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The effect of some hormonal variables on the blood serum of men recovered from COVID-19 in Fallujah city

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Abstract---The current study aimed to know the effect of some physiological and hormonal variables in the blood serum of 60 samples of recovered men from COVID-19, their ages range from 20 to 49 years old, some of them were infected once and some of them were infected more than once, while the control group included 30 samples of healthy men, The samples were collected in Fallujah Teaching Hospital and from some private laboratories in the city of Fallujah. The sample collection period was from 10/15/2021 to 7/2/2022, during which blood samples were collected and variables were measured with various modern devices.

Keywords---PSA, testosterone, FSH, LH.

Introduction

Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome (SARS CoV2)) is one of the most prevalent diseases, so it is called a pandemic that affected the world (Unnikrishnan and Misra, 2021). This disease affects the respiratory system, the heart, the blood vessels, the digestive system, the nervous system and the genitourinary system, (Çayan *et al.*, 2021). The coronavirus disease (COVID-19) appeared in late December 2019 in Wuhan, China, and spread to different regions of the world, causing a panic attack, and it affected the various economic, social and health fields (Subhi *et al.*, 2020)

The most common symptoms at the onset of the disease are fever, dry cough, fatigue, shortness of breath and muscle pain, and the least common symptoms are dizziness, headache, diarrhea, vomiting and nausea (Chatterjee *et al.*, 2020), and on March 11, 2020, the World Health Organization (WHO) announced that

the severe acute respiratory syndrome coronavirus (SARS-CoV-2) that causes the coronavirus disease (COVID-19) has become a pandemic, and it turns out that men are more likely to be infected with COVID-19 disease, even with a higher death rate compared to women, and thus from Importance Determining whether SARS-CoV-2 infection can affect male fertility as an immediate or long-term consequence of the disease (Dutta and Sengupta, 2021). The COVID-19 affects on the testis and affects on the male reproductive function, and there are many other factors such as fever, inflammation, and HPG axis dysregulation may also decrease the testosterone secretion or sperm production (Ma *et al.*, 2021).

Material and Methods

Collection of blood sample

Blood samples were collected by withdrawing 5 ml of venous blood using a sterile medical syringe for one time only. The blood was divided into two tubes (Gel tube, EDTA tube, and the blood in EDTA was used directly to measure the complete blood picture, then a tube was placed Gel tube was centrifuged at 3000 rpm for 15 minutes in order to obtain blood serum, then the serum was withdrawn by micropipette, and the serum was divided into several parts and distributed in Eppendorf tube and kept at a temperature of -20°C , until it was used to measure variables Biochemical and hormonal (Kale *et al.*, 2012)

Determination of PSA

The concentration of the tPSA test was estimated using the ready-made kit from the American company Monobind, and it was measured using ELISA technology.

Working Principle

The basic reagents required for the enzyme immunoassay include antibodies of high affinity and quality (enzyme and stabilizer). In this procedure, fixation occurs during the examination on the surface of a small plate through the process of interaction between Streptavidin that covers the inner surface of the small pits and the Biotinylated monoclonal antibody (anti-PSA). When the biotinylated monoclonal antibody, the enzyme labeled antibody, and the antigen present in the serum of the sample are mixed, it results in an interaction between the antigens and antibodies without competition or hindrance and thus forms a complex in the form of a Sandwich sandwich. The high affinity and affinity between Streptavidin and Biotinylated antibody, and then the pits are washed to get rid of the unbound substances, and then the base material that binds to the enzyme bound to the antibody is added, and the result is that the solution turns blue, which stops when the stop solution is added. Then the absorbance is measured at a wavelength of 450 nm, as the intensity of the color is directly proportional to the amount of hormone in the sample (Suryasa *et al.*, 2021).

Determination of Testosterone

Working Principle

The main reagents required for measuring the concentration of testicular lipotropic hormone in the ELISA technique include the competition between a fixed amount of Enzyme-Antigen Conjugate, with the original antigen present in the sample, and when mixing a fixed amount of biotinylated antibody reagent with each of the Enzyme-antigen conjugate and containing serum. On the native antigen, a competitive reaction is produced between the biotinylated antibody and the material that coats the inner surface of the pits, streptavidin, to form a Sandwich Complex bound to the inner surface of the pits. With the enzyme conjugated with the antibody and thus the result is obtained where the solution turns blue and this color stops when the stop solution is added and the absorbance is measured at a wavelength of 450 nm. The intensity of the color formed is directly proportional to the amount of enzyme present, and inversely to the amount of testicular fat hormone in the sample.

Determination of FSH, LH

The concentration of hormones (FSH, LH) was estimated using ready-made kit from the American company Monobind and it was measured using ELISA technology.

Working principle

A good test subject to undergo testing, this procedure is in place between Streptavidin covering the inner surface of the micro-pits and Biotinylated monoclonal (anti-FSH, anti-LH). When mixing a biotinylated monoclonal antibody and an antibody called an antienzyme that contains an enzyme that is present in the reaction due to the high-image related interaction between Streptavidin and the biotinylated antibody, then the pits are washed to get rid of immaterial substances, and then the pits are washed to get rid of general substances, Then add the base substances to the classifications of antibody-bound substances, the result by the antibody-bound antibody, and the result. Then the absorbance is measured at the wavelength of 450 nm, as it is proportional to the colored region from the amount of hormone in the region.

Statistical analysis

All study results were analyzed using the Statistical Package for the Social Sciences (SPSS) program. Using the Completely randomized design (CRD) system, the arithmetic mean was compared by using the t-test to determine the variance between two groups of studied traits at the level of probability ($P \leq 0.05$). (Steel and Torrie, 1980)

Results and Discussion

Prostate Specific Antigen Concentration

The results in Table (1) showed that there was no significant difference between the results of the group who recovered from COVID-19 and the results of the control group at a significant level $P \leq 0.05$. These results are in agreement with the results of (Cinislioglu, Demirdogen, *et al.*, 2022), where the results of his study in 91 people showed no statistical difference between the PSA level in the blood that was measured before the people were infected with COVID-19 and the PSA level in the blood measured after recovery from the disease. his study showed an increase in PSA results during the infected period. In a previous study conducted by the researcher (Di Vincenzo *et al.*, 2021) assessed serum PSA levels in 24 patients with COVID-19 and found that PSA levels in serum of COVID-19 patients were within the normal range.

Table (1) The PSA concentration of the Recovered group and the control group

Variable	Groups	No.	Mean± Std.
PSA (ng/ml)	Recovered	60	1.35± 0.43 N.S
	Control	30	1.32± 0.36

FSH Concentration

Results from Table (2), showed a significant increase in FSH hormone in the group who recovered from COVID-19 compared to the control group at a significant level of $P \leq 0.05$. The highest values were in people who had recently recovered from COVID-19, as shown in Table (3). The results of this study agree with (Cinislioglu, *et al.*, 2022) in infected persons due to a recent recovery from COVID-19 in the first weeks after infection, so the virus has an effect on the gonads due to fever, inflammation or medications and may also effect on the axis of Hypothalamus - pituitary gland - male gonads (Ma *et al.*, 2021)

Table (2) FSH concentration in the Recovered group and the control group

Variable	Groups	No.	Mean± Std.
FSH (mlU/ml)	Recovered	60	11.20±2.28 *
	Control	30	6.08±2.97

Table (3) Distribution of follicle-stimulating hormone results for the group of recovered patients according to the recovery period

Recovery Duration	Normal range FSH (mlU/ml)			Total
	< 0.1	1.0 – 14.0	> 14	
1-4 week	0	12	8	20
1-4 month	0	16	3	19
4-7 month	0	18	3	21
Total	0	46	14	60

Luteinizing hormone(LH) Concentration

The results of Table (4) showed that there was a significant increase in the group of recovered patients compared to the control group at a significant level of $P \leq 0.05$. The highest values were in people who had recently recovered from COVID-19, as shown in Table (5). The results of this study agree with (Tiwari *et al.*, 2021) and (Kadihasanoglu *et al.*, 2021) and that the increase in these results may be due to the recent recovery from COVID-19 due to the rise in this hormone during infection, as their results showed an increase in LH values. While these results did not agree with (Abbas, 2022) in a study conducted on the recovered, where the results were a little increase, that's mean there is no significant difference, and the reason may be taking samples after months of recovery

Table (4) LH concentration in the recovered group and the control group

Variable	Groups	No.	Mean± Std.
LH (mIU/ml)	Recovered	60	9.52± 2.92 *
	Control	30	5.26± 1.69

Table (5) Distribution of Luteinizing Hormone (LH) results among the group of recovered patients according to the recovery period

Recovery Duration	Normal range LH (mIU/ml)			Total
	< 0.7	0.7 - 7.4	> 7.4	
1-4 week	0	11	12	23
1-4 month	0	13	3	16
4-7 month	2	14	5	21
Total	2	38	20	60

Testosterone Concentration

The results showed in Table (6) that there was a significant decrease among the recovered group compared to the control group at a significant level $P \leq 0.05$. The results of this study are in agreement with (Abbas, 2022) and (Ma *et al.*, 2021), which showed a significant decrease in people recovering from COVID-19 in a study to evaluate sex hormones and semen characteristics of those recovered. The drop in the testosterone hormone may be due to the late pneumonia that people suffer from at the end of the recovery stage (Aboelnaga *et al.*, 2021), or it may be due to the high level of the prolactin hormone after recovery, which was proven by the Egyptian study (Salama and Belgozah, 2021).) and the Chinese study (Xu *et al.*, 2021)

Table (6) the concentration of testicular lipid hormone in the recovered group and the control group

Variable	Groups	No.	Mean± Std.
Testosterone (ng/ml)	Recovered	60	5.64± 1.20 *
	Control	30	8.51± 2.24

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