The Relation between the Plasma Level of Testosterone Hormone and the Severity of COVID-19 in Iraqi Patients

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Abstract

Coronavirus disease 2019 discovered in December 2019, Wuhan, China. It was transmitted globally producing the present COVID-19 pandemic. Concerns have been raised about the potential impact of COVID-19 on male reproductive organs and male fertility as the number of infections in the male community has increased. The objectives of current study are studying the relationship between the plasma levels of testosterone and the markers of immune reaction with the severity and mortality in a sample of COVID-19 patients. A cross section study included NO= 103 male patients affected by SARS- CoV-2 pneumonia, diagnosed by PCR and chest CT scan, (≥ 18 years old), and recovered in the respiratory intensive care unit (RICU). Several biochemical risk factors were determined Free Testosterone, sex hormone binding globulin (SHBG) were measured by Enzyme-Linked Immunosorbent Assay(ELISA), D-dimer, Ferritin, CRP, Urea, Creatinine were measured by automated method by using Abbott Architect c4000 and Complete Blood Count(CBC). The results show that the serum free testosterone and SHBG levels a significant lower in non-survivor patients than survivor patients with COVID-19. While the other biomarkers (D-dimer, Ferritin, Urea, Creatinine) were significant higher in non-survivor patients than survivor patients. The CRP, WBC and lymphocyte showed that no significant between the both group of patients. In conclusion the study showed that lower free testosterone and SHBG levels enable significant role in increasing risk of COVID-19 mortality amongst adult male patients.

Keywords: *COVID-19 ,Testosterone level, Severity.*

Introduction

In the second half of 2019, a pneumonia with an unclear etiology was discovered in Wuhan, Hubei Province, China. The pathogen was quickly isolated and named 2019 Novel Coronavirus (2019-nCoV) on12 January, 2020 [(1)]**.** The virus has spread to Italy and other European countries, as well as the United States, after being contaminated in China and causing thousands of deaths, and the number of new confirmed cases is rising every day. Owing to the wide spread infectivity and high transmission rate, the World Health Organization(WHO) called the disease coronavirus disease 2019 (COVID-19) and declared it a pandemic. Human coronaviruses typically cause respiratory and enteric infections [(2)]. To data, there have been over 140 Million confirmed cases of COVID-19 and over 3 Million deaths across

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the globe. The COVID-19 begins when COVID-19 is transmitted from one person to another through inhalation or oral ingestion of virus-containing droplets. COVID-19 spike protein binding to the angiotensin-converting enzyme 2 (ACE2) receptor may allow the virus to penetrate epithelial cells in the nasal or oral cavities[3]. The COVID-19 is more common in men than in women, according to epidemiological data from China and Europe. A meta-analysis of 39 observational studies found that males (57.4%) were more likely than women to require hospitalization as a result of COVID-19 infection[4]. However, there are gender disparities in the prevalence of COVID-19, and it is an unexplained phenomenon why men appear to be more susceptible to COVID-19 infection than women, with a greater fatality rate. The COVID-19 pathogenesis is aided by the angiotensin-converting enzymes 2 (ACE2) receptor, which aids direct host cell destruction. Binding of COVID-19 virus with the ACE2 receptors enables its cellular entry and replication. As a result, cells with increased ACE2 expression may appear to be more sensitive to COVID-19 infection. Having considered these reports, it is a breakthrough revelation in male fertility research that testes have the highest ACE2 mRNA and protein expressions levels of all body tissues. The seminiferous duct cells, spermatogonia, Leydig cells, and Sertoli cells are the four main testicular cell types that express ACE2 [5].

Materials and Methods

This study was conducted at the Department of Biochemistry, College of Medicine, University of Baghdad, at Khalis General Hospital and Baquba Teaching Hospital in Diyala during the period from November 2020 to January 2021. It involved 103 COVID-19 male patients who were diagnosed by PCR and chest CT scan. Their age ≥ 18 years old. Patients who taking testosterone and tamoxifan drugs, taking sports stimulants, suffer from excessive obesity and the females were excluded from the study.

Blood Samples:

About 6 ml of blood samples were obtained from veins for COVID-19 patients. Each blood samples divided into three parts:

1. The first part 1ml of whole blood for measuring CBC.

2. The second part 2ml put in anticoagulant tube separated by centrifugation at 3000 rpm for 10 min for obtained plasma to measuring D-Dimer.

3. The third 3ml in plane tube separated by centrifugation at 3000 rpm for 10 min, the resulting serum divided into aliquot for:

Aliquot 1: Biochemistry measurement for ferritin, CRP, urea and creatinine.

Aliquot 2: The rest were stored at (-20 ºC) until assayed for serum free testosterone and sex hormone binding globulin(SHBG).

Results

The current study is cross sectional study that involve 103 COVID-19 male patient, with mean± SE of age 45.7 ± 1.7 years. Enrolled patient have been tested for several biomarker level to evaluate their clinical condition and the severity of disease that include CBC, S. ferritin, plasma D-Dimer, CRP, S. urea and creatinine. In order to evaluate association of disease severity and gender difference free testosterone and SHBG were measured.

all those details are presented in table 1.

Table 1: Study laboratory characteristics of participants, frequency and percentage.

	Reference range		Frequency	Percent
Age group		below 35	19	18.4%
		age range 35-55	43	41.7%
	0.5≥	above 55	41	39.8%
CRP level (mg/dl)		normal level	2	1.9%
		high CRP level	101	98.1%
lymphocyte level (10^3/uL)	1.00 – 3.70	Low lymphocyte count	55	53.4%
		Normal lymphocyte count	48	46.6%
WBC level (10^3/uL)	3.00 – 15.00	normal WBC count	21	20.4%
		Leukocytosis	82	79.6%
D-dimer level(ng/ml)	198	normal D-dimer level	2	1.9 %
		high D-dimer level	101	98.1%
Urea (mmol\l)	< 50 yrs(3.2- 7.4) >50 yrs(3.0 -9.2)	High Urea level	51	49.5%
		Normal Urea level	51	49.5%
		Low Urea level	1	0.97%
Creatinine(umol\l)	63.6 -110.5	Normal creatinine level	49	47.5%
		High creatinine level	34	33%
		Low creatinine level	20	19.4%
Ferritin level (ng\ml)	30 – 300	normal ferritin level	11	10.7%
		high ferritin level	92	89.3%
Testosterone level (pg/ml)	19-55 yrs(1.00-28.28) >55yrs (0.70-21.45)	normal or low testosterone level	73	70.9%
		high testosterone level	30	29.1%
SHBG level (nmol/l)	16.8 - 113.2	low or normal SHBG	98	95.1%
		high SHBG	5	4.9%
Fate				
		Alive	63	61.2%
		Dead	40	38.8%
		Total	103	100.0%

In table 2, the patient is divided into two groups according to their fate, the mean ±SE of biomarker for reach group was measured

Table 2: Mean ± SE of the studied biomarkers.

	Alive(n= 63)	**Dead(n= 40)**	**p-value**
	Mean ± SE	**Mean ± SE**	
age yrs.	52.77 ± 2.04	57.80 ± 2.93	0.15
CRP (mg/dl)	182.91 ± 27.83	194.29 ± 23.005	0.77
Ferritin(ng\ml)	1182.7 ± 142.45	1777.8 ± 126.02	0.005*
WBC (103/uL)	14.27 ± 0.70	16.18 ± 0.93	0.10
LYMPH(103/ul)	0.98 ± 0.09	0.82 ± 0.07	0.24
D-dimer(ng/ml)	1857.50 ± 291.21	3985.42 ± 1224.95	0.04*
Urea(mmol\l)	9.92 ± 1.07	15.10 ± 2.07	0.01*
Creatinine(umol\l)	96.17 ± 8.54	174.61 ± 29.92	0.003*
Free Testosterone (pg/ml)	18.81 ± 0.92	11.77 ± 1.35	0.001*
SHBG (nmol/l)	62.49 ±3.87	44.10 ± 4.88	0.001*

*Significant p-value ≤ 0.05

To evaluate the correlation between free testosterone and SHBG with other biomarker in both dead and alive group, Pearson correlation coefficient r was measured and as showed that in table 3 and table 4.

Table 3: The correlation between free testosterone with other markers.

	Alive (n= 63)		**Dead (n= 40)**	
	r	**p-value**	**r**	**p-value**
WBC(103/uL)	0.25	0.04*	0.01	0.918
Lymphocyte(103/uL)	0.04	0.75	-0.01	0.94
CRP (mg/l)	0.06	0.63	-0.08	0.62
Ferritin (ng/ml)	-0.03	0.8	-0.008	0.96
D-Dimer (ng/ml)	-0.17	0.18	-0.03	0.83
Urea (mmol/l)	-0.06	0.62	-0.12	0.43
Creatinine (µmol/l)	-0.19	0.23	-0.11	0.38
SHBG (nmol/ml)	0.47	0.001*	0.21	0.19

Table 4: The correlation between SHBG with other marker in both groups(dead and alive) .

	Alive (n= 63)		Dead (n= 40)	
	r	p-value	r	p-value
WBC (103/µl)	0.16	0.19	0.15	0.3
Lymphocyte(103/µl)	0.07	0.56	-0.12	0.43
CRP (mg/l)	0.23	0.06	0.01	0.95
Ferritin (ng/ml)	0.03	0.79	-0.008	0.96
D-Dimer (ng/ml)	-0.14	0.25	0.45	0.003*
Urea (mmol/l)	-0.08	0.51	-0.07	0.65
Creatinine (µmol/l)	-0.08	0.52	-0.11	0.49
Free testosterone (pg/ml)	0.47	0.00*	0.21	0.91

Discussion

The current findings showed that there was significant increased (98.1%) in CRP levels and (79.6%) in leukocyte count in COVID-19 patients, these results in agreement with [6]. These increment of levels might be linked to the overproduction of inflammatory cytokines in patients with COVID-19 levels [7]. And the current results showed that no significant different in the non-survivors and survivors patients in CRP levels and WBC count , these results was disagreement with [8]. The results of present study showed that the levels of lymphocyte count in blood of COVID-19 patients were decreased in (53.4%) of patients. These results in agreement with [6,9]. Lower lymphocyte count might be associated with severe COVID-19, need for Intensive Care Unit and increased mortality[8]. These results disagree with the present study that showed the non-survivors with confirmed COVID-19 had lower lymphocyte count when compared with survivors but no significant difference between the survivors and non-survivors patient group. The current findings showed that (89.3%) of the enrolled patients have high levels of ferritin ,these results were in agreement with [6]. Patients with severe COVID-19 have been reported to have elevated ferritin levels as well as a cytokine storm. Because hyperferritinemia has been linked to inflammatory states in COVID-19 infection, ferritin could be a valuable marker for predicting disease severity and the scope of the cytokine storm [10]. The present results found that a significant differences between non-survivors group which is higher than the survivors group of patient, which in turn is a higher than the normal level this results was in agreement with [11].

D-dimer which is the fragments produced when plasmin cleaves fibrin to break down clots was high in about (98.1%) of patients. This increment in D-dimer levels is linked to venous thromboembolism (VTE) and acute pulmonary embolism (APE), both of which are associated with a high fatality rate [12]. The current study found that D-dimer a significant

differences between dead group which is higher than the alive group of patients which was higher than reference range this results was in agreement with [12]. Higher D-dimer levels in COVID-19 patients ,hinting coagulation abnormalities [10]. Testosterone level was taken as an additional marker for disease severity markers in the present study and showed that (70.9%) of the participants have normal or low testosterone level , which comes in agreement with [6], this study found that COVID-19 has the potential to lower testosterone levels. Lower testosterone levels at baseline were associated with a significantly increased risk in term ICU admission and mortality. The current findings showed that free testosterone levels was lower significant in COVID-19 non-survivor patients compared with survivor patients , which was also lower than normal range , this results was in agreement with [6]. Low Testosterone levels appear to be associated with increased susceptibility of respiratory diseases, as well as a predictor for adverse outcomes and mortality from COVID-19 [13]. Çayan et al [13] showed that COVID-19 could decrease circulating testosterone levels, and lower testosterone levels at baseline were linked to a higher risk of severe symptoms requiring ventilator support because of the ACE2 receptor upregulation in respiratory cells ,elevated risk of lung damage and mortality. Testosterone is linked to the immune system of respiratory organs, and low testosterone levels might increase the risk of respiratory infections. The ACE2 is a constitutive product of adult type Leydig and Sertoli cells. In addition, it plays a critical function in lung protection [14]. Coronavirus disease 2019 can penetrate epithelial respiratory cells because ACE2 is activated and down-regulated by the virus's spike protein [15]. As a result, viral binding to the ACE2 receptor may reduce its expression causing degradation in a lung protective pathway, and might affect testosterone production in male COVID-19 patients , resulting in an increase in pro-inflammatory cytokines in COVID-19 infected patients [16]. The current findings also showed that (95.1%) have low or normal sex hormone binding globulin (SHBG). Sex hormone binding globulin levels in the current study showed that low significant in non- survivor patients compared with survivor patients , which was also lower than normal range. This decrease in the levels of SHBG might be due to it's direct relation to testosterone. Although COVID-19 primarily affects the respiratory system, it has also been found to damage multiple organs, such as the kidneys . Because the kidneys have the highest level of ACE2 expression [17]. The present study showed that (49.5%) of patients having high level of urea. While (33%) of patients having high level of creatinine. And showed that both urea and creatinine are significant differences between non-survivors group which were higher significant than survivors group. These results were in agreement with (18) who found that (35%) of patients with COVID-9 had increase in urea and creatinine levels. These data suggest that COVID-19 mediated impaired kidney function may be one of the main causes final death in COVID-19 patients.

The current findings showed that no significant correlation between the free testosterone with other biomarkers in both groups. But found that a significant positive correlation between SHBG and free testosterone in the survivors group might be normal due to it's direct relation to testosterone. And found that positive significant correlation between WBC count and free testosterone in the survivors group, further more studies are needed to understand the correlation between WBC and free testosterone in survivors patients. The findings in the present study showed that no significant correlation between SHBG with other biomarkers in both groups. But found that a significant positive correlation between SHBG and free testosterone in the survivors group, and a significant positive correlation between SHBG and D-dimer in the non-survivors group.

Conclusions

Free testosterone and SHBG levels decrease in all patients with COVID-19 and it was more clear in non-survivors patients compared with survivor patients might be good taking a marker of severity.

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Ethical Clearance: Not required

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