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CHANGES IN MALE REPRODUCTIVE HORMONES IN PATIENTS WITH COVID-19

Muataz Lafta Jabbar[1], Majed A Mohammad[2], Ali Malik Tiryag[3]*

[1]*Assistant professor, Dean of College of Nursing, University of Basrah, Iraq.*
[2]*Department of Surgery, College of Medicine, University of Basrah, Iraq.*
[3]*Fundamentals of Nursing Department, College of Nursing, University of Basrah, Iraq.*

Abstract.

Background: COVID-19 is a global, highly contagious, predominantly respiratory viral illness caused by coronavirus 2 (SARS-CoV2). Although COVID-19 is mainly a respiratory disease, it can affect other organ systems causing a lot of extrapulmonary manifestations and multiple organ dysfunctions.
Aim: The aim of the study is to discover if COVID-19 affects male reproductive hormones (testosterone and luteinizing hormone) or not, and whether this effect (if present) is negatively associated with the severity of the disease.

Patients and methods: In this prospective case-control study we recruited 120 reproductive-aged male patients, they represent group (A) which is subdivided into 3 groups according to the severity of the disease (mild, moderate, and severe). A control group consisting of another 120 age-matched males was randomly selected and they represent group (B). The hormone profile of the diseased group is then compared to that of the control group. The testosterone/LH ratio of both groups was calculated and compared.

Results: The age of cases had a mean of 41.96 ± 10.18 and that of the control was 41.85 ± 10.2 years. The age distribution of both groups did not show significant statistical differences (0.937). Patients with COVID-19 exhibited considerably lower levels of total blood testosterone (P value = 0.043) and T/LH ratio (P value 0.0001) compared to the control group, although serum LH levels were significantly higher in the COVID-19 group (P value 0.0001).

Conclusions: In addition to the hematological, biochemical, inflammatory, and immune biomarkers abnormality in patients with COVID-19, the current study demonstrated that total testosterone level, LH level, and T/LH ratio might be affected by the disease according to severity.

Recommendations: Due to the novelty of COVID-19, only a few studies about its effect on male reproductive hormones are available worldwide, and no similar study in our locality. Further studies are required for a better understanding of this issue.

Key words. Male, reproductive hormones, patients, Covid-19.

Introduction.

COVID-19 emerged in China in December 2019 and showed a high rate of infection and mortality. Patients developed pneumonia similar to SARS-CoV [1,2]. The International Committee on Taxonomy of Viruses renamed the virus as SARS- CoV2 and declared as a pandemic by the WHO on 11 March 2020 [3]. The origin is unknown, but it is assumed that it originates in the animal market in China [1]. Old age patients more than 65 years developed more severe disease, among them males showed higher mortality rates than females [4,5]. Data from 29 countries showed clear sex-specific mortality with a higher mortality rate among men compared to women [6]. Previous studies showed that many viruses may attack the testis and induce orchitis, which in turn affects the male reproductive function and may cause infertility, these viruses include influenza, mumps virus, HIV, hepatitis B, hepatitis C, CoxsacKie virus, zika virus, and SARS-COV [7,8]. Apart from the direct damage of the viruses on the testis, other factors such as inflammation, fever, and dysregulation of the HPG axis could impair male reproductive function [7,9].

SARS-CoV2 uses ACE2 receptors and the trans-membrane serine protease (TMPRSS) as an entry into the host cells [10]. ACE2 is expressed in Sertoli cells, Leydig cells, and spermatogonia, while TMPRSS is found more in Sertoli cells as reported by Wang and colleagues [11]. The high expression of ACE2 in the major cells of the testis enhances the virus entry and colonization, which may negatively impact spermatogenesis and reproductive hormones [12], so, for a better understanding of SARS-CoV2 effect on male reproductive hormones, the male reproductive hormone profile (total serum testosterone hormone, luteinizing hormone, and T/LH ratio).

Testosterone hormone is the major male sex and anabolic hormone, it is essential for the development and maintenance of reproductive tissues in human males such as testis, epididymis, seminal vesicles, prostate, and penis as well as male secondary sexual characteristics such as the growth of body hair and increased bone and muscle mass [13]. The testis secretes more than 95% of testosterone (about 6-7 mg per day), and a small amount of testosterone is produced by the adrenal cortex [13]. Several metabolic steps in about 500 million Leydig cells in the testis are responsible for the production of testosterone from cholesterol, which is converted into inactive metabolites in the liver, then it acts by binding to and activating androgen receptors [14].

The process of testosterone production is regulated by a finely controlled system called Hypothalamic-Pituitary-Gonadal (HPG) Axis, in this axis a hormone called Gonadotropin-Releasing Hormone (GnRH) is secreted by the hypothalamus in the brain in a pulsatile manner into the blood circulation, then GnRH stimulates the anterior pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the peripheral circulation, LH triggers the synthesis of testosterone and other sex hormones by the Leydig cell in the testis, on the other hand, FSH acts on Sertoli cells to regulate seminiferous tubules and spermatogenesis in the testis [15]. When testosterone level increases, it acts on the anterior pituitary gland and hypothalamus through a negative feedback loop to inhibit the secretion of GnRH and in turn FSH and LH [16].

Like all other steroids, cholesterol is the precursor substance for the synthesis of testosterone. The first step in the biosynthesis of testosterone is a side-chain cleavage of cholesterol and loss of 6 carbon atoms by the effect of cholesterol side-chain cleavage enzyme (P450scc, CYP11A1) to yield pregnenolone. Pregnenolone loses two carbon atoms to produce a variety of C19 steroids by the effect of 17α- hydroxylase/17,20-lyase enzyme in the endoplasmic reticulum [17]. In the next step, 3β-hydroxysteroid dehydrogenase oxidize the 3β-hydroxyl group to produce androstenedione, which in turn is converted by 17β-hydroxysteroid dehydrogenase into testosterone [18].

In men aged between 19-39 years, the total testosterone level is 264-916 ng/dl [19] with a mean of about 630 ng/dl [20], while it is about 32.6 ng/dl in adult females [21]. Circulating testosterone has three main fractions: free testosterone, sex hormones binding globulin (SHBG) bound, and albumin-bound testosterone [22]. Free or unbound testosterone represents 1.5-2% of total testosterone, while the plasma protein bound represents 98-98.5% [23].

Patients and Methods.

Study design:

The present prospective case-control study was done in Al-Basrah Teaching

Hospital under the supervision of the Scientific Council of Urology during the period between November 10, 2020, and June 2021.

Study population:

We recruited 120 reproductive-aged (median age 36.5 years, ranging from 18-55) male patients for testosterone and luteinizing hormone analysis, who were admitted to Al-Basrah Teaching Hospital, and they represent group (A), a proper sampling strategy was utilized. COVID-19 patients were divided into three subgroups: Group 1: represents 33 patients with mild COVID-19. Group2: represents 41 patients with moderate COVID-19. Group 3: represents 46 patients with severe COVID-19.

The degree of COVID-19 severity [mild (which is defined as those who exhibit any of the different COVID-19 symptoms such as fever, cough, sore throat, malaise, headache, muscular pain, nausea, vomiting, diarrhea, and loss of taste and smell), but who do not exhibit shortness of breath, dyspnea, or abnormal chest imaging) moderate, severe] was determined according to the National Institutes of Health (NIH) classification of severity [24]. A control group consisting of another 120 age-matched males (median age 36.5 years, ranging from 18-55) were randomly selected as consecutive volunteers whose ages almost matched the ages of the patients and they had neither preexisting illness nor other viral infection. Written informed consent was obtained from them and they represent a group (B). The blood samples collected from patients in group A on the 1st day of hospital admission.

Ethical consideration: They provided written informed permission and are a group, therefore (B).

Procedure.

Records from patients with COVID-19 were used to gather clinical data, laboratory results, and radiographic characteristics. The blood samples were obtained from the patients for medical purposes, after the necessary lab tests were finished, the remaining serum was gathered for hormone profile assessment (total serum testosterone, luteinizing hormone, and T/LH ratio). A written informed agreement was acquired for this portion of the trial since the remaining serum samples were often discarded as medical waste and the technique did not place an extra burden or damage on the participants.

The blood levels of luteinizing hormone and testosterone hormone were measured in the control group, and the T/LH ratio was computed. The age-matched control group's men were all detected in the morning due to the sex-related hormones' diurnal pattern. Only blood samples taken in the morning were chosen for the COVID-19 group.

According to the manufacturer's instructions, electro chemiluminescent immunoassays (Cobas e411; Roche, Switzerland) were used to measure the total levels of serum LH and testosterone. The results of the patient's hormone profile and T/LH ratio were compared to that of the age-matched control group.

Statistical analysis: All statistical analysis was performed using SPSS (26-All platforms); continuous variables were expressed as means±standard deviations. The student's t-test results were used to compare the two groups' differences. Differences between multiple groups or sub-groups were analyzed by one-way ANOVA test with Bonferroni correction (Bonferroni Post hoc test). Statistical significance was defined as a P value of < 0.05.

Results.

This study involved 120 patients with COVID-19 of various severities (group A) and carefully matched an equal number of healthy individuals (group B). The age of group A had a mean of 41.96 ± 10.18 and that of group B was 41.85 ± 10.2 years. The age distribution of both groups did not show significant statistical differences (P value = 0.937). Compared to group B, COVID-19 patients had significantly lower total serum testosterone levels (P value = 0.043), and T/LH ratio (P value < 0.0001), whereas serum LH level was significantly elevated in the COVID-19 group (P value < 0.0001). (Table 1).

By comparing the COVID-19 group as subgroups (mild, moderate, and severe) to the age-matched control group, a similar result was found significantly higher LH (P value < 0.0001), lower testosterone (P value = 0.006), and significantly lower T/LH ratio (P value < 0.0001).

For a more detailed comparison between each COVID-19 sub-group with the control group and the other COVID-19 sub-groups, Post hoc analysis (Bonferroni correction) is used, and the results were as follows:

Regarding testosterone hormone, there was no statistical difference when comparing the control group to the mild (P value = 1.000) or moderate group (P value = 0.202), but the difference was significant between the control group and severe group (P value = 0.045). No significant statistical difference was found in serum testosterone between mild and moderate groups (P value = 0.116), but it was significantly lower in the severe group compared to the mild group (P value = 0.036). No statistical difference between moderate and severe groups was found (P value =1.000) (Table 3).

Table 1. *Male reproductive hormones profiles and age in the COVID-19 group and the control group.*

Variables	Cases	Control	P value*
Age	41.96 ± 10.18	41.85 ± 10.2	0.934
Testosterone	4.25 ± 1.3	4.58 ± 1.22	0.043
LH	4.39 ± 1.75	2.91 ± 0.64	0.0001
T/LH ratio	1.11 ± 0.54	1.63 ± 0.48	0.0001

**Student's t-test*

Table 2. *Comparing hormone profiles in COVID-19 subgroups and control group.*

Variables	Control (N= 120)	Mild (N= 33)	Moderate (N=41)	Severe (N=46)	P value*
Testosterone	4.58 ± 1.22	4.79 ± 0.86	4.10 ± 1.12	4.01 ±1.59	0.006
LH	2.91 ± 0.64	2.73 ± 0.57	4.93 ± 1.28	5.10 ± 1.91	0.0001
T/LH ratio	1.63 ± 0.48	1.81 ± 0.45	0.85 ± 0.23	0.84 ± 0.33	0.0001

**One-way ANOVA*

Table 3. *Comparing testosterone of each COVID-19 sub-group with that of the control group and the other sub-groups.*

Group		Mean difference	P value*
Control	Mild	-0.20481	1.000
	Moderate	0.48133	0.202
	Severe	0.58270*	0.045
Mild	Moderate	0.68614	0.116
	Severe	0.78751*	0.036
Moderate	Severe	0.10137	1.000

**Bonferroni correction (Post hoc test)*

Table 4. *Comparing LH of each COVID-19 sub-group with that of the control group and the other sub-groups.*

Group		Mean difference	P value*
Control	Mild	0.17839	1.000
	Moderate	-2.02287*	0.0001
	Severe	-2.18520*	0.0001
Mild	Moderate	-2.20126*	0.0001
	Severe	-2.36360*	0.0001
Moderate	Severe	-0.16233	1.000

**Bonferroni correction (Post hoc test)*

Table 5. *Comparing the T/LH ratio of each COVID-19 sub-group with that of the control group and the other sub-groups.*

Control	Mild	-0.18183	0.168
	Moderate	0.77280*	0.0001
	Severe	0.78230*	0.0001
Mild	Moderate	0.95463*	0.0001
	Severe	0.96413*	0.0001
Moderate	Severe	0.00950	1.000

**Bonferroni correction (Post hoc test)*

Regarding LH, no statistical difference was found between the mild group and the control group (P value =1.000). Serum LH was significantly higher in moderate and severe groups as compared to control or mild groups (P value < 0.0001). No significant difference was identified between the LH of the moderate group and that of the severe group (P value = 1.000) (Table 4).

Regarding the T/LH ratio, no statistical difference was found between the mild group and the control group (P value =0.168). The T/LH ratio was significantly lower in moderate and severe groups as compared to control or mild groups (P value < 0.0001). No significant difference was seen between the T/LH ratio in the moderate group and that in the severe group (P value = 1.000) (Table 5).

Discussion.

The result of this study showed that neither T nor LH in the mild group was significantly changed as compared to the control group; hence the T/LH ratio was not affected. In the moderate disease group, T is not remarkably decreased, LH is significantly increased, and the T/LH ratio is significantly decreased. In the severe disease group, T is significantly decreased, LH is significantly increased, and T/LH ratio is significantly decreased.

Several studies investigated the impact of COVID-19 on male sex hormones, the first evidence showing disturbance in male reproductive hormones in COVID-19 patients was reported in China by Ma et al. [25], showing that testosterone is not remarkably changed, LH is significantly increased, while T/LH ratio is significantly reduced. Also, another study reported there is reduced testosterone/LH ratios have been seen in COVID-19 individuals, suggesting probable subclinical impairment to male gonad function [26] which agrees with the current study.

Another study in China in 2020 showed similar results [27], the results of our study agreed with these findings with the exception that testosterone levels are significantly decreased in the severe COVID-19 group. Rastrelli et al. in Italy reported that low testosterone levels are observed in the most severe cases of COVID-19 [28], the current study agrees with this result. In a study in Germany Schroeder et al reported that testosterone is low in the majority of COVID-19 patients [29], as found in this study. Another study done in Mersin City, Turkey 2020, also reported that testosterone might be decreased in COVID-19 patients [30], the present study showed similar findings. The virus attack on the endocrine glands, cytokines storm, and medication used to manage COVID-19 may disrupt the normality of endocrine coordination.

Conclusion.

The limitations of this study were the lack of long-term follow-up and also development of many variants of the virus, This study demonstrated that total testosterone level, LH, and T/LH ratio might be affected by the disease according to severity. In the severe disease group, T is decreased, LH is significantly increased, and T/LH ratio is significantly decreased. In the moderate group, there is no remarkable decrease in T and a significant increase in LH, while the T/LH ratio is significantly decreased. On the other hand, the hormone profile in the mild group is not affected as compared to the control group. Preexisting medical illnesses, age, viral load, and severity of symptoms all may affect the duration of hormone changes.

Recommendations.

Due to the novelty of COVID-19, only a few studies about its effect on male reproductive hormones are available worldwide and no similar study in our locality. Further studies are required for a better understanding of this issue.

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