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Investigation of pituitary functions after acute coronavirus disease 2019

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Abstract. Although coronavirus disease 2019 (COVID-19) mainly involves the lungs, it also affects many systems. The hypothalamic/pituitary axis is vulnerable to hypoxia, hypercoagulation, endothelial dysfunction and autoimmune changes induced by COVID-19 infection. Given that there is no extensive investigation on this issue, we investigated the pituitary functions three to seven months after acute COVID-19 infection. Forty-three patients after diagnosis of COVID-19 infection and 11 healthy volunteers were included in the study. In addition to the basal pituitary hormone levels, growth hormone (GH) and hypothalamo-pituitary adrenal (HPA) axes were evaluated by glucagon stimulation test (GST) and low-dose adrenocorticotropic hormone (ACTH) stimulation test, respectively. The peak cortisol responses to low-dose ACTH test were insufficient in seven (16.2%) patients. Twenty (46.5%) and four (9.3%) patients had inadequate GH and cortisol responses to GST, respectively. Serum insulin-like growth factor-1 (IGF-1) values were also lower than age and sex-matched references in four (9.3%) patients. The peak GH responses to GST were lower in the patient group when compared to the control group. Other abnormalities were mild thyroid-stimulating hormone elevation in four (9.3%) patients, mild prolactin elevation in two (4.6%) patients and central hypogonadism in four (9.3%) patients. Mean total testosterone values were lower in male patients when compared to male controls; however, the difference was not significant. These findings suggest that COVID-19 infection may affect pituitary functions, particularly the HPA and GH axes. These insufficiencies should be kept in mind in post-COVID follow-up. Long-term data are needed to determine whether these deficiencies are permanent or not.

Key words: COVID-19 (Coronavirus disease 2019), SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2), Pituitary, Growth hormone, Cortisol

IN NOVEMBER 2019, coronavirus disease 2019 (COVID-19) was first identified in Wuhan city of China and it exhibited a quick spread worldwide. As a result, the World Health Organisation declared it a pandemic in March 2020 [1, 2]. The causal agent of the disease, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a novel RNA virus from the coronavirus family. Both SARS and SARS-CoV-2 enter the target cell *via* angiotensin-converting enzyme 2 (ACE2) receptors and these viruses show structural similarities [3, 4]. The clinical presentation has a wide spectrum from being asymptomatic to progressive pneumonia leading to acute respiratory distress syndrome (ARDS). Specific molecular tests of respiratory samples and computed tomography of the thorax, if necesssary, are used in the diagnosis [5].

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ACE2 is a transmembrane metalloproteinase first identified in 2003 as the cell surface receptor for SARS. It is well-known that numerous tissues and systems have ACE2 receptors [6]. For SARS-associated infection to occur, the target cell must contain the ACE2 receptor. Lung injury is the most prominent pathophysiology of this infection; however, tissues overexpressing the ACE2 receptor could be potential targets of COVID-19 infection [2]. The presence of ACE2 receptors has been shown in many tissues, such as the colon, small intestine, liver, heart, kidney, salivary gland, pancreas, testis, ovary, brain, skin, spleen, bladder, adipose tissue, thyroid gland, adrenal glands, pituitary, and hypothalamus [7-9]. Gastrointestinal, cardiovascular, neurological, and genital system involvement may also be seen in the course of COVID-19 infection [1, 10-12]. Moreover, newly diagnosed diabetes mellitus due to pancreatic beta-cell damage associated with COVID-19 infection have been reported [13]. COVID-19 infection may affect many organ systems, since the disease behaves like systemic vasculitis considering the involvement of arterial and

venous small vessels [8, 14]. Moreover, it may lead to endothelial dysfunction and thromboembolic hypercoagulable condition by microvascular injury due to vascular infiltration [15, 16]. Apart from acute infection, in the post-COVID period, there may be persistent symptoms such as fatigue, myalgia, mood disturbances, anorexia, and vomiting [17-19].

The pituitary gland has a rich blood supply and is considered an important regulator of the endocrine system mediating a number of physiological processes. The pituitary gland is highly vulnerable to hypoxia, ischaemia, and hypovolemia [20]. Pathological conditions, such as encephalitis, meningitis, ischaemic stroke, subarachnoid haemorrhage, trauma, and sepsis, are known to cause hypothalamo-pituitary dysfunction *via* increased intracranial pressure, vascular injury, autoimmune, and inflammatory changes [21-24].

Furthermore, SARS was previously suggested to affect the pituitary gland after recognition of central hypothyroidism and central hypoadrenalism in some patients [25]. Autopsy examinations of patients infected with SARS showed ACE2 receptor overexpression in the hypothalamus and significant effects were seen in the adenohypophysis [26, 27]. Thus, SARS may probably affect pituitary functions through direct viral damage and/or lead to hypophysitis with autoimmune mechanisms [8, 9].

The pituitary gland and hypothalamus are potential targets of the virus due to the abundance of the ACE2 receptor. However, the relationship between COVID-19 infection and pituitary function/dysfunction is yet to be extensively investigated. Therefore, this study investigated pituitary functions, particularly the growth hormone (GH) and hypothalamic pituitary adrenal (HPA) axes, at least three months after acute COVID-19 infection.

Patients and Methods

Study participants

The Ethics Committee of Erciyes University Medical School approved this study (Date: 8th July 2020, Approval number: 2020/363). Informed consent was obtained from all participants. The presence of SARS-CoV-2 was confirmed by real-time reverse transcription-polymerase chain reaction test (rRT-PCR) using oropharyngeal and nasopharyngeal swab samples.

Patients were consecutively involved in the study after at least three months (mean period of 5.6 ± 1.3 months) of the diagnosis of COVID-19 infection. Exclusion criteria were the presence of stress factors, such as infection other than COVID-19, medications affecting the HPA axis, history of hypothalamic-pituitary disease and head trauma, malignancy, liver and kidney failure and pregnancy at the time of evaluation.

According to the Chinese Clinical Guidance of COVID-19 Pneumonia, the severity of COVID-19 infection was divided into four groups [28]. Mild disease was diagnosed as the presence of mild clinical symptoms without any pneumonia. Moderate disease referred to the presence of respiratory symptoms with fever and pneumonia was diagnosed. Severe disease was diagnosed as the presence of any of these criteria: respiratory rate at or higher than 30 breaths per minute, resting oxygen saturation at or lower than 93% or more than 50% increase in pulmonary lesions on imaging within 24–48 hours. Critical disease was diagnosed as the presence of any of these criteria: need of mechanical ventilation for respiratory failure, shock or need of intensive care service. During the treatment of COVID-19 infection, 20 patients used favipiravir, 33 patients used hydroxychloroquine, 11 patients used oseltamivir, 7 patients used glucocorticoid, one patient used tocilizumab and 32 patients used low molecular weight heparin.

Assessment of pituitary functions

Basal hormone levels, including prolactin, total testosterone, estradiol, luteinising hormone (LH), follicle-stimulating hormone (FSH), free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), insulin-like growth factor-1 (IGF-1), and sex hormone-binding globulin were measured between 08.00 and 09.00 h in all participants.

Gonadotropin deficiency was diagnosed by low levels of estradiol (in females) or testosterone (in males) in the context of inappropriately normal levels of gonadotropins. TSH deficiency was diagnosed by low levels of free T4 when TSH levels were low or normal. Hyperprolactinemia was diagnosed by serum prolactin levels above the normal reference range and samples were obtained in stress-free conditions at least two hours after awakening. Seven patients used glucocorticoid treatment in the acute phase of COVID-19 infection. Five of patients used 40 mg/day methylprednisolone for three days, two patients used 80 mg/day methylprednisolone for seven days. Evaluation of the patients was done at least three months after the last dose of glucocorticoids. Thus, the probability of the iatrogenic adrenal insufficiency is very low. HPA axis was evaluated using basal cortisol/adrenocorticotropic hormone (ACTH) levels and low-dose ACTH stimulation test. IGF-1/GH axis was evaluated with GST and serum IGF-1 level. Cortisol responses to GST were also evaluated. These stimulation tests were done on different days after overnight fasting. Blood samples were obtained for cortisol response measurement at baseline and at 30, 60, 90, 120 minutes (min)

after intravenous administration of 1 μg of ACTH (Novartis Pharma, Synacthen, Lion, France). 1 μg of ACTH was prepared by using 0.25 mg of ACTH mixed 250 mL of 0.9% NaCl. The details are presented elsewhere [29, 30]. Peak cortisol response higher than 12.5 μg/dL was considered to be sufficient for the low-dose ACTH stimulation test. We have previously defined the lower limit of cortisol response to low-dose ACTH stimulation test in healthy individuals using high-dose ACTH stimulation test as reference. In other words, cut-off cortisol values for low-dose ACTH test was compatible with adequate cortisol response to standard dose ACTH test [30, 31]. We did not perform an additional high-dose ACTH test, since we used the appropriate cut-off levels determined for low-dose ACTH test. Blood samples were obtained for GH at baseline and at 90, 120, 150, 180, 210, and 240 minutes after intramuscular administration of 1 mg (1.5 mg if patients >90 kg) glucagon (Denmark, Bagsvaerd, Novo Nordisk). The peak GH value >1.07 μg/L was considered an adequate response to GST [32] . Cortisol response lower than 10.7 μg/dL was considered an inadequate response to GST [30].

Analytical methods

Serum TSH, FSH, LH, prolactin, ACTH, cortisol, free T3, estradiol, testosterone, GH, IGF-1, and free T4 were measured using the electrochemiluminescence immunoassay (ECLIA) technique with commercially available assay kits (Cobas; Roche Diagnostics, Mannheim, Germany).

Statistical analysis

IBM SPSS version 15.0 (IBM Inc, USA) was used for the statistical analyses. Data were presented as mean ± standard deviation (SD) or median (25%–75%). The distribution pattern of the data was determined by Shapiro-Wilk test. Two independent samples *t*-tests were used to analyse normally distributed data and Mann-Whitney U test was used for data that were not normally distributed. Chi-square test was used analyse qualitative variables. One-way ANOVA was used to compare the infection severity groups of COVID-19. In post hoc analyses, Games-Howell test was used when the variances were not normally distributed, while Tukey HSD test was used when the variances were normally distributed. *P* values <0.05 were considered statistically significant.

Results

Forty-three patients (24 men, 19 women) and 11 controls (6 men, 5 women) were included in the study. Body mass index (BMI) and mean age of the patients was 31.0 ± 5.9 kg/m^2 and 44.2 ± 10.7 years (range: 21–64), respectively, while BMI and mean age of the controls was 29.9 ± 3.2 kg/m^2 and 44.1 ± 12.4 years (range: 29–64), respectively. There were no significant differences between the two groups in terms of gender, BMI and age. Thirteen patients gained weight (median 6 kg, range: 2–12 kg) during the recovery phase, where 5 of them reported that they re-gained the weight they lost during the infection period. Four patients lost a median weight of 6 kg (range: 4–8 kg) during the recovery phase. The others did not report any significant change in their weights. The underlying conditions were as follows: Hypertension in six patients, hyperlipidemia in three patients, diabetes mellitus in four patients (two of them were on insulin therapy and the others were on oral hypoglycaemic agents). Twelve patients were obese (BMI >30 kg/m^2). According to the Chinese Clinical Guidance on COVID-19 Pneumonia, the patients were categorised as having mild (2 patients), moderate (15 patients), severe (22 patients), and critical (4 patients) disease [28].

Due to COVID-19 infection, five (11.6%) of the 43 patients were followed-up in the intensive care unit. No significant differences were detected for pituitary functions between patients with or without a history of intensive care unit admission. During the acute COVID-19 infection, 10 (23.2%) patients had impaired kidney function, 13 (30.2%) patients had impaired liver function, 26 (60.4%) patients had fever, and 7 (11.6%) patients had used glucocorticoid therapy less than three weeks. Twenty-two (51.1%) of the 43 patients had oxygen requirement due to COVID-19 infection. Statistical analysis investigating the effect of impaired liver/kidney function tests, fever and use of glucocorticoid therapy on pituitary hormone levels were not significant. Although the patients had some symptoms, there were no apparent post-COVID symptoms.

Mean free T3 and ACTH levels were significantly higher in the patient group (p = 0.04 and p = 0.002, respectively). No significant differences between the two groups were detected for other basal hormone values (Table 1).

Mild TSH elevation was detected in 4 (9.3%) out of the 43 patients. Three (12.5%; age: 42, 43 and 47 years, respectively) of the 24 male patients and 1 (5.2%; age: 40 years) of the 19 female patients had central hypogonadism. Mean total testosterone levels were lower in the male patients when compared to the male controls, although the difference was not statistically significant. Mild hyperprolactinemia was detected in 2 (4.6%) out of the 43 patients and these patients had normal thyroid function tests.

In the low-dose ACTH stimulation test, the peak cortisol values were insufficient in 7 (16.2%) out of the 43

Table 1 Basal hormone levels in patients and control subjects

	Normal Range	Patient Group ($N = 43$)	Control Group ($N = 11$)	p Value
Age (years)		44.28 ± 10.76	44.18 ± 12.41	0.907
Gender		24 men, 19 women	6 men, 5 women	0.94
BMI (kg/m^2)		31.04 ± 5.92	29.92 ± 3.29	0.63
TSH	0.27–4.20 μU/mL	1.83 (1.43–2.67)	2.66 (1.70–3.10)	0.14
Free T4	0.93–1.97 ng/dL	1.16 ± 0.12	1.20 ± 0.16	0.36
Free T3	2–4.4 pg/mL	3.46 ± 0.38	3.21 ± 0.28	0.04
IGF-1	ng/mL (all ages and gender specific)	154.22 ± 45.10	151.09 ± 40.95	0.83
	–2 SD and +2 SD	mean: –0.3 SD, range –3.3 SD/1.5 SD	mean: –0.3 SD, range –1.4 SD/2 SD	0.84
ACTH	7.2–63.3 pg/mL	31.4 (20.2–51.4)	14.7 (13–21.1)	0.002
Prolactin	Female 2.8–29.2 ng/mL Male 2.1–17.7 ng/mL	11.93 ± 4.94	11.31 ± 3.77	0.69

BMI, Body mass index; TSH, Thyroid-stimulating hormone; T4, Thyroxine; T3, Triiodothyronine; IGF-1, Insulin-like growth factor 1; SD, Standard deviation; ACTH, Adrenocorticotropic hormone.

patients. Before enrolment in the study, three patients had a moderate history of COVID-19 infection, while four patients had a severe history of COVID-19 infection. Six (13.9%) out of the 43 patients had insufficient peak cortisol and GH responses to the stimulation tests. The peak cortisol values were adequate in all the controls. The peak GH values were lower in patients with inadequate peak cortisol response to the ACTH stimulation test than in patients with sufficient peak cortisol response to the ACTH test ($p = 0.016$). Four (9.3%) out of the 43 patients showed inadequate cortisol response to GST.

Twenty (46.5%) out of 43 the patients had insufficient GH response to GST; 2 patients, 5 patients, 12 patients and 1 patient had mild, moderate, severe and critical COVID-19 disease, respectively. Serum IGF-1 levels were lower than the gender and age-specific reference range in 4 (9.3%) out of the 43 patients. In addition, the IGF-1 SD score of these 4 patients was also lower than –2 SD for age and gender. Among them, 1 patient, 2 patients and 1 patient had moderate, severe and critical COVID-19 disease, respectively and 3 of these 4 patients had GH peak values lower than the cut-off value. The peak GH responses were significantly lower in the patients than in the controls during GST (Tables 2 and 3).

Patients were grouped and compared according to their sufficient cortisol and GH responses, respectively (Tables 4 and 5). There was no significant difference between GH-deficient and GH-sufficient patients in any variable. The peak GH values were significantly lower in the cortisol-deficient patients than in the cortisol-sufficient patients.

Discussion

The present study revealed an insufficient cortisol response to low-dose ACTH stimulation test in 16.2% and and inadequate GH response to GST in 46.5% of the patients with a history of COVID-19 infection at least 3 months ago.

It is well-known that COVID-19 infection affects multiple organs and systems, including the endocrine system. Although new data for COVID-19 infection are accumulating rapidly, the involvement of the endocrine system in patients with acute infection or thereafter is yet to be completely elucidated. The viral genome was shown in the cerebrospinal fluid of a patient with COVID-19 infection, which suggests that the virus may invade the central nervous system [33]. The involvement of the hypothalamic/pituitary gland during COVID-19 infection has been confirmed by autopsy findings. The hypothalamus was considered an important target of COVID-19, considereing its high expression of ACE 2 receptor and transmembrane protease serine 2. These expressions may play a role in pituitary dysfunction, in addition to the direct invasion of the hypothalamus-pituitary region [34]. It has been previously shown that SARS infection may lead to central hypocortisolism and hypothyroidism, thus suggesting the involvement of the hypothalamic and pituitary gland [35]. Although there are data on the course of acute infection of COVID-19 [36, 37], the pituitary functions have not been extensively investigated after recovery from the disease.

In the present study, the frequency of HPA axis insufficiency was 16.2% after COVID-19 infection according to low-dose ACTH stimulation test. Generally, although

Table 2 Stimulation tests in patients and control subjects

Variables	Patient Group ($N = 43$)	Control Group ($N = 11$)	p Value
ACTH Stimulation Test			
0. Min Cortisol (μg/dL)	12.45 ± 3.87	13.58 ± 4.60	0.40
30. Min Cortisol	17.81 ± 5.03	18.57 ± 3.98	0.64
60. Min Cortisol	13.92 ± 4.21	13.85 ± 3.89	0.96
90. Min Cortisol	11.46 ± 3.64	10.95 ± 3.56	0.67
120. Min Cortisol	9.63 ± 3.16	9.09 ± 3.50	0.62
Peak Cortisol	18.23 ± 4.21	18.62 ± 3.91	0.78
Glucagon Stimulation Test			
0. Min GH (ng/mL)	0.06 (0–0.29)	0.08 (0.03–0.30)	0.49
90. Min GH	0.05 (0–0.17)	0.14 (0.03–0.63)	0.22
120. Min GH	0.09 (0–0.25)	0.34 (0.06–0.54)	0.11
150. Min GH	0.22 (0.05–1.08)	0.93 (0.04–1.39)	0.49
180. Min GH	0.46 (0.08–4.29)	2.80 (0.22–5.66)	0.31
210. Min GH	1.16 (0.16–3.06)	3.88 (1.30–5.70)	0.03
240. Min GH	0.85 (0.13–2.48)	3.00 (0.75–6.15)	0.01
Peak GH	1.63 (0.32–4.91)	5.40 (3.00–7.89)	0.02

ACTH, Adrenocorticotropic hormone; GH, Growth hormone.

Table 3 Patients with insufficient peak response and their disease severity

	Patient Group ($N = 43$)
Insufficient peak cortisol in ACTH test peak value <12.5 μg/dL considered as insufficient	7 (%16.2) Moderate: 3 Severe: 4
Insufficient peak GH in GST peak value <1.07 ng/mL considered as insufficient	20 (%46.5) Mild: 2 Moderate: 5 Severe: 12 Critical: 1
Both insufficient peak cortisol and GH values	6 (%13.9) Moderate: 3 Severe: 3
Low IGF-1 levels and lower than –2 SD	4 (%9.3) Moderate: 1 Severe: 2 Critical: 1
Both low IGF-1 levels and insufficient GH peak value	3 (%6.9) Severe: 2 Critical: 1

ACTH, Adrenocorticotropic hormone; GH, Growth hormone; GST, Glucagon stimulation test; IGF-1, Insulin-like growth factor 1; SD, Standard deviation.

not widely used in comparison to ACTH stimulation tests, cortisol responses to GST was also evaluated and 9.3% of the patients showed inadequate response. From this point of view, we may say that 9–16% of the patients had cortisol responses, thus suggesting adrenal insufficiency. Leow *et al.* evaluated survivors of SARS three months after recovery from the infection and 40% of the patients had evidence of adrenal failure confirmed by low-dose ACTH test. Most of them were resolved within 12 months. Direct viral damage to the pituitary/hypothalamus and triggering of hypophysitis were suggested as the causes of cortisol deficiency [25]. In addition, molecular mimicry of host ACTH structure with viral antigens may lead to the binding of host antibodies against the virus to host ACTH, thereby resulting in secondary hypocortisolism [38]. On the other hand,

Table 4 Comparison of GH deficient and sufficient patients

	GH-Deficient Patients ($N = 20$)	Gh-Sufficient Patients ($N = 23$)	p Value
Gender	14 men, 6 women	10 men, 13 women	0.81
Age (years)	44.40 ± 10.37	44.17 ± 11.32	0.94
BMI (kg/m^2)	30.3 (27.7–36.4)	29.3 (24.8–33.6)	0.11
Albumin (g/dL)	4.69 ± 0.23	4.58 ± 0.23	0.12
IGF-1 (ng/mL)	140.5 (118.2–169.5)	159 (137–175)	0.12
ACTH (pg/mL)	37 (21.1–53.5)	26.4 (19.9–41.8)	0.41
TSH (μU/mL)	1.67 (1.37–2.48)	1.99 (1.43–3.11)	0.30
free T4 (ng/dL)	1.18 ± 0.11	1.14 ± 0.12	0.32
free T3 (pg/mL)	3.59 ± 0.42	3.36 ± 0.32	0.05
Peak cortisol (μg/dL) in ACTH test	17.26 ± 4.46	19.07 ± 3.89	0.16

GH, Growth hormone; BMI, Body mass index; IGF-1, Insulin-like growth factor 1; ACTH, Adrenocorticotropic hormone; TSH, Thyroid-stimulating hormone; T4, Thyroxine; T3, Triiodothyronine.

Table 5 Comparison of cortisol deficient and sufficient patients

	Cortısol-Deficient Patients ($N = 7$)	Cortisol-Sufficient Patients ($N = 36$)	p Value
Gender	4 men, 3 women	20 men, 16 women	0.63
Age (years)	45.2 ± 2.7	44.08 ± 11.4	0.79
BMI (kg/m^2)	29.4 (28.4–35.9)	30.3 (25.9–35.6)	0.46
Albumin (g/dL)	4.82 (4.74–5.01)	4.61 (4.47–4.77)	0.04
IGF-1 (ng/mL)	145 (134–158)	155.5 (119.5–181)	0.37
ACTH (pg/mL)	35.1 (23.7–50.9)	30.1 (20.1–52)	0.93
TSH (μU/mL)	1.89 (1.56–2.67)	1.78 (1.37–2.64)	0.56
free T4 (ng/dL)	1.18 ± 0.07	1.16 ± 0.12	0.62
free T3 (pg/mL)	3.54 ± 0.52	3.45 ± 0.36	0.59
Peak GH (ng/mL) in GST	0.32 (0.04–0.62)	2.33 (0.49–5.7)	0.01

BMI, Body mass index; IGF-1, Insulin-like growth factor 1; ACTH, Adrenocorticotropic hormone; TSH, Thyroid-stimulating hormone; T4, Thyroxine; T3, Triiodothyronine; GH, Growth hormone; GST, Glucagon stimulation test.

relatively higher serum ACTH levels were detected in our patient group; however, there is no clear explanation for this finding. We can speculate that it may be the recovery of the 'relative adrenal insufficiency', which can be seen in some severe clinical conditions. Another factor that may lead to the suppression of the HPA axis during COVID 19 infection is the use of exogenous steroids in the management of COVID-19. However, in the present study, none of the patients with insufficient peak cortisol response to the low-dose ACTH stimulation test had a history of glucocorticoid use. Since these patients were hemodynamically stable and did not have overt clinical findings of adrenal insufficiency, they were not prescribed glucocorticoids, considering the possible reversibility of the clinical condition and their close contact with the treating physician.

The pituitary gland may be affected by several ischaemic, infectious, and inflammatory diseases [21, 22, 39, 40]. Isolated GH deficiency was shown in 4 (28.6%) out of 14 patients 6–48 months after acute meningitis [23]. We detected insufficient GH response to GST in almost half of the patients with a history of COVID-19 infection. The peak GH values were also significantly lower in the patients than in the controls. Although the patient group was overweight/obese, there were no differences between the GH and/or cortisol-sufficient/insufficient groups. Thus, we think that although obesity can affect GH/IGF-1 axis, this issue did not affect the results. We preferred GST to ITT, since it is a safe and simple test procedure in terms of GH axis evaluation [41, 42]. In a study of 216 participants, a peak GH value lower than 1.07 ng/mL in GST was suggested to be 100% sensitive

and specific for GH deficiency [32]. In the present study, we preferred these cut-off values which are more specific to define insufficiency. A lower than normal IGF-1 value may be an indicator for GH deficiency, but a normal serum IGF-1 value does not rule out GH deficiency and the serum IGF-1 value does not indicate GH deficiency alone [43, 44]. In the present study, four out of the 43 patients showed lower serum IGF-1 levels and three of them also showed inadequate GH response to GST. It is currently unknown how these patients will evolve, since it has been shown that hormone deficiencies, particularly GH, may recover after the events affecting the central nervous system, such as head trauma [45].

Regarding the evaluation of GH deficiency, an important issue is the effect of hypo or hypercortisolism on GH secretion. It has been shown that glucocorticoids are one of the most prominent hormones modulating the secretion of GH by different actions on the hypothalamus, pituitary gland, and the liver. Several studies have shown that glucocorticoids are both stimulators and inhibitors of GH secretion depending on hormonal concentrations and exposure time [46, 47]. Patients with glucocorticoid deficiency show functional and reversible inhibition of GH secretion which is called "Giustina effect". The Giustina effect is reversible during glucocorticoid replacement therapy and indicates that GH deficiency should be retested after appropriate glucocorticoid treatment. However, it is important to note that if patients with hypoadrenalism receive too much cortisol, GH secretion might to continue to be functionally suppressed [48]. We have both GH and cortisol deficient patients and considering the effect of glucocorticoids on GH secretion and the reversibility of the situation, none of the patients received GH replacement therapy.

It has been shown that GH is the most affected pituitary hormone due to hemodynamic changes in the hypothalamo-pituitary region, such as ischaemia and hypovolemia [40, 49]. Remarkably, in the present study, GH deficiency was detected as the most common pituitary dysfunction irrespective of the severity of the COVID-19 infection. Therefore, these results suggest that COVID-19 may cause direct viral damage in the central nervous system and/or affect cerebrovascular dynamics.

In the immunohistochemical examinations of autopsy specimens of 5 patients with SARS infection, GH, ACTH and TSH immunostaining were lower and PRL, LH, and FSH were higher than the control cases [27]. Sixty-one SARS survivors were investigated three months after acute infection in 2005 and central hypocortisolemia was detected in 24 (39.3%) of survivors in whom 62.5% resolved within one year. The central hypothyroidism was found in 5% of the survivors. The authors concluded that pituitary dysfunction might be caused by direct viral damage or reversible hypophysitis [25].

In other autopsy examinations of patients with SARS infection, degeneration of adrenal cortical cells and neuronal degeneration in the hypothalamus and overt destruction of thyroid follicular and parafollicular cells have been demonstrated [50, 51]. In a study of 274 COVID-19 patients, free T3 and TSH levels were significantly lower in patients who died from COVID-19 than in recovered patients [52]. In 48 SARS patients, low free T4 and low free T3 levels were detected in 46% and 94% of patients during acute infection and in 38% and 90% of patients during the post-recovery period, respectively [53]. It would be more accurate to consider the change in thyroid function tests during acute infection as a component of sick euthyroid syndrome rather than a specific effect of COVID-19 infection. In contrast to the some published data, during acute infection, free T3 and T4 values were within the normal range in all our patients, since we examined them after resolving the acute infection period. Patients had relatively higher serum free T3 levels; however, the results were still within normal limits and we think that they are probably adaptive changes that lack clinical meaning. Therefore, it seems that COVID-19 infection does not cause overt thyroid dysfunction, at least, in the early post-infection period.

The cases of acute orchitis associated with both COVID-19 and SARS infection have been reported [54, 55]. In a study including 81 male patients with COVID-19 infection, although not statistically significant, total testosterone levels were lower when compared to control subjects [56]. We detected central hypogonadism in 3 (13%) out of 23 male patients. However, although total testosterone levels were mildly lower in patients than in controls, the difference was not statistically significant. The men with hypogonadism will be followed for the recovery from hypogonadism without administering any therapy.

One of the limitations of the present study is its lack of ITT, which was not preferred due to unwanted effects. The pituitary magnetic resonance imaging could not be performed due to limited conditions during the pandemic. It will be performed if the changes persist in the clinical follow-up. A small number of control subjects is an another limitation of the study, although we have previously elucidated the cut-off values for GH and cortisol to corresponding tests.

In conclusion, we detected insufficient GH responses to GST in nearly half of the patients and insufficient cortisol responses in 9%–16.2% of the patients with at least 3 months history of COVID-19 infection. The hypothalamus/pituitary dysfunction, particularly in HPA

and GH axes are not uncommon and may be involved in the aetiology of post-COVID 19 symptoms. The effects of these dysfunctions on the post-COVID 19 period and recovery of the dysfunctions in the follow-up require further investigations.

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Data Availability

The datasets in this study are available by reasonable request from the corresponding author.

Ethical Approval

All procedures in the studies with human participants followed the 1964 Helsinki Declaration the ethical standards of the national or institutional research committee and its later amendments or comparable ethical standards. Therefore, Ethics Committee of Erciyes University approved this study (Approval Date: 08.07.2020, Approval Number: 2020/363).

Disclosure

All authors declare no conflict of interest in the reported research.

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