Assessment of Neuroendocrine Changes and the Hypothalamo-Pituitary Autoimmunity in Patients With COVID-19

Mustafa Sait Gonen
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Annamaria De Bellis
University of Campania Luigi Vanvitelli: Universita degli Studi della Campania Luigi Vanvitelli
Emre Durcan
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Giuseppe Bellastella
University of Campania Luigi Vanvitelli: Universita degli Studi della Campania Luigi Vanvitelli
Paolo Cirillo
University of Campania Luigi Vanvitelli: Universita degli Studi della Campania Luigi Vanvitelli
Lorenzo Scappaticcio
University of Campania Luigi Vanvitelli: Universita degli Studi della Campania Luigi Vanvitelli
Miriam Longo
University of Campania Luigi Vanvitelli: Universita degli Studi della Campania Luigi Vanvitelli
Basak Ecem Bircan
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Serdar Sahin
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Cem Sulu
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Hande Mefkure Ozkaya
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Dildar Konukoglu
Cerrahpasa Medical School: Istanbul Universitesi-Cerrahpasa Cerrahpasa Tip Fakultesi
Fatma Ferda Kartufan
Yeditepe University School of Medicine: Yeditepe Universitesi Tip Fakultesi
Fahrettin Kelestimur (fktimur@erciyes.edu.tr)
Yeditepe University School of Medicine: Yeditepe Universitesi Tip Fakultesi https://orcid.org/0000-0002-2861-4683

Research Article

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Abstract

Purpose: The SARS-CoV-2 may affect the hypothalamic-pituitary axis and pituitary dysfunction may occur. Therefore, we investigated neuroendocrine changes, particularly, secondary adrenal insufficiency using a dynamic test and the role of autoimmunity in pituitary dysfunction in the patients with COVID-19.

Methods: The single-center, prospective, case-control study included PCR-confirmed COVID-19 patients and healthy controls. Basal hormone levels were measured and ACTH stimulation test was performed. Anti-pituitary (APA) and anti-hypothalamic antibodies (AHA) were also determined.

Results: We examined a total of 49 patients with COVID-19 and 28 healthy controls. The frequency of adrenal insufficiency in patients with COVID-19 was found as 8.2%. The patients with COVID-19 had lower free T_3, IGF-1, total testosterone levels, and higher cortisol and prolactin levels when compared with controls. We also, demonstrated the presence of APA in three and AHA in one of four patients with adrenal insufficiency.

Conclusions: The COVID-19 may result in adrenal insufficiency, so the routine screening of adrenal functions is these patients is needed. Endocrine disturbances in COVID-19 are similar to those seen in acute stressful conditions or infections. Also, pituitary or hypothalamic autoimmunity may play a role in neuroendocrine abnormality in COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has already affected more than 226 million people and caused over 4 million deaths worldwide since the first case was confirmed, in Wuhan, China, in late December, 2019 [1]. SARS-CoV-2 is known to primarily cause disease in the lungs. However, studies show that its effects are not limited to the lungs, but they also expand to other organs such as the brain, heart, kidneys, skin, gastrointestinal and endocrine organs [2–5]. The disease can progress in different severities, from asymptomatic to severe respiratory distress that may require mechanical ventilation [6, 7]. Sepsis may develop very quickly in some patients, which can lead to organ failure and death [6].

The SARS-CoV-2 enters the body through the respiratory system and infects the host pneumocytes via the angiotensin converting enzyme 2 (ACE-2) receptor. In addition, viral ribonucleic acid (RNA) has also been isolated from plasma or serum of patients with COVID-19 [8]. This indicates that virus may interact with ACE-2 receptors expressed in other tissues apart from pneumocytes. As a matter of fact, it is also possible that SARS-CoV-2 causes endocrine disorders due to its interaction with ACE-2 receptors expressed in a number of endocrine organs such as the pituitary, adrenal glands, thyroid, pancreas, testis and ovary [9]. The SARS-CoV-2 can cause variable endocrine abnormalities including hypothalamic-pituitary-adrenal (HPA) insufficiency, euthyroid sick syndrome (ESS), decreased sex steroids, and worsening in diabetes and in obesity [10–16].

The SARS-CoV-2 may affect the hypothalamic-pituitary axis directly or through an immune-mediated way and various clinical pictures due to a deficiency of anterior pituitary hormones may occur [17]. In our previous study, we assessed pituitary functions in adults with acute bacterial or viral meningitis and demonstrated a considerable risk of hypopituitarism during in the acute phase and 6 and 12 months after acute bacterial and/or viral meningitis. Moreover, we suggested that pituitary dysfunction might occur after acute bacterial or viral meningitis associated with the presence of anti-pituitary antibodies (APA) and anti-hypothalamus antibodies (AHA) in these patients [18]. The occurrence and the roles of APA and AHA in patients with COVID-19 have not been reported, so far.

Although, there are a few studies in the literature, neuroendocrine changes due to the COVID-19 have not been investigated in detail [19–23]. In the present study, we aimed to investigate neuroendocrine changes, particularly the presence of adrenal insufficiency using a dynamic test in the patients with COVID-19. The correct diagnosis of adrenal insufficiency is crucially important to decide whether the patient need or not glucocorticoid treatment. Moreover, we intended to investigate whether autoimmune mechanisms could play a role in pituitary dysfunction due COVID-19 for the first time in the literature.

Materials And Methods

Participants

The single-center, prospective, case-control study included 49 patients with real-time reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed COVID-19 who were consecutively hospitalized to the COVID-19 inpatient clinic of Istanbul University-Cerrahpasa, Cerrahpasa Medical School between June 18th and 28th, 2020. As control group with similar age and sex, 28 COVID-19 negative healthy volunteers with no known chronic disease in the same study period were included in the study.

Participants with any of the following characteristics were excluded from the study: endocrine disorders related to the hypothalamic-pituitary-target organ axes; use of any drugs that might affect hypothalamic–pituitary function such as antidepressants, psychostimulants, oral contraceptives, anti-epileptics, and oral, inhaled or topical steroids; pregnancy.

Diagnostic Method

Combined pharyngeal and nasopharyngeal swab samples were obtained for the RT-PCR assay. RNA was extracted using a commercial kit (BioSpeedy Nucleic Acid extraction kit; Bioeksen R&D Technologies Ltd., Istanbul, Turkey), followed by the detection of COVID-19 RNA using a commercial RT-PCR kit (Bio-Speedy COVID-19 RT-qPCR kit; Bioeksen R&D Technologies Ltd., Istanbul, Turkey) that targeted the RdRP gene of COVID-19 in the samples. Both kits were used according to the manufacturer’s guidelines. The RT-PCR test was performed using a 20-µL final volume using the following protocol: 5 min RT-PCR at 52°C, 10 sec initial denaturation step at 95°C, followed by 40 cycles of 1 sec at 95°C, and 30 sec at 60°C. The Rotor-Gene Q 5plex HRM platform was used for amplification and detection.

Case Definitions

The disease severity of COVID-19 was defined according to the Diagnosis and Treatment Protocol for COVID-19 Patients (Tentative 8th Edition) [24]. COVID-19 was categorized as mild, moderate, severe, and critical according to the disease severity. Mild was defined as mild symptoms and no pneumonia on imaging. Moderate was defined as having respiratory tract symptoms and imaging with pneumonia. Severe was defined as any of the following items: (a) respiratory distress and respiratory frequency ≥ 30/min; (b) blood oxygen saturation ≤ 93% at rest; (c) PaO2/FiO2 ratio ≤ 300 mmHg; and(d) Lung infiltrates > 50% within 24–48 hours. Critical was defined when any of the following items were satisfied: (a) respiratory failure and requiring mechanical ventilation, (b) shock, (c) COVID-19 combined with other organ failure and requiring intensive care unit monitoring and treatment.

Biochemical Analysis

Biochemical parameters such as adrenocorticotropic hormone (ACTH), basal cortisol, thyroid-stimulating hormone (TSH), free T_3, free T_4, growth hormone (GH), insulin-like growth factor (IGF-1), prolactin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone in men and estradiol in women were collected in a fasting state in the morning. The venous blood samples were centrifuged at 3200 rpm and serum samples were separated, which were stored at -20°*C* until required for analysis. All blood samples collected at the initial and follow-up visits were analyzed at the same time.

Venous blood samples from each study participant were collected into vacutainer tubes with or without anticoagulant. Serum ACTH, basal cortisol, TSH, free T_3, free T_4, GH, IGF-1, prolactin, FSH, LH, total testosterone and estradiol levels were assayed using a electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas e system (Roche, Cobas e 602, Roche Diagnostics GmbH, Mannheim, Germany).

Evaluation of Basal Serum Hormone Levels

The normal serum reference ranges were 0–46 pg/mL for ACTH; 6.2–19.4 μg/dL for cortisol; 0.27–4.2 μIU/mL for TSH; 2-4.4 pg/*m*L for free T_3; 0.93–1.7 ng/dL for free T_4; 0.03–2.47 ng/mL in males and 0.126–9.88 ng/mL in females for GH; 4.1–15.2 ng/mL in males and 4.3–23.3 ng/mL in females for prolactin; 1.6–12.4 mIU/mL for FSH; 0.8-6 mIU/mL for LH; 280–800 ng/dL for total testosterone. The estradiol reference ranges were as follows: for premenopausal women, 12.5–166 pg/mL in the follicular phase; 43.8–211 pg/mL in the luteal phase, and < 54.7 pg/mL for postmenopausal women. IGF-1 levels were assessed according to age- and sex-adjusted reference ranges [25]. Euthyroid Sick Syndrome (ESS) was described as normal or low TSH and free T_4 levels accompanied by low free T_3 [26].

The Low Dose (1 mcg) ACTH Stimulation Test (LDST)

0.25 mg intravenous tetracosactrin [1–24] (Synacthen, Novartis, Switzerland) was used for LDST. Tetracosactrin (0.25 mg) was mixed with 250 mL 0.9% NaCl solution and preserved at + 4°*C* for no more than 4 weeks; 1 mL of this mixture contained 1 μg ACTH. Blood samples for the measurement of cortisol

were obtained in the basal state and 30 min and, 60 min after the administration of 1 μg intravenous ACTH [27].

Assessment of Adrenal Insufficiency

We used the minimum peak cortisol levels of the healthy controls after the LDST for the assessment of adrenal insufficiency in patients with COVID-19. An adrenal insufficiency was considered for those who had peak cortisol levels below this level. Follow-up visits for patients with adrenal insufficiency were scheduled approximately 6 months later.

Immunologic evaluation

All patients were tested for APA and AHA at the Endocrinology and Metabolic Unit, Department of Advanced Medical and Surgical Sciences, University of Campania Luigi Vanvitelli, Naples, Italy, using a simple indirect immunofluorescence method on cryostat sections of young baboon pituitary gland and young baboon hypothalamus supplied by Halifax spa (Polverara, Pordenone, Italy) and Biomedis Srl (Rome, Italy), respectively, as previously described [28]. Unfixed cryostat sections of young normal baboon pituitary and hypothalamus were initially incubated with the sera. Then, serum samples were subsequently tested with fluorescein isothiocyanate-conjugated goat antihuman immunoglobulin (Ig) to detect the presence of APA and AHA. To improve the sensitivity and specificity of this method, we considered the sera positive starting from a titer of 1:8 and, as regards to APA, onwards and with immunostaining involving a few isolated pituitary cells (type 1 pattern) but not with a diffuse immunostaining pattern involving all cells in the pituitary section (type 2 pattern) [29]. Finally, we considered sera positive for APA and AHA at low titer at a dilution of 1:8, at middle titer at dilution 1:16/1:32, at high titer at dilution > 1:32). APA and AHA were evaluated by two different operators in a double-blind manner. The collaborators performing the immunologic evaluation were also blinded by a possible pituitary impairment in these patients.

Data Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 21.0). Data were first analyzed for normality using the Kolmogorov– Smirnov test. Continuous variables are expressed as mean ± standard deviation (SD) and/or medians [interquartile range (IQR)]. Student's t-test or analysis of variance (ANOVA) were used to compare means between groups with normal data distribution. Medians were compared using the Mann–Whitney U test and the Kruskal–Wallis test. Spearman's rank-order test and Pearson's correlation test were used to calculate the correlation coefficients between continuous variables. Frequencies were compared using Pearson's and Fisher's exact tests. The results were evaluated at a 95% confidence interval, and p-values < 0.05 were considered statistically significant.

Results

We examined a total of 49 patients with COVID-19 and 28 healthy controls. The mean age was 54.3 ± 16.6 years in the patients with COVID-19 and 54 ± 5 years in the controls. Twenty-seven (55.1%) of the patients with COVID-19 and 14 (50%) of the controls were female. There was no significant difference between the groups in terms of age and sex ($p = 0.905$ and $p = 0.666$, respectively).

Serum hormone profiles in the patients with COVID-19 and healthy controls

In all participants, ACTH levels were within the normal range. The median basal cortisol levels were higher in the patients than in the control group (11.74 [IQR: 8.11–16.84] vs. 8.89 [IQR: 6.64–12.1], $p = 0.012$). Free T_4 levels were within the normal limits in both groups. Fourteen (28.6%) of the patients had free T_3 levels lower than the reference range, and four (8.2%) had TSH levels lower than normal reference; all controls had normal free T_3 and TSH levels ($p = 0.001$ and $p = 0.049$, respectively). Euthyroid sick syndrome was diagnosed in 14 (28.6%) patients with COVID-19. There was no significant difference between the groups in terms of GH levels. The IGF-1 levels were lower than the reference range in 18 (36.7%) patients, all controls had normal IGF-1 levels ($p < 0.001$). The median IGF-1 levels were lower in the patients than in the control group (96.45 [56.98-128.45] vs. (128.2 [103.5-151.2, $p = 0.003$). Of the 49 patients, 20.4% had prolactin levels higher than normal reference, while prolactin levels were higher than reference range in only one control case ($p = 0.042$). The median levels of prolactin were higher in patients with COVID-19 than in the controls (14 [9.26–20.2] vs. 10.52 [7.61–12.52]); $p = 0.013$). Fifteen (68.2%) of the male patients had total testosterone levels lower than the reference range, whereas this rate was 14.3% (only two cases) in the male controls ($p = 0.002$). On the other hand, the median total testosterone levels were lower in the male patients than in the male controls (151.2 [57.22–365] vs. 388.3 [312.5–553], $p = 0.001$). The anterior pituitary and other hormone profiles in the patients with COVID-19 and the controls are shown in Table 1.

Table 1
Comparison of the anterior pituitary and other hormone profiles in the patients with COVID-19 and healthy controls

Hormones	**COVID-19 (n = 49)**	**Controls (n = 28)**	
	median (IQR 25–75)		P
ACTH, *pg/mL*	10.36 (5.78–17.89)	16.39 (7.24–26.7)	0.095
Basal Cortisol, *µg/dL*	11.74 (8.11–16.84)	8.89 (6.64–12.1)	**0.012**
TSH, *µIU/mL*	1.3 (0.76–2.07)	1.59 (1.2–2.1)	0.282
Free T_3, *pg/mL*	2.34 (1.9–2.87)	3.21 (2.74–3.34)	**< 0.001**
Free T_4, *ng/dL*	1.32 (1.13–1.48)	1.34 (1.12–1.47)	0.966
GH, *ng/mL*	0.45 (0.15–1.16)	0.26 (0.07–0.84)	0.179
IGF-1, *ng/mL*	96.45 (56.98-128.45)	128.2 (103.5-151.2)	**0.003**
PRL, *ng/mL*	14 (9.26–20.2)	10.52 (7.61–12.52)	**0.013**
FSH, *mIU/mL*	6.87 (3.85–14.8)	6.42 (4.71–9.07)	0.885
LH, *mIU/mL*	5.61 (4.83-15)	5.59 (4.76–7.57)	0.490
Total Testosterone*, *ng/dL*	151.2 (57.22–365)	388.3 (312.5–553)	**0.001**
Estradiol*, *pg/mL*	8.48 (3.95–45.3)	6.65 (5-55.16)	0.406

ACTH: adrenocorticotropic hormone, *TSH*: thyroid stimulating hormone, *Free T_3*: free triiodothyronine, *Free T_4*: free thyroxine, *GH*: growth hormone, *IGF*-1: insulin-like growth factor, *PRL*: prolactin, *FSH*: follicle stimulating hormone, LH: luteinizing hormone

* Total testosterone was evaluated only in males and estradiol was evaluated only in females.

Clinical course of the patients with COVID-19

While the clinical course of COVID-19 was severe in 36.7% of the patients, no patient was in the critical disease group. The disease severity classification in the patients with COVID-19 are shown in Table 2. All of the patients with COVID-19 were followed at the inpatient clinic. The median hospitalization time was 7 (IQR: 5–12) days. Four patients were considered died due to COVID-19-related acute respiratory failure (21st, 23rd, 46th and 92nd days after diagnosis). In addition, in the 5th month of the study, three patients who were died at different times (16th, 80th and 90th days after discharge) according to the national death reporting system. Two of these patients had been diagnosed as having lung cancer before they died.

Table 2
The disease severity classification in patients with COVID-19 (n = 49)

Variables	*n (%)*
Disease severity of COVID-19	
Mild	13 (26.5)
Moderate	18 (36.7)
Severe	18 (36.7)

Evaluation of adrenal insufficiency

After the LDST, the minimum peak cut-off level of cortisol was 14.88 μg/dL in the control group. According to this cut-off, adrenal insufficiency was detected in four (8.2%) patients.

Patients with adrenal insufficiency and their follow-up visits

We examined a total of four patients with adrenal insufficiency. One patient who died while being followed up could not be re-evaluated. Also, three patients were called by phone to come to the hospital, but one could not be reached. Finally, the presence of adrenal insufficiency was investigated using the LDST in a total of two patients. After about 6 months, adrenal insufficiency had disappeared in all of two in the follow-up visit. The clinical and follow-up characteristics, and biochemical parameters at baseline and in the recovery period of COVID-19 of all four participants who had adrenal insufficiency are summarized in detail in Table 3.

Table 3

Comparison from baseline APA and AHA evaluation and the clinical, follow-up characteristics and biochemical parameters at baseline and recovery period of the COVID-19 in the patients with adrenal insufficiency

Variables	**Case 12**	**Case 17***	**Case 23**	**Case 42**
Age, *yrs.* / Sex	78 / Male	43 / Male	82 / Male	59 / Male
Disease severity	severe	moderate	severe	mild
Hospitalization, *day*	28	7	21	5
Exitus / when after diagnosis, *day*	no	no	yes / 20	no
Control visit after diagnosis, *day*	169	no visit	no visit	157
Baseline APA titer	1/64••	1/256•	Absent	1/256••
Baseline AHA titer	Absent	Absent	1/128	Absent
Evaluation of AI†				
ACTH, *pg/mL*	16.43	4.23	1.9	18.77
Baseline, *peak cortisol*	12.48	13.04	11.82	13.96
Recovery, *peak cortisol*	20.43	na	na	16.46
Abnormal hormone profiles at baseline	fT3 ↓ (ESS) IGF-1, T.Testo ↓ FSH, LH, PRL ↑	FSH, LH ↑ T.Testo ↓	fT3 ↓ (ESS) IGF-1, T.Testo ↓ PRL ↑	fT3 ↓ (ESS) T.Testo ↓
Abnormal hormone profiles at recovery	IGF-1, T.Testo ↓ FSH, LH ↑	na	na	normal levels
Poor prognostic biomarkers *(first / end)*				

APA: anti-pituitary antibodies, *AHA*: anti-hypothalamus antibodies, *AI*: adrenal insufficiency, *ACTH*: adrenocorticotropic hormone, *fT_3*: free triiodothyronine, *ESS*: euthyroid sick syndrome, *IGF*-1: insulin-like growth factor, T.Testo: total testosterone, *FSH*: follicle stimulating hormone, LH: luteinizing hormone, *PRL*: prolactin, na: no assessment, *CRP*: C-reactive protein, *LDH*: lactate dehydrogenase, *CPK*: creatine-phosphokinase. *Trop T*: Troponin T,

* Case 17 was reached but he didn't come to visit.

† ACTH stimulation test

Type 1 immunostaining pattern • Type 2 immunostaining pattern ••

Lympho < 800 *μL*	- / -	- / na	- / na	- / -
D-dimer >1000 *ng/L*	+ / +	+ / na	+ / na	- / -
Ferritin >500 *μg/L*	+ / -	+ / na	- / na	- / -
CRP > 100 mg/L	+ / -	- / na	+ / na	- / -
LDH > 245 *IU/L*	- / -	- / na	+ / na	- / -
CPK > 340 *IU/L*	- / -	- / na	- / na	+ / -
Trop-T > 0.028 *ng/mL*	+ / -	- / na	+ / na	- / -

APA: anti-pituitary antibodies, *AHA*: anti-hypothalamus antibodies, *AI*: adrenal insufficiency, *ACTH*: adrenocorticotropic hormone, fT_3: free triiodothyronine, *ESS*: euthyroid sick syndrome, *IGF*-1: insulin-like growth factor, T.Testo: total testosterone, *FSH*: follicle stimulating hormone, LH: luteinizing hormone, *PRL*: prolactin, na: no assessment, *CRP*: C-reactive protein, *LDH*: lactate dehydrogenase, *CPK*: creatine-phosphokinase. *Trop T*: Troponin T,

* Case 17 was reached but he didn't come to visit.

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Type 1 immunostaining pattern • Type 2 immunostaining pattern ••

Serum hormone profiles according to disease severity of COVID-19

Euthyroid sick syndrome has was found to be more common in patients with severe disease when compared to the other groups (50% for severe; 5.6% for moderate; 30.8% for mild, p = 0.013). The IGF-1 levels in patients with severe disease were lower than the reference range than in all the other groups (61.1% for severe; 27.8% for moderate; 15.4% for mild, p = 0.021). The median levels of IGF-1 in patients who had severe disease were lower than in patients who had mild disease (65.45 [36.33–93.87] vs. 120.9 [93.34–143.7], p = 0.015) and moderate disease (65.45 [36.33–93.87] vs. 116.1 [57.18-142.98], p = 0.043). Also, the median total testosterone levels in patients with severe disease were lower than in patients with moderate disease (53.2 [46.55–193.4] vs. 319.65 [159–432], p = 0.029).

The presence of APA and/or AHA in patients with COVID-19

APA was present in 25 out of 49 (18.4%) patients, nine (36%) at a titer ≤ 1:32, and in 16 (64%) patients at a titer > 1:32. Regarding the immunostaining pattern, four patients had a type 1 (selective) immunostaining pattern, and in 21 patients had a type 2 immunostaining pattern (Table 4). AHA was present in 15 out of 49 (31%) patients, five (33.3%) at a titer > 1:32 and in 10 (66.7%) patients at a titer < 1:32. APA and AHA were not correlated with disease severity. Regarding the behavior of the baseline APA and AHA in the four patients with hypocortisolism (Table 3), APA was present in 3 out of 4 patients (titer > 1:32 in the all of three patients). Concerning the immunostaining pattern, type 1 APA was observed in one

patient and type 2 in the remaining two patients. AHA was also present in one patient (titer 1:128) and absent in another three patients (Table 3). Moreover, in two patients in whom adrenal functions recovered, APA was present at a titer > 1:32 (with immunostaining type 2), and AHA was absent in both. Finally, in seven patients without hypocortisolism with low basal GH and IGF1 levels, baseline APA was also present (titer > 1:32) in five, and AHA in two.

Table 4
Frequency of APA titer and immunostaining pattern

Total APA positive *(n = 25)*			
	Total	**Type 1 immunostaining pattern**	**Type 2 immunostaining pattern**
APA ≤ 1/32, n (%)	9 (36)	0 (0)	9 (100)
APA > 1/32, n (%)	16 (64)	4 (25)	12 (75)

Discussion

In the present study, we found that the frequency of adrenal insufficiency is 8.2% in patients with COVID-19. After about six months, in the follow-up visits, we observed that adrenal insufficiency disappeared in two patients who were still alive. On the other hand, the patients with COVID-19 had lower free T_3, IGF-1, total testosterone levels, and higher cortisol and prolactin levels when compared with healthy volunteers. Similarly, we determined that free T_3, IGF-1, and total testosterone levels were lower in patients who had severe disease than all the other disease severity groups. Also, we detected that euthyroid sick syndrome was more common in patients with COVID-19 than controls, especially in those with severe group. Finally, we demonstrated the presence of APA in three and AHA in one of four patients with adrenal insufficiency.

In patients who had severe disease, it has been shown that cytokines affect deiodinase activity in thyroid tissue and can mimic the acute stress response of the thyroid axis [30, 31]. This change result in euthyroid sick syndrome accompanied by distinctly low free T_3 [26]. Similarly, in the current study, the frequency of euthyroid sick syndrome characterized by reduced free T_3 levels was higher in patients who had severe disease. On the other hand, when all patients with COVID-19 and healthy controls are compared, regardless of disease severity, the patients had lower TSH and T_3 levels, whereas all controls had normal levels. Leow et al. showed that 5% of survivors of the SARS outbreak had central hypothyroidism [32]. In another study, free T_3, free T_4 and TSH were detected to be lower in patients with SARS compared with the controls. In the same study, the authors found a positive association between disease severity and low free T_3 [33]. In several studies conducted on patients with COVID-19, authors reported similar results to studies performed during the SARS outbreak [34–36].

We found that the IGF-1 levels were lower in the patients who had severe disease than all the other disease severity groups. When we evaluated the patients with COVID-19 and controls regardless of

disease severity, the patients had lower IGF-1 levels compared with controls. In the early stage of illness, decreased negative feedback as a result of low IGF-1 levels may cause abundant GH release [37]. Inhibition of the IGF-mediated anabolic effects and stimulation of the GH effects is an adaptive response against the illness, which protects the organism. In this way, energy-consuming anabolic activities are reduced [38, 39]. On the other hand, because the prognosis of COVID-19 has been reported to depend on sex and age, Lubrano et al. stated that the decrease in GH levels in old age and men was an important factor in the course of COVID-19 [40].

Reproductive hormone levels change significantly in an acute severe disease. Although LH levels increase as a result of acute physical stress, serum testosterone levels continue to decrease. The reduction in testosterone, which is an anabolic hormone, is a life-saving response for the organism to reduce energy consumption [41]. On the other hand, high levels of ACE-2 expression are seen in the testicles, which is almost the highest production of testosterone site in the human body. In an earlier study, SARS-CoV was shown to cause orchitis and widespread germ cell destruction in human testicles [42]. Also, a decrease in serum testosterone was shown in male mice infected with SARS-CoV in an animal study [43]. However, serum testosterone levels in COVID-19 need to be interpreted with caution because any acute severe disease can lead to suppression of the hypothalamic-pituitary-testicular axis [44]. In our study, male patients with severe disease had lower levels of total testosterone than male patients with moderate disease. When all patients with COVID-19 and controls are compared, regardless of disease severity, the rate of male patients with COVID-19 who had total testosterone, lower than reference range was higher than in the controls. Similar to our findings, in a recent study including 81 males with COVID-19, the authors showed that serum total testosterone was lower compared with the controls, although it was not statistically significant [44]. Also, we observed that prolactin levels were higher in patients with COVID-19 than in controls. Hyperprolactinemia is known to develop in response to many stressors, including infections [45]. Ma et al. showed that prolactin levels were significantly higher in patients with COVID-19, as reported by Gu et. al [44, 46].

It would be expected that HPA axis would have been affected in COVID-19. Gu et al. showed that ACTH levels were increased in patients with COVID-19 compared with healthy controls. By contrast, ACTH levels were within the normal range in all participants in our study [46]. The basal cortisol levels were also higher in the patients with COVID-19 than in controls. Similarly, in a short report, Tan et al. described that patients with COVID-19 had higher cortisol levels than patients without COVID-19 [22]. As expected, patients with high basal cortisol levels had lower median survival times [47]. Moreover, in Tan et al.'s study, failure to perform cortisol analysis after adjustment for disease severity may not reflect the true predictive potential of cortisol [22]. Conflictingly, in another study involving 28 patients with COVID-19, no robust response was observed in cortisol levels in any patients. In fact, cortisol levels were close to the lower end of the reference range [19]. According to an interesting hypothesis, SARS-CoV expresses an amino acid sequence that has a molecular homology with ACTH, it can block the stress-induced host's cortisol response as a result of antibodies against ACTH [48]. This hypothesis can also be considered for the new virus because SARS-CoV and SARS-CoV-2 share 90–99% homology in their proteins. Also, the hypothalamus and pituitary were shown to express ACE-2 and SARS genomes in autopsy specimens.

Therefore, this novel coronavirus might also cause acute adrenal insufficiency by affecting the HPA axis [32, 49]. Although there is no evidence of the direct hypothalamic-pituitary effect of COVID-19, Leow et al. reported findings of hypothalamic-pituitary involvement in 61 post-SARS survivors [32].

In our study, we detected the presence of adrenal insufficiency using the LDST. Actually, in non-critically ill patients, it is debated whether the 1 mcg or 250 mcg ACTH stimulation test is more useful in the diagnosis of central adrenal insufficiency [50–53]. We chose the LDST of which results are more concordant than 250 mcg-ACTH test in comparison with the insulin tolerance test (ITT) in an acute situation [54]. In our study, we determined a cut-off value for the LDST based on the healthy controls because of all these interpretations. In present study, we revealed that frequency of adrenal insufficiency was 8.2% in patients with COVID-19. In Leow et al.'s study which investigated the function of the HPA axis in 61 SARS survivors, 39.3% of patients had hypocortisolism, and among these, 83.3% had central adrenal insufficiency using basal cortisol (< 5 μg/dL) and/or peak cortisol response on LDST (20 μg/dL). Forty percent of these people had evidence of central hypocortisolism and most resolved within a year [32]. Therefore, we considered all cases as secondary adrenal insufficiency. Also, in Leow et al.'s study, most of the patients with hypocortisolism detected in the 3rd month recovered in the 1st year. We observed that adrenal insufficiency disappeared in two patients who were still alive after about six months. Similar to our results, in a recent study, the authors found that adrenal function is preserved 3 months after admission with COVID-19 [55].

In the present study, a possible autoimmune mechanism in the hypothalamic-pituitary region in patients with COVID-19 has been observed due to the presence of APA and AHA in patients with secondary adrenal insufficiency, suggesting for the first time that secondary hypocortisolism may have been due to autoimmune hypophysitis in three out four patients and autoimmune hypothalamitis occurring in one of them. Another important result is that the basal presence of a type of immunostaining is perfectly correlated to the evolution of adrenal insufficiency is observed in patients who achieve remission over time despite having a high APA titer but with type 2 immunostaining. This has been demonstrated in previous studies in a large cohort of patients with autoimmune polyendocrine syndrome suggesting that not only the presence of APA at high titer both in patients with normal pituitary function and in those with early stage of hypophysitis with subclinical impairment (ACTHD, GHD) not yet requiring therapy but also that APA positive patients with type 1 immunostaining pattern had a worsening of pituitary dysfunction with respect to those with type 2 immunostaining pattern, who, on the contrary, showed spontaneous remission [29]. Based on these observations, we suggest that in some patients with COVID-19, the initial autoimmune involvement of the pituitary and/or hypothalamus can be reversible, and the basal presence of anti-pituitary and anti-hypothalamus antibodies, their titer, and immunostaining of APA in secondary adrenal insufficiency is able to predict the possible evolution of the disease in subsequent observations. It would be interesting to re-evaluate these antibodies over time (study in progress). Finally, the presence of APA in five patients and AHA in two at high titers suggests that an autoimmune hypophysitis or hypothalamitis seems to be the cause of GH /IGF1 axis impairment.

The limitations of our study can be summarized as follows: our study was a case-control study and had a limited number of patients and controls. This situation can be explained by the inclusion of only steroid-naive patients and the addition of corticosteroids to the routine treatment protocol in many patients with COVID-19 in the later period of the outbreak. Even so, for more precise results, our findings need to be tested with larger numbers of patients. After dividing the patients into groups according to disease severity, it made number of patients low in some groups, which makes it difficult to generalize the results.

In conclusion, we demonstrated that most of the anterior hypothalamic-pituitary-target hormone changes seen in patients with COVID-19 are characterized by physiologic responses to acute disease. The COVID-19 may result in adrenal insufficiency, so the routine screening of adrenal functions is these patients is needed. The presence of AHA and APA positivity in patients with COVID-19 was demonstrated for the first time. Further perspective studies are needed to clarify the role of autoimmunity in pituitary function in the acute and chronic phases of COVID-19.

Declarations

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Conflict of Interest:

The authors declare no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Ethical Approval:

The study was approved by the local ethics committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty (Decision No: 73186 dated 16 June 2020). All procedures performed in studies involving human participants were conducted in accordance with ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent:

Informed consent was obtained from all participants included in the study.

Authors' Contributions:

All authors made substantial contributions to conception and design, and/or acquisition of data, and/oranalysis and interpretation of data; participated in drafting the article or revising it critically for important intellectual content; and gave final approval of the version to be submitted.

Availability of Data and Material:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Johns Hopkins University, (n.d.).
2. J. Baj, H. Karakuła-Juchnowicz, G. Teresiński, G. Buszewicz, M. Ciesielka, E. Sitarz, A. Forma, K. Karakuła, W. Flieger, P. Portincasa, and R. Maciejewski, J. Clin. Med. **9**, 1753 (2020).
3. C. M. Roberts, M. Levi, M. McKee, R. Schilling, W. S. Lim, and M. P. W. Grocott, Br. J. Anaesth. **125**, 238 (2020).
4. W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, and W. Tan, JAMA - J. Am. Med. Assoc. **323**, 1843 (2020).
5. K. Yuki, M. Fujiogi, and S. Koutsogiannaki, Clin. Immunol. **215**, (2020).
6. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, and B. Cao, Lancet **395**, 497 (2020).
7. B. Li, J. Yang, F. Zhao, L. Zhi, X. Wang, L. Liu, Z. Bi, and Y. Zhao, Clin. Res. Cardiol. **109**, 531 (2020).
8. L. Chang, Y. Yan, and L. Wang, Transfus. Med. Rev. **34**, 75 (2020).
9. F. Liu, X. Long, W. Zou, M. Fang, W. Wu, W. Li, B. Zhang, W. Zhang, X. Chen, and Z. Zhang, MedRxiv (2020).
10. M. Marazuela, A. Giustina, and M. Puig-Domingo, Rev. Endocr. Metab. Disord. **21**, 495 (2020).
11. M. D. Lundholm, C. Poku, N. Emanuele, M. A. Emanuele, and N. Lopez, J. Endocr. Soc. **4**, (2020).
12. N. P. Somasundaram, I. Ranathunga, V. Ratnasamy, P. S. A. Wijewickrama, H. A. Dissanayake, N. Yogendranathan, K. K. K. Gamage, N. L. de Silva, M. Sumanatilleke, P. Katulanda, and A. B. Grossman, J. Endocr. Soc. **4**, (2020).
13. L. M. Mongioì, F. Barbagallo, R. A. Condorelli, R. Cannarella, A. Aversa, S. La Vignera, and A. E. Calogero, Endocrine **68**, 467 (2020).
14. R. Pal and M. Banerjee, J. Endocrinol. Invest. **43**, 1027 (2020).
15. E.-J. Rhee, J. H. Kim, S. J. Moon, and W.-Y. Lee, Endocrinol. Metab. **35**, 197 (2020).
16. M. Puig-Domingo, M. Marazuela, and A. Giustina, Endocrine **68**, 2 (2020).
17. S. Chiloiro, E. D. Capoluongo, T. Tartaglione, A. Giampietro, A. Bianchi, A. Giustina, A. Pontecorvi, and L. De Marinis, Trends Endocrinol. Metab. **30**, 590 (2019).
18. F. Tanriverdi, A. De Bellis, H. Teksahin, E. Alp, A. Bizzarro, A. A. Sinisi, G. Bellastella, V. A. Paglionico, A. Bellastella, K. Unluhizarci, M. Doganay, and F. Kelestimur, Pituitary **15**, 579 (2012).

19. A. S. Alzahrani, N. Mukhtar, A. Aljomaiah, H. Aljamei, A. Bakhsh, N. Alsudani, T. Elsayed, N. Alrashidi, R. Fadel, E. Alqahtani, H. Raef, M. I. Butt, and O. Sulaiman, Endocr. Pract. **27**, 83 (2021).
20. M. Hashim, S. Athar, and W. H. Gaba, BMJ Case Rep. **14**, e237690 (2021).
21. M. Heidarpour, M. Vakhshoori, S. Abbasi, D. Shafie, and N. Rezaei, J. Med. Case Rep. **14**, 134 (2020).
22. T. Tan, B. Khoo, E. G. Mills, M. Phylactou, B. Patel, P. C. Eng, L. Thurston, B. Muzi, K. Meeran, A. T. Prevost, A. N. Comninos, A. Abbara, and W. S. Dhillo, Lancet Diabetes Endocrinol. **8**, 659 (2020).
23. Y. Mao, B. Xu, W. Guan, D. Xu, F. Li, R. Ren, X. Zhu, Y. Gao, and L. Jiang, Front. Endocrinol. (Lausanne). **11**, (2021).
24. (n.d.).
25. M. Bidlingmaier, N. Friedrich, R. T. Emeny, J. Spranger, O. D. Wolthers, J. Roswall, A. Körner, B. Obermayer-Pietsch, C. Hübener, J. Dahlgren, J. Frystyk, A. F. H. Pfeiffer, A. Doering, M. Bielohuby, H. Wallaschofski, and A. M. Arafat, J. Clin. Endocrinol. Metab. **99**, 1712 (2014).
26. E. Fliers, A. C. Bianco, L. Langouche, and A. Boelen, Lancet Diabetes Endocrinol. **3**, 816 (2015).
27. G. Dickstein, J. Clin. Endocrinol. Metab. **82**, 322 (1997).
28. G. Patti, E. Calandra, A. De Bellis, A. Gallizia, M. Crocco, F. Napoli, A. M. E. Allegri, H. F. Thiabat, G. Bellastella, M. I. Maiorino, M. L. Garrè, S. Parodi, M. Maghnie, and N. di Iorgi, Front. Endocrinol. (Lausanne). **11**, (2020).
29. G. Bellastella, M. Rotondi, E. Pane, A. Dello Iacovo, B. Pirali, L. Dalla Mora, A. Falorni, A. A. Sinisi, A. Bizzarro, A. Colao, L. Chiovato, A. De Bellis, M. R. Ambrosio, E. Arvat, P. Beck-Peccoz, C. Betterle, S. Cannavò, E. Degli Uberti, R. Giordano, E. Ghigo, G. Lombardi, M. Maghnie, F. Mantero, L. Persani, A. Spada, F. Santeusanio, and M. Delvecchio, J. Clin. Endocrinol. Metab. **95**, 3750 (2010).
30. M. Michalaki, J. Clin. Endocrinol. Metab. **86**, 4198 (2001).
31. L. Croce, D. Gangemi, G. Ancona, F. Liboà, G. Bendotti, L. Minelli, and L. Chiovato, J. Endocrinol. Invest. (2021).
32. M. K.-S. Leow, D. S.-K. Kwek, A. W.-K. Ng, K.-C. Ong, G. J.-L. Kaw, and L. S.-U. Lee, Clin. Endocrinol. (Oxf). **63**, 197 (2005).
33. W. Wang, Y. Ye, and H. Yao, J Chin Antituberculous Assoc **25**, 232 (2003).
34. M. Chen, W. Zhou, and W. Xu, Thyroid **31**, 8 (2021).
35. T. Chen, D. Wu, H. Chen, W. Yan, D. Yang, G. Chen, K. Ma, D. Xu, H. Yu, H. Wang, T. Wang, W. Guo, J. Chen, C. Ding, X. Zhang, J. Huang, M. Han, S. Li, X. Luo, J. Zhao, and Q. Ning, BMJ m1091 (2020).
36. W. Gao, W. Guo, Y. Guo, M. Shi, G. Dong, G. Wang, Q. Ge, J. Zhu, and X. Zhou, J. Endocrinol. Invest. (2020).
37. J. Arnold, I. T. Campbell, T. A. Samuels, J. C. Devlin, C. J. Green, L. J. Hipkin, I. A. MacDonald, C. M. Scrimgeour, K. Smith, and M. J. Rennie, Clin. Sci. **84**, 655 (1993).
38. R. C. Baxter, Best Pract. Res. Clin. Endocrinol. Metab. **15**, 421 (2001).
39. L. Langouche and G. Van den Berghe, in *Handb. Clin. Neurol.* (2014), pp. 115–126.

40. C. Lubrano, D. Masi, R. Risi, A. Balena, M. Watanabe, S. Mariani, and L. Gnessi, Obesity **28**, 2038 (2020).
41. S. C. Gilliver, J. Steroid Biochem. Mol. Biol. **120**, 105 (2010).
42. J. Xu, L. Qi, X. Chi, J. Yang, X. Wei, E. Gong, S. Peh, and J. Gu, Biol. Reprod. **74**, 410 (2006).
43. R. Channappanavar, C. Fett, M. Mack, P. P. Ten Eyck, D. K. Meyerholz, and S. Perlman, J. Immunol. **198**, 4046 (2017).
44. L. Ma, W. Xie, D. Li, L. Shi, Y. Mao, Y. Xiong, Y. Zhang, and M. Zhang, MedRxiv (2020).
45. L. Vilar, J. Abucham, J. L. Albuquerque, L. A. Araujo, M. F. Azevedo, C. L. Boguszewski, L. A. Casulari, M. B. C. Cunha, M. A. Czepielewski, F. H. G. Duarte, M. D. S. Faria, M. R. Gadelha, H. M. Garmes, A. Glezer, M. H. Gurgel, R. S. Jallad, M. Martins, P. A. C. Miranda, R. M. Montenegro, N. R. C. Musolino, L. A. Naves, A. Ribeiro-Oliveira, C. M. S. Silva, C. Viecceli, and M. D. Bronstein, Arch. Endocrinol. Metab. **62**, 236 (2018).
46. W. T. Gu, F. Zhou, W. Q. Xie, S. Wang, H. Yao, Y. T. Liu, L. Gao, and Z. B. Wu, Endocrine **72**, 340 (2021).
47. B. Aygen, M. Inan, M. Doğanay, and F. Keleştimur, Exp. Clin. Endocrinol. Diabetes **105**, 182 (2009).
48. R. Wheatland, Med. Hypotheses **63**, 855 (2004).
49. R. Pal, Endocrine **68**, 251 (2020).
50. F. Tanriverdi, Z. Karaca, K. Unluhizarci, and F. Kelestimur, Stress (2007).
51. Z. Karaca, A. Lale, F. Tanriverdi, M. Kula, K. Unluhizarci, and F. Kelestimur, Pituitary **14**, 134 (2011).
52. Y. Simsek, Z. Karaca, F. Tanriverdi, K. Unluhizarci, A. Selcuklu, and F. Kelestimur, Clin. Endocrinol. (Oxf). **82**, 45 (2015).
53. Z. Karaca, A. Grossman, and F. Kelestimur, Rev. Endocr. Metab. Disord. **22**, 179 (2021).
54. H. S. Dökmetaş, R. Çolak, F. Keleştimur, A. Selçuklu, K. Ünlühizarci, and F. Bayram, J. Clin. Endocrinol. Metab. **85**, 3713 (2000).
55. S. A. Clarke, M. Phylactou, B. Patel, E. G. Mills, B. Muzi, C. Izzi-Engbeaya, S. Choudhury, B. Khoo, K. Meeran, A. N. Comninos, A. Abbara, T. Tan, and W. S. Dhillo, J. Clin. Endocrinol. Metab. (2021).