hospital laboratory, Babol, Iran. The cost of the tests was determined by Babol University of Medical Sciences (12, 13). The inclusion criteria for the study included patients over 18 years old hospitalized with severe (oxygen saturation (SpO2) ≤93% at rest, computed tomography (CT) (>50% lung imaging progress in the short term within 24–48 h), respiratory failure and mechanical ventilation required, shock, combining other organ failures that were hospitalized in the intensive care unit (ICU) (12). Exclusion criteria included no clinical trials in the file, incomplete treatment and discharge with the personal consent of the patient, and sending to centers outside the province. Also, patients who have been treated with biological drugs and with a history of rheumatic disease were excluded (14-16). Data were collected for demographic features by interview and clinical examination. The association between increased serums TNF-α with sex and age was determined using the student t-test and analysis of variance (ANOVA) test. Multiple logistic regression analysis was used to determine independent association. All analyses were performed using the statistical package for the Social Sciences (SPSS) software version 18 ($p < 0.05$) (3, 4).

Results

A total of 91 (38 women, 41.75%, and 53 men, 58.24%) patients entered the study. The mean serum TNF-α concentration in total patients was 9.9 + 0.87 picograms per milliliter (pg/mL). In all 91 (100%) patients, the TNF-α serum level was more than the normal limit (based on the kit; detection limit of 10 pg/mL), but no significant difference was observed (P=0.95). Eighty-seven (95.60%) patients suffered severe COVID-19 pneumonia, with a TNF-a serum level of 10.20+8.40 pg/mL, but no significant difference was observed between severe COVID-19 pneumonia and TNF-α serum level (P=0.87). Mean serum TNF-α values in women and men were 11.37+8.6 pg/ml and 8.8+8.07 pg/ml, respectively (P = 0.17) (table 1, figure 1). Patients aged 61+1.5 years entered the study. Fifty (54.94%) patients aged > 50 years old. In the age group of > 70 years (11.30 + 8.10 pg/ml), serum TNF-α concentration was higher than in other age groups, but the difference did not reach a significant statistical level (p>0.05) (table 2, figure 2).

Table1. Frequency of normal and higher than normal values of serum TNF-α in the patient with COVID-19

Sex	Normal value (< 7 pg/mL) No/Total (%)	High values No/Total (%)	P-values
Men	26/53 (49.05%)	27/53 (50.94%)	
Women	14/38 (36.84%)	24/38 (63.15%)	0.54
Total	40/91 (43.95 %)	51/91 (56.04%)	

* No= number, pg/ml= picograms per milliliter, $p < 0.05$

Table 2. TNF-α (pg/ml) value according to age in hospitalized patients with COVID-19

Age group (no, %), years	Mean+SD	P-values
< 50 (22, 24.17%)	8.80+7.60	-
50 -69 (38, 41.75%)	9.50+8.90	0.63
> 70 (31, 34.06%)	11.30+8.10	0.51
Total (91, 100%)	9.90 + 8.30	-

* no= Number, SD= standard deviation, compared with < 50 years using ANOVA test, $p < 0.05$

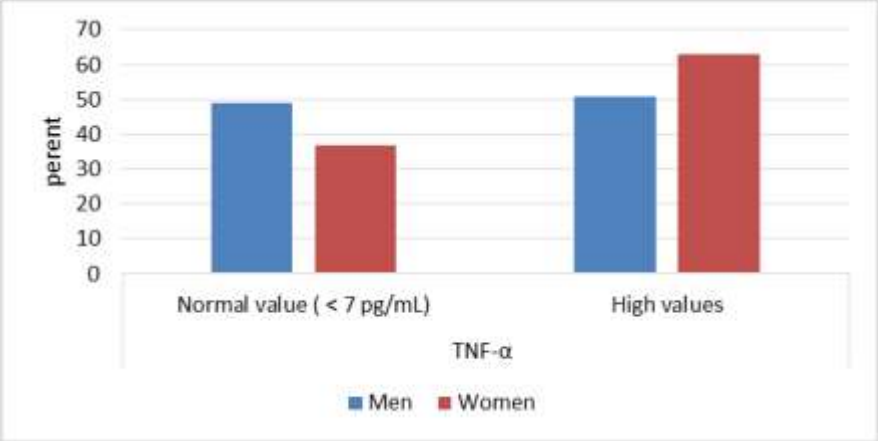

Figure 1. Comparison of normal (< 7 pg/mL) and higher than normal values of serum TNF-α in men and women with COVID-19, vertical; percentage (%) of patients, horizontal; values of TNF-α (pg/mL)

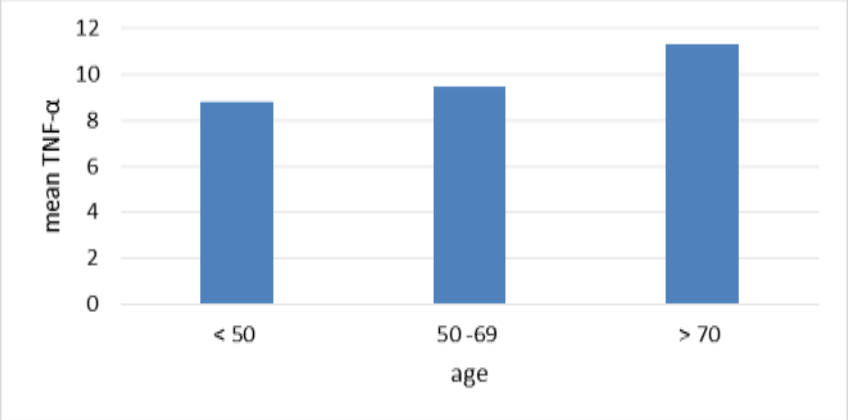

Figure 2. Comparison of TNF-α (pg/ml) value according to age in men and women with COVID-19, vertical; mean of TNF-α, horizontal; age of patients (years)

Discussion

Tumor necrosis factor-alpha (TNF-α) plays a key role in the pathogenic effects of COVID-19 (1). The results of this study indicate a high proportion of patients (51 patients; 56.04%) with increased serum TNF-α levels in COVID-19. Although women's mean serum TNF-α (11.37+8.6 pg /ml) concentration and proportion of patients (63.15%) with an increased level of TNF-α was higher than men's (8.8+8.07 pg/mL, 50.94%) the difference did not reach a statistical level. Bernardi et al., reported that gender can affect the levels of cytokines. A study on 104 adults showed that TNF-α was significantly higher in men than in women (in our study, women's mean serum TNF-α was higher than men's). All of them were related to testosterone

and the ratio of testosterone to estradiol. Gender appears to influence cytokine levels. Therefore, gender differences can play an important role in vulnerability to infections. In general, the production of TNF-α is higher in men than in women. However, autoimmune diseases, microbial load, immunoglobulin levels, innate immune responses, and disease severity can affect TNF-α serum levels in different patients based on different studies (3). Furthermore, in this study increased serum TNF-α was not associated with age groups, but in the age of > 70 (34.06%, 11.30 + 8.10), the TNF-α serum level was higher than other age groups (< 50, 24.17%, 8.80 +7.60 and 50 -69, 41.75%, 9.50 + 8.90). Martínez de Toda et al., have shown that immune aging, causes inflammatory disease, especially during illness. In older people, the aging immune system cannot protect the body against infections. The immune system in older people creates a chronic disease state and produces inflammatory mediators to eliminate infectious and other diseases. T cells in elderly people are prone to differentiate into pro-inflammatory cytokines and maintain chronic and persistent inflammatory lesions in many organ systems (4). Petrescu et al., reported that inhaled cigarette smoke can induce the production of TNF-α by alveolar macrophages, which in turn may increase the production of metalloproteinases, thereby causing airway inflammation and lung destruction. The TNF-α serum level was significantly higher in smokers (to 45 pg/mL) compared to non-smokers (to 9 pg/mL). There was a positive correlation between TNF-α serum level and exposure to tobacco smoke. Therefore, smokers can be exposed to high severity of the diseases (17). In a similar study performed by Akbari et al., serum TNF-α increased in the severe group of patients with COVID-19, compared to the non-severe group. So, the elevation of serum TNF-α level can be associated with COVID-19 severity and used as an indicator of severe COVID-19 (6). Huang et al., in another similar research, reported that most of the patients (median age was 49 years) with COVID-19 were men (30/41 (73%)); in our study also the most of patients were men (41.75% women, 58.24% men). Huang et al., also showed that in patients admitted to ICU, serum TNF-α level was higher than non-ICU patients. COVID-19 caused SARS-CoV-2 and was associated with ICU admission and high mortality (7). However, existing data indicate several factors including the severity of COVID-19 infection such as the presence of pneumonia, the severity of radiographic score, and the presence of underlying comorbidities such as diabetes, and obesity are associated with increased serum TNF-α concentration (6-8). Del Valle et al., reported that of 1484 hospitalized patients with COVID-19 who were followed up to 41 days after admission, TNF-α levels at the time of hospitalization were strong and independent predictors of survival after adjustment for disease severity (18). TNF-α is a marker of inflammation that raises together with other parameters of inflammation like ferritin, procalcitonin, and CRP not only in patients with COVID-19 (6-10) but also in other inflammatory conditions (19-21). Tzanavari et al., also showed that even in an obesity state, serum TNF-α increases because adipose tissues are sources of proinflammatory cytokines (19). High levels of inflammation in patients due to viral infections resulted from the creation of immunological mechanisms in different tissues such as lymph nodes and the digestion system, as well as increasing the amount of lipopolysaccharides. Also, simultaneous infections (that cause the continuation of the activity of the immune system and inflammation in the patient's body) and the patient's immune system, the type of medicines prescribed to different patients, can affect the serum TNF-α (2). In our study, there were no significant differences between women, men, and age. It appears that adjuvant treatment during the disease can lower the serum TNF-α (22).

The present study has several limitations including an absence of a control group, lack of data regarding other markers of inflammation, and severity of COVID-19. Although an assessment of serum TNF-α was done at the time of hospitalization, however, the impact of treatment before hospitalization could not be ignored. Due to the absence of a control group serum, TNF-α in this study was compared with normal values from other studies, as well as reference values from the manufacturer. The findings of this study indicate high serum TNF-α levels in a significant proportion of patients with COVID-19. Since increased serum level of TNF-α may be a predictor of COVID-19 severity, requiring more serious treatment measures. Therefore, assessment of TNF-α concentration at the time of hospitalization provides both treatment and prognostic information, this issue needs further studies with a larger sample size.

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