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ORIGINAL RESEARCH

Assessing the Relationship between Serum Testosterone Levels and COVID-19 Outcomes in Admitted Patients

Amir Reza Abedi[1], Mohammad Dashti[1], Seyyed Pooria Salehi Mashhadsari[2], Amirreza Eghbaldoost[2], Roshanak Mehravar[2], Erfan Ghadirzadeh[3], Reza Dashti[1], Farzad Allameh[1]*

1. Men's Health & Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
3. School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

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Abstract: **Introduction:** With the emergence of the worldwide COVID-19 pandemic, a high toll of mortality and morbidity was imposed on Iran. Despite endeavors to control the disease using the social distancing protocols, isolations, quarantine and vaccination, COVID-19 is still afflicting individuals by mutation and development of new variants. Studies suggest higher morbidity and mortality among men compared to women, and in men with underlying diseases. Studies suggest low serum testosterone level as a risk factor for more severe diseases. We aimed to assess the relation of testosterone level with laboratory investigation and its effect on COVID-19 disease outcomes.

Methods: Seventy patients were selected from those referred to Shohada-e-Tajrish hospital during February 2022 till July 2022. The patients were further categorized in two groups regarding their need for respiratory support: patients who did not need invasive respiratory support and patients who needed invasive respiratory support and were admitted to the intensive care unit. Blood samples were collected for laboratory examination. Each patient was followed for one month, and the outcomes were recorded.

Results: The mean age of the participants was 64.44 years including 33 men and 37 women. Mean serum total and free testosterone level was 0.45 nmol/L and 1.22 nmol/L, respectively. Demised patients were significantly older and had significantly lower total testosterone levels. D-dimer was significantly higher in demised patients.

Conclusions: Serum total testosterone levels can be used as a prognostic factor to predict COVID-19 patients' prognosis. Serum total testosterone is inversely associated with disease severity, and lower serum total testosterone level is significantly associated with higher mortality. Moreover, inflammatory markers such as D-dimer can be used to determine prognosis and severity.

Keywords: Androgen, COVID-19, Mortality, Prognosis, Testosterone

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1. Introduction

With the identification of new kinds of pneumonia in Wuhan, China in December 2019, a new strain of the beta corona virus was discovered, resulting in a pandemic with about 518 billion cases and 7 billion and 228 thousand fatalities by May 13, 2022. Despite numerous attempts to manage this illness by social distancing protocol, isolation, quarantine, and immunization, this disease led to more illness due to mutation and the production of new strains (1).

The SARS-CoV-2 virus disrupts the operation of several bodily systems, which is the cause of the sickness. The most frequent side effects of this illness are respiratory difficulties and symptoms (2). Recent studies revealed that the pathophysiology of this illness is mostly focused on the Angiotensin converting enzyme (ACE2) receptor allowing viruses to enter cells (3). Other complications of COVID-19 include pulmonary involvement, digestive involvement, and neurological system involvement, among others. The reproductive system is one of these systems affected by COVID-19(4).

***Corresponding Author:** Farzad Allameh; Address: Department of Urology, Shohada-e-Tajrish Hospital, Tajrish Sq., Tehran, Iran. Tel: +989123885545, Email: farzadallame@gmail.com.

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The blood barrier protects testicular cells, which confers immunological privilege on these cells (safe from immune system attack). Any breach in this barrier will result in testicular inflammation and destruction to germ cells. In certain articles on COVID-19, it was discovered that this virus is present in the testis and sperm, indicating its entry into the reproductive system (5). Other studies have shown that the virus' entrance into the reproductive system impairs the function of the testis and its cells. On Leydig and Sertoli cells, ACE2 and TMPRSS2 may function as a viral entry point (6). Leydig cells are the primary source of testosterone production in men governed by Luteinizing hormone (LH). According to a number of studies, COIVD-19 may reduce LH levels in the body (7).

Recent research indicates that this condition is substantially more lethal and intense in men than in women. According to their calculations, this rate is between 1.7 to 3 times greater in men compared to women (7, 8). These data demonstrate that sexuality has a considerable impact on this illness. Moreover, this percentage is much greater among older men than among younger men, and among men with underlying illness than among healthy men. Blood testosterone levels fall by around 0.8% to 2% each year with age (4). This decline is more prevalent among men with underlying illness. This is the primary reason why men with background illness have a higher death and morbidity rate, and low total testosterone levels have been identified as one of the most significant risk factors for developing a severe form of this disease(9). When evaluating gonad function, the ratio of testosterone to LH is more reliable than testosterone level since typical blood testosterone levels vary across groups (5). Men with hypogonadism make up between 2% and 9.5% of the male population, and they have an increased chance of developing serious diseases (4).

Low testosterone is not only a risk factor for severe Corona illness, but COVID-19 infection also reduces testosterone levels. Decreased testosterone levels are the result of increased peripheral aromatization of androgens and dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis (6).

2. Material and Methods

2.1. Participants

We included 70 patients according to the inclusion and exclusion criteria. First of all, patients who were enrolled in this study should have positive COVID PCR test, or their chest Computed tomography (CT) scan was consistent with COVID-19 pneumonia. The second criterion was that patients must be admitted to hospital because of COVID 19 infection. Patients who were younger than 18 years of age, took antiandrogen drugs, were on testosterone therapy, underwent orchiectomy, were diagnosed with testis cancer, prolactinoma, Kallmann syndrome, Klinefelter syndrome, or diseases that alter testosterone level were excluded from the study.

In accordance with the exclusion and inclusion criteria, 70 patients were included in the research from February 2022 till July 2022. This research was conducted at Shohada-e Tajrish Hospital, and patients were selected among those who referred to the hospital throughout the study period. There were two main inclusion criteria: confirmed COVID-19 infection and requiring admission.

2.2. Study groups

Patients were categorized into two groups: the first group was hospitalized in departments where patients did not need mechanical ventilation, and used only supplemental oxygenation with a low-flow system via nasal cannula. The second group is the patients who were admitted to intensive care units (ICUs) where patients need mechanical ventilation.

The treatment for COVID-19 infection was based on the most recent edition (8th version) of Iran's health ministry's guideline.

According to this guideline, two distinct groupings are distinguished. There are both suspected and confirmed instances. The suspected cases consist of individuals with acute respiratory infections whose symptoms have emerged during the last 10 days or patients with clinical symptoms and an epidemiological condition. Clinical signs such as abrupt fever or cough, sudden onset of at least three symptoms such as fever (at least 38°C, oral temperature), cough, exhaustion, headache, muscular pain, sore throat, runny nose, difficulty breathing, nausea/vomiting, lack of appetite, and decreased awareness. Site of residence, employment, or travel to a crowded location (such as conferences, ceremonies, etc.) within 14 days are examples of epidemiologic variables. For a definitive diagnosis of COVID-19, laboratory test results such real-time PCR are required. Certain criteria were crucial for clinical decisions to admit the patients, including: 1-O2 saturation of less than 90% and, depending on the patient's condition, the necessity for breathing assistance, such as oxygen treatment. 2-decrease in degree of awareness Three-systolic pressure below 90 mmHg 4-dehydration and inability to tolerate PO (per os) during outpatient therapy. 5-physician decisions based on the patient's situation.

2.3. Study method

Physicians took the patients' history and examined the patients who were admitted to the hospital and asked for some routine laboratory tests such as cell blood count, kidney and liver function tests, coagulation tests (PT, PTT, INR), D-Dimer, erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) tests. Upon admission, treatment strategies started. On the second day, between 7:00 to 9:00 a.m., routine

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Table 1: Comparison of patients' characteristics admitted to internal departments with patients admitted to intensive care units (ICU).

Variables	Patients in internal department (n = 60)	Patients in ICU (n = 10)	p-value
Age (years)	61.81 ± 16.57	78.54 ± 5.80	< 0.05*
Sex, Male (%)	27(45.7)	6(54.5)	0.83
Total Testosterone (nmol/L)	0.47 ± 0.43	0.39 ± 0.35	0.56
Free Testosterone (nmol/L)	1.21 ± 1.27	1.26 ± 0.73	0.90
WBC* (per microliter)	10.57 ± 2.39	8.74 ± 2.01	0.02*
CRP** (mg/dl)	33.25 ± 16.60	24.36 ± 12.30	0.09
D-Dimer (ng/ml)	180.40 ± 76.89	157.81 ± 82.60	0.37

*: white blood cell, **: C-Reactive Protein.

Table 2: The mortality rate.

Variables	Patients in internal department	Patients in ICU	P-value
discharge	46	13	< 0.05*
death	3	8	

laboratory blood tests were repeated, and total testosterone (TT), free testosterone (FT), estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), and progesterone were ordered. All of these hormone levels were measured using the ELISA technique, and the Vermeulen formula was used to determine modified free testosterone (cFT).
The patients were observed for one month, and the findings were gathered. The primary criteria for COVID-19 patients' release were the absence of fever for at least two or three days, evidence of improvement on a chest CT scan, and clinical improvements in respiratory health.

2.4. Analysis techniques

Continuous variable data are reported as mean standard deviation, while categorical variable values are summarized as percentages in the tables. The Mann-Whitney U test and the t test are used to compare the length of hospitalization across groups. The serum levels are compared across groups using a one-way ANOVA test. Other Total Testosterone results are used to examine the association between the laboratory and clinical Pearson's correlation test. An odds ratio with a 95% confidence interval (%ci) is used to forecast illness prognosis, such as the probability of ICU admission and mortality, using variables such as the likelihood of ICU admission and death. The Chi-square test is used to compare mortality rates between men and women.

3. Results

3.1. Information on demographics

This research was conducted on 70 patients with a mean±SD age of 64.44±16.53 years including 33 (47.1%) men and 37 (52.9%) women.

3.2. Total testosterone

The average testosterone level was 0.45±0.41nm/l. Testosterone level was more than 0.1 nm/l in one fourth of the patients and less than 0.67 nm/l in 75% of patients.

3.3. Free testosterone

The mean±SD free testosterone level was 1.22±1.2 nmol/l.

3.4. CRP

The mean±SD CRP level was 31.86 mg/dl±16.26 mg/dl. CRP level was more than 17 mg/dl in 25% of patients and less than 46 mg/dl in 75% of the patients.

3.5. D-dimer

The mean±SD D-dimer level was 176.9 ng/dl±77.63 mg/dl. D-dimer level was more than 112 ng/dl in 25% of patients and less than 245 ng/dl in 75% of the patients.

3.6. Fundamental characteristics of hospitalized patients

Table 1 compares patients' characteristics admitted to internal departments with patients admitted to ICUs. Approximately 85.7% of the patients were hospitalized in the internal department, and 14.3% of the patients were admitted to the ICU. The patients who were admitted to the ICU were significantly older than those admitted to the internal department. Additionally, WBC count of patients who were hospitalized in the internal department was much greater than WBC count of the patients admitted to the ICU.

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Table 3: Comparison of variables based on mortality.

Variables	discharged (n = 59)	death (n = 11)	P-value
Age (years)	61.24 ± 16.67	71.90 ± 13.85	0.01*
Sex, Male (%)	24 (48.9)	9 (42.8)	0.83
Total Testosterone (nmol/L)	0.54 ± 0.42	0.25 ± 0.31	< 0.05*
Free Testosterone (nmol/L)	1.32 ± 1.35	0.98 ± 0.72	0.17
WBC *(per microliter)	10.50 ± 2.46	9.78 ± 2.29	0.25
CRP** (mg/dl)	32.59 ± 16.91	30.14 ± 14.88	0.56
D-Dimer (ng/ml)	165.65 ± 72.53	203 ± 84.50	0.06*

*: white blood cell, **: C-Reactive Protein.

3.7. Outcomes

The mortality rate in this study was 15.7% (11 patients). Most of them were admitted to the ICU (8 patients). The average mortality rate of patients admitted to the ICU was 38% that was much higher than the average mortality rate of patients admitted to the internal departments (6.1%). (Table 2)

3.8. Testosterone as a prognostic factor

The patients who passed away were older than the patients who recovered (P=0.01). The average testosterone level was significantly lower in patients who passed away (0.25 nm/dl) as a result of COVID-19 infection compared with patients who recovered (0.54nm/dl) (P<0.05). The average D-dimer level was higher in patients who passed away (203 ng/dl) as a result of COVID-19 infection compared with patients who recovered (166.65ng/dl), but it is not statistically significant. (P=0.06, Table 3).

4. Discussion

Since the emergence of the COVID-19 pandemic in December 2019, a considerable number of men and women have been admitted to hospitals with a range of complications, including respiratory and gastrointestinal issues. Due to the endurance of the pandemic and the emergence of new pathogenic versions of the virus, while adhering to preventive measures and using the benefits of vaccination, it is vital to investigate the disease's distinguishing characteristics and create novel treatment strategies. The male mortality rate was 1.7 to 3 times more than women, showing that male gender is a risk factor for the severity of the disease (7, 8). Thus, the primary causes of mortality in male patients are underlying diseases including hypertension and diabetes mellitus (10). Men have a higher mortality rate than women, according to studies. Sex hormones are thus likely to have a role in this process. Serum levels of sex hormones such as testosterone and estrogen may help explain the higher mortality rate among male patients compared to females (11, 12). Generally, the parenchymal tissue of the testis has two primary cells with distinct functions: Sertoli cells and Leydig cells. Leydig cells produce and secrete testosterone in response to LH, while Sertoli cells are principally responsible for spermatogenesis (9).

In viral infectious diseases, as opposed to bacterial infectious diseases, the testis may be a location of infection. The virus may be found in a patient's sperm, and testosterone levels in the blood may fluctuate. There are many androgenic receptors on different cells of the body (including immunological and pulmonary cells), and a change in the blood levels of these hormones may indicate their action. According to the investigations, those with low testosterone levels had greater concentrations of inflammatory cytokines (13). In acute diseases, a reduction in testosterone levels may be seen, according to the results of scientific studies. Low testosterone levels and total testosterone levels below 100 ng/dl are also indicative of an unfavorable prognosis for patients. In studies done by Lancer et al., testosterone levels below 100 ng/dl were related with an 18-fold higher mortality risk (9).

Additionally, decreased blood testosterone levels accompany an increase in pro-inflammatory cytokines. In study done by Zhang et al., it was discovered that testosterone levels are negatively associated to IL-6 and CRP levels. Through activating T cells, altering lymphocyte activity, and other communication pathways, testosterone may have a direct influence on these immune system changes (8).

The correlation between total Testosterone and cFT levels and sickness severity was inverse in COVID-19 patients hospitalized with pneumonia. Testosterone levels diminish with age. As a result, the blood testosterone levels of senior men are lower than those of younger men; yet, research does not indicate that aging has a substantial impact on testosterone level decline (4, 7, 9).

The severity of the illness is proportional to the patient's presentation and its intensity. Consequently, the number of viruses that enter a cell has a significant effect on the severity of a disease. SARS-COV-2 employs two kinds of receptors, membranous angiotensin-converting enzyme 2

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(mACE2) and Transmembrane protease, serine 2 (TMPRSS2), for cell entry. TMPRSS2 is a protein that is reliant on an androgenic receptor (AR), which raises the production of TMPRSS2 and, as a result, promotes viral entrance into the cell. Due to the interaction between the spike protein of the virus and mACE2, the release of soluble ACE2 (sACE2) rises. Consequently, especially in light of the presence of Androgenic Receptors on pulmonatry cells in men with low testosterone levels, there is a greater risk of an increase in the virus's frequency of more severe manifestations and the virus's rate of entry(12). During hospitalization, and as a result of viral entrance into the cell, alterations in enzyme and hormone levels that indicate cell dysfunction may be measured. A decrease in testosterone levels is caused by viral infection of the testis cells and a reduction in testosterone synthesis, which may be caused by the presence of high amounts of ACE2 on the testicular cells, which acts as a conduit for the virus to enter the cell. Research demonstrates that Angiotensin 2 has a detrimental influence on the basic and LH-stimulated amount of testosterone synthesis, and that ACE2, by destroying Angiotensin 2, may decrease the disease's severity. Thus, ACE2 in Leydig Cells may influence the microcirculatory blood flow in the local region (7). Measuring the blood levels of the estrogenic hormones, in addition to testosterone, may aid in identifying the reasons of greater COVID-19 mortality and morbidity in men compared to women. Different levels of estradiol (E2) in men and women are correlated with a poor prognosis and a favorable prognosis, respectively, which seems to be related to the biphasic effect of estradiol at different concentrations. High blood levels of estradiol are associated with anti-inflammatory cytokine inhibition, according to studies. It promotes the synthesis of inflammatory cytokines, including IL-6, TNF-a, and IFN-y, at low blood concentrations, as shown in males (10).

The effect of testosterone on respiratory muscles is one of the possible mechanisms of its action. Studies indicate that testosterone has a good effect on the metabolism of respiratory muscles, as in patients with low testosterone levels, respiratory muscle weakening may result in the need for mechanical ventilation and hospitalization in the intensive care unit(8). The level of estrogen hormone in males is typically determined by the aromatase enzyme's conversion of androgenic hormones to estrogen hormone. This enzyme transforms testosterone to estrogen in testis, adipose tissue, bone tissue, brain, and breast. In acute inflammatory diseases such as covid-19, an increase in extratesticular aromatase hormone activity is observed, particularly in adipose tissue. This leads to a decrease in serum testosterone level and an increase in serum estrogen level, both of which are associated with a poor prognosis in patients (5, 9, 12).

According to the studies, a low testosterone level is directly related to the severity of the illness and the necessity for intensive care unit hospitalization.

Studies reveal that low testosterone levels are associated with a rise in inflammatory cytokines, according to studies. On the other hand, recovering patients exhibit a rise in testosterone levels (4). Testosterone Level is a marker that displays the levels of androgenic hormones in males, as well as the function of the testes, and may be used as a prognostic factor. However, studies indicate that testosterone to estradiol ratio (T/E2) is preferable(5). In this research, the average age of the patients was 64.44 years old, with 33 males and 37 women comprising the sample. From 70 patients, 10 were admitted to the intensive care unit and 60 to the internal department. 11 fatalities were recorded at the conclusion of the study, including 3 internal department patients and 8 ICU patients. Patients hospitalized in the ICU were significantly older than those treated in the internal department. Thus, internal department patients had a larger leukocyte count than ICU patients, however there was no significant difference in testosterone and cFT levels. Compared to discharged patients, deceased patients were substantially older. Thus, testosterone levels in died patients were substantially lower than in individuals who were discharged (P-value < 0.05). However, there was no significant difference in the cFT levels of the two groups (P=0.17). Also, D-dimer levels were greater in deceased patients than in discharged patients, although this difference was not significant (P=0.06).

In the research conducted by Pagano et al., greater levels of inflammatory markers such as D-dimer and ferritin suggested a more severe type of illness and correlated negatively with Serum Testosterone Level. In addition, regardless of gender, the blood level of D-dimer correlated with the severity of the illness in our study, as it did in earlier research. Also, In the research by Pagano et al., the severity of the illness was connected with a low lymphocyte count in males and a high neutrophil count in women (10).

In our research, a patient's older age was associated with a greater likelihood of having an illness that necessitates admission in ICU and a greater risk of mortality. According to studies, the blood testosterone level decreases with age, however the serum testosterone level decreases independently of age in acute disorders such as COVID-19 (7, 9).

In our research, there was a substantial association between leukopenia and illness severity, such that ICU patients had a lower WBC count than internal patients. In the research by Infante et al., there was an inverse association between testosterone Level and Neutrophil to Lymphocyte Ratio (NLR), indicating that NLR rises in patients with lower testosterone levels (9).

Overall, Serum testosterone Levels can be used as a prognostic factor for critically ill patients. Serum testosterone level is inversely related to disease severity, and mortality is much greater in individuals with low serum testosterone lev-

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els. Evaluation of the levels of inflammatory markers such as D-dimer is thus beneficial for determining the disease's severity and predicting its prognosis. The levels of D-dimer and serum testosterone were significantly inversely correlated.

5. Conclusion

Serum total testosterone levels can be used as a prognostic factor to predict COVID-19 patients' prognosis. Serum total testosterone is inversely associated with the disease severity, and lower serum total testosterone level is significantly associated with more mortality. Moreover, inflammatory markers such as D-dimer can be used in determining the disease prognosis and severity.

6. Appendix

6.1. Acknowledgment

None.

6.2. Conflict of interest

None.

6.3. Funding support

None.

6.4. Author's contributions

None.

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