Hormonal trends in patients suffering from long COVID symptoms

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Abstract. Symptoms of long COVID are complex and long-lasting, and endocrine dysfunction might be involved in the underlying mechanisms. In this study, to clarify the hormonal characteristics of long COVID patients, laboratory data for patients who visited the outpatient clinic for long COVID were evaluated. A retrospective analysis was performed for patients who visited Okayama University Hospital during the period from Feb 2021 to Dec 2021 with focus on the interrelationships between major symptoms and endocrine data. Information and laboratory data were obtained from medical records for 186 patients. The patients had various symptoms, and the most frequent symptoms were general malaise, dysosmia/dysgeusia, hair loss, headache, dyspnea, and sleeplessness. Patients who were suffering from fatigue and dysosmia/dysgeusia were younger, while hair loss was more frequent in older and female patients. As for the characteristics of patients suffering from general fatigue, the scores of depression and fatigue were positively correlated with serum levels of cortisol and free thyroxin (FT4), respectively. Also, patients suffering from general fatigue had lower levels of serum growth hormone and higher levels of serum FT4, while patients with dysosmia/dysgeusia had a significantly lower level of serum cortisol. Serum thyrotropin (TSH) levels were higher and the ratios of FT4/TSH were lower in the initially severe cases, suggesting occult hypothyroidism. In addition, the ratios of plasma adrenocorticotropin to serum cortisol were decreased in patients with relatively high titers of serum SARS-CoV-2 antibody. Thus, hormonal changes seem to be, at least in part, involved in the persistent symptoms of long COVID.

Key words: Fatigue, Growth hormone, Hypothalamo-pituitary-adrenal (HPA) axis, Long COVID, Thyroid

THE NOVEL coronavirus disease 2019 (COVID-19) pandemic has been continuing for more than 2 years since the declaration of a pandemic by the World Health Organization (WHO) [1]. Besides the acute-phase symptoms induced by viral infection, COVID-19 can also cause prolonged sequelae, which have recently been termed long COVID or post-acute sequelae of SARS-CoV-2 infection (PASC) and have recently been defined as post-COVID-19 condition by WHO [2-4]. Various symptoms including general malaise, dysgeusia, dysosmia, low-grade fever, headache, and alopecia have been reported [3, 5-8] in approximately one-third of COVID-19 patients [9, 10]. However, the pathophysiology of long COVID has remained unclear and an effective treatment strategy for long COVID has yet to be established.

Expression of the angiotensin-converting enzyme 2 (ACE2) receptor, which is the receptor for SARS-CoV-2 virus, has been shown in a wide variety of endocrine organs including the hypothalamus, pituitary, adrenal gland, thyroid, testes, and pancreatic islets, indicating the involvement of the endocrine system during and after recovery of the disease [11]. Since long COVID symptoms partly mimic the symptoms caused by hormonal insufficiency, long COVID syndrome is likely to have a certain relationship with the endocrine system. The ACE2 receptor, by which the virus enters cells, is
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abundantly expressed in many endocrine glands [12]. Therefore, endocrine tissues are likely to be involved not only in the acute phase of the disease but also in the long COVID condition. It has been reported that the adrenocortical response was impaired in patients with COVID-19 infection in the acute phase and that a large percentage of patients had plasma cortisol and adrenocorticotropin (ACTH) levels that were consistent with central adrenal insufficiency [13]. As for the involvement of COVID-19 in the hypothalamo-pituitary-adrenal (HPA) axis, it has been reported that evidence of the viral genome, edema, and neuronal degeneration was found in the hypotalamus in autopsies of patients who died from COVID-19 [14]. However, little is known about the impact of COVID-19 on the endocrine system.

Since the symptoms of long COVID tend to be complex and long-lasting, endocrine dysfunction might be potentially involved. To clarify the hormonal characteristics of long COVID patients, laboratory data for patients who visited the outpatient clinic of our university hospital for patients with long COVID were evaluated.

**Patients and Methods**

**Patients’ characteristics**

A retrospective analysis was performed for patients who visited Okayama University Hospital. Medical records for 186 patients who visited our COVID-19 aftercare clinic (CAC) due to long COVID during the period from Feb 2021 to Dec 2021 were carefully reviewed. Long COVID was defined as symptoms that persist for more than one month after the onset of COVID-19 [2-4]. Information was obtained from medical records for age, gender, body mass index (BMI), underlying conditions (current smoking and alcohol drinking habits), hospitalization due to COVID-19, therapeutic use of oxygen or a corticosteroid during the acute phase, date of visiting the CAC after the onset of COVID-19, severity of COVID-19, history of COVID-19 vaccination, clinical symptoms of long COVID, and Self-rating Depression Scale (SDS) for assessment of the severity of depression [15, 16] and Fatigue Assessment Scale (FAS) for assessment of the fatigue condition [17] as validated self-reporting instruments.

**Analysis of endocrine data**

The decision to examine serum and plasma hormones for evaluation of the possibility of some endocrine disorders was made by each physician. Blood samples were collected in a sitting position at the time when the patients visited our clinic at late morning time. Information on the following biochemical parameters was also obtained: ACTH, cortisol, growth hormone (GH), insulin-like growth factor (IGF)-I, free thyroxin (FT4), and thyrotropin (TSH). The levels of those parameters were determined by using the auto-analyzer system Cobas 8000 (F. Hoffmann-La Roche AG, Basel, Switzerland) at the Central Laboratory of Okayama University Hospital. Plasma ACTH and serum cortisol were measured by an electro-chemiluminescence immunoassay (ECLIA) method using Elecsys ACTH and Elecsys Cortisol II kits (F. Hoffmann-La Roche AG), respectively. Serum GH and IGF-I were measured using Elecsys GH and Elecsys IGF-I kits (F. Hoffmann-La Roche AG), respectively, and IGF-I levels were shown by the standard deviation (SD) values [18]. Serum FT4 and TSH were determined by Elecsys FT4 III and Elecsys TSH kits (F. Hoffmann-La Roche AG), respectively.

**Analysis of serum anti-SARS-CoV-2 antibody**

Measurement of serum anti-SARS-CoV-2 antibody was performed using the Elecsys Anti-SARS-CoV-2 S (S300) electrochemiluminescence (ECLIA) kit (Roche Diagnostics, Rot-kreuz, Switzerland). Ranges for the measurement are set from 0.40 U/mL to 25,000 U/mL (by 1:100 dilution) and concentrations less than 0.80 U/mL were considered as negative reactions. Necessity of blood tests for serum antibodies was determined by each physician to evaluate the past infectious condition and the vaccination status. The arbitrary thresholds of the antibody titers were set to 100, 200 and 500 U/mL to stratify the patients based on previous reports [8, 19].

**Statistical analysis**

EZR, version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria), was used in all statistical analyses [20]. It is modified from R commander, which is designed to add frequently used functions in biostatistics. The data were analyzed by the Mann-Whitney U-test or chi-square test to determine significant differences between groups. The data were also analyzed by performing a linear regression analysis and by obtaining Spearman’s rank correlation coefficients to determine relationships between parameters. All tests were performed as two-sided, and *p < 0.05 and **p < 0.01 were regarded as statistically significant.

**Ethics**

Information regarding the present study was provided on our hospital wall and on the website of our hospital, and patients who wished to opt out were offered that opportunity. Informed consent from the patients was not
necessary due to the anonymization of data. This study was approved by the Ethics Committee of Okayama University Hospital (No. 2105-030) and adhered to the Declaration of Helsinki.

Results

Data for all of the 195 patients who visited our CAC during the study period were obtained from medical records. Patients with insufficient data ($n = 9$) were excluded from the study, and data for the remaining 186 patients were analyzed. Clinical backgrounds of the patients who visited our CAC are shown in Table 1. The 186 patients included 74 males (40%) and 112 females (60%). The median age of all patients was 40 years (interquartile range [IQR]: 25–51 years), and the median ages of male and female patients were 39 years (IQR: 25–51 years) and 42 years (IQR: 26–51 years), respectively. The median BMI of all patients was 22.7 (IQR: 20.4–25.8), and there was a significant difference in median BMI between the male and female patients: 24.4 (IQR: 21.4–26.6) vs. 21.8 (IQR: 19.7–24.8) (**$p = 0.00228$). There were 77 patients (41%) with a smoking habit and 80 patients (43%) with a drinking habit. Fifty-four patients (29%) were admitted to hospital due to COVID-19, and 31 patients (17%) had received oxygen and/or steroid therapy. Based on the severity of COVID-19 defined by the Ministry of Health, Labour and Welfare in Japan [21], the numbers (proportions) of patients with mild, moderate-I, moderate-II, and severe states were 141 (76%), 15 (8%), 27 (14%), and 3 (2%), respectively. The numbers (proportions) of patients who had received COVID-19 vaccination (either BNT162b2 [Pfizer/BioNTech] or mRNA-1273 [Moderna]) were 22 (12%) for one dose and 44 (24%) for two doses, and 120 patients (64%) had not received vaccination. The median duration after onset of COVID-19 to visiting our CAC clinic was 83 days (IQR: 56–117 days).

The number of patients with each long COVID symptom at the initial visit is shown in Fig. 1A. There were more than 10 symptoms of long COVID, and the five most frequent symptoms were general fatigue (97 patients, 52.2%), dysosmia/dysgeusia (80 patients, 43.0%), hair loss (46 patients, 24.7%), headache (37 patients, 19.9%), dyspnea (29 patients, 15.6%) and insomnia (27 patients, 14.5%). The three most frequent symptoms, that is, general fatigue, dysosmia/dysgeusia and hair loss, were analyzed by gender and age (Fig. 1B). Regarding the gender proportions of these complaints, hair loss was a more frequent complaint in female patients than in male patients (*$p = 0.0182$), while there was no gender difference in general fatigue ($p = 0.0764$) or dysosmia/dysgeusia ($p = 0.314$). The median age of patients complaining of general fatigue was significantly younger than that of patients without general fatigue (45 years vs. 38 years; *$p = 0.0485$).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Backgrounds of patients visiting the COVID-19 aftercare outpatient clinic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Number of patients</td>
<td>186</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>40 (25–51)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>22.7 (20.4–25.8)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>77 (41%)</td>
</tr>
<tr>
<td>Alcohol drinking habit</td>
<td>80 (43%)</td>
</tr>
</tbody>
</table>

COVID-19-related clinical information

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>54 (29%)</td>
<td>22 (30%)</td>
<td>32 (29%)</td>
<td>0.996</td>
</tr>
<tr>
<td>Steroid and/or $O_2$ therapy</td>
<td>31 (17%)</td>
<td>15 (20%)</td>
<td>16 (14%)</td>
<td>0.384</td>
</tr>
<tr>
<td>Severity</td>
<td>Mild</td>
<td>141 (76%)</td>
<td>54 (73%)</td>
<td>87 (78%)</td>
</tr>
<tr>
<td>Moderate-I</td>
<td>15 (8%)</td>
<td>5 (7%)</td>
<td>10 (9%)</td>
<td></td>
</tr>
<tr>
<td>Moderate-II</td>
<td>27 (14%)</td>
<td>12 (16%)</td>
<td>15 (13%)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>3 (2%)</td>
<td>3 (4%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>COVID-19 vaccination</td>
<td>None</td>
<td>120 (64%)</td>
<td>51 (69%)</td>
<td>69 (62%)</td>
</tr>
<tr>
<td>1 dose</td>
<td>22 (12%)</td>
<td>7 (9%)</td>
<td>15 (13%)</td>
<td></td>
</tr>
<tr>
<td>2 doses</td>
<td>44 (24%)</td>
<td>16 (22%)</td>
<td>28 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

Days after onset to visit, median (IQR) | 83 (56–117) |

Values are shown as $n$ (%) and were statistically analyzed by the Mann-Whitney U-test and chi-square test as appropriate. Significant level was set at *$p < 0.05$ and **$p < 0.01$. BMI: body mass index, COVID-19: coronavirus disease 2019, IQR: interquartile range.
Similarly, the median age of patients suffering from dysosmia/dysgeusia was significantly younger than that of patients without dysosmia/dysgeusia (44 years vs. 39 years; \( p = 0.032 \)), while patients afflicted with hair loss were older than patients without hair loss (39 years vs. 46 years; \( p = 0.0135 \)).

The scores of FAS and SDS in long COVID patients are shown in Fig. 2A. SDS and FAS scores were positively correlated with serum levels of cortisol (\( r = 0.173; \ *p = 0.0179 \)) and FT4 (\( r = 0.206; \ **p = 0.00486 \)), respectively, whereas these scores were not significantly correlated with basal levels of ACTH, GH and TSH. We further analyzed the characteristics of the two groups of high scores of SDS (\( \geq 50 \)) and others (SDS <50). As shown in Fig. 2B, there were no significant differences between the two SDS groups in the levels of ACTH, cortisol, GH, IGF-I, FT4 and TSH, suggesting that the psychiatric status of neurosis or depression is not directly involved in the changes of endocrinological abnormality in long COVID conditions.

The levels of hormones in patients with and those without general fatigue are shown in Fig. 3A. Patients who were suffering from general fatigue had lower levels of serum GH (median: 0.32 ng/mL vs. 0.16 ng/mL; \( **p = 0.00444 \)) and higher levels of serum FT4 (median: 1.24 ng/dL vs. 1.31 ng/dL; \( **p = 0.00548 \)). Levels of other hormones including ACTH, cortisol, IGF-I, and TSH were not different between patients with and those without general fatigue. The levels of hormones in long COVID patients with and those without dysosmia/dysgeusia and in patients with and those without hair loss are shown in Fig. 3B and Fig. 3C, respectively. Patients with dysosmia/dysgeusia had a significantly lower level of serum cortisol (median: 7.65 μg/dL vs. 6.20 μg/dL; \( p = 0.0175 \)) without alteration of plasma ACTH levels. Otherwise, there were no significant differences in hormone levels between patients with and those without hair loss.

Moreover, we evaluated the alteration of endocrine data in relation to the severity of initial infection. As shown in Fig. 4A, the levels of plasma ACTH and serum cortisol and the ratios of ACTH/cortisol were not significantly different between the mild severity group and the moderate/high severity group. The level of serum TSH was significantly higher and the ratio of FT4/TSH was lower in the moderate/high severity group than in the mild group, suggesting the possibility of “occult hypothyroid” status in severe conditions of initial infection.
We further analyzed the influence of anti-SARS-CoV-2 antibody on the ACTH/cortisol ratio, which is an indicator of relative responsiveness of the HPA axis, and on FT4/TSH ratio, which is a sensitive indicator of an occult hypothyroid condition. Data with serum anti-SARS-CoV-2 antibody titers set at 100, 200 and 500 U/mL were evaluated on the basis of previous reports [8, 19]. As shown in Fig. 4B, it was revealed that a relatively high titer of serum antibody (higher than 500 U/mL) decreases the ratios of ACTH/cortisol and FT4/TSH, indicating the possibilities of impaired function of ACTH in response to cortisol feedback and a certain level of latent hypothyroid status.

Discussion

The present study revealed that there are various long COVID symptoms including general malaise, dysosmia/dysgeusia, hair loss, headache, dyspnea, and sleeplessness. Fatigue and dysosmia/dysgeusia were more frequent in younger patients, while hair loss was more frequent in older female patients. Of interest, depression score (SDS) and fatigue score (FAS) were significantly correlated with serum levels of cortisol and FT4, respectively. Also, patients suffering from general fatigue had lower levels of serum GH and higher levels of serum FT4. Patients with dysosmia/dysgeusia had a significantly lower level of serum cortisol, but there were no significant differences in hormone levels between patients with and those without hair loss. Furthermore, long COVID with initially severe symptoms were apt to fall into hypothyroidism, and patients with relatively high titers of serum antibody showed decreased ratios of ACTH/cortisol and FT4/TSH, indicating the possibilities of impaired ACTH response and latent hypothyroid status.

The major symptoms of long COVID tend to overlap with symptoms due to a low cortisol level [22] including fatigue and muscle or joint pain. This condition may be related to suppression of the HPA axis caused by physical stress stimulated by the virus and the following immune response and/or by glucocorticoid treatment. Expression of ACE2 and transmembrane protease serine 2 (TMPRSS2) was detected in the zona fasciculata and reticularis of the adrenal cortex, suggesting that the
Adrenocortical portion can be a target of SARS-CoV-2, leading to impairment of steroidogenesis [23, 24]. Furthermore, given the finding that SARS virus can express amino acids that mimic ACTH residues [25], SARS-CoV-2 infection may give rise to cross-reacting antibodies that can inactivate endogenous ACTH molecules and/or their secretion. Considering that the ratios of ACTH/cortisol levels were lowered in patients with relatively high titers (≥500 U/mL) of serum SARS-CoV-2 antibody in the present study, it is possible that the antibody affects the sensitivity of the feedback system of the HPA axis in response to circulating cortisol.

**Fig. 3** Hormonal levels in long COVID patients with general fatigue, dysosmia/dysgeusia and hair loss. The details of each panel are shown in the legend of Fig. 1. The levels of hormones including adrenocorticotropin (ACTH), cortisol, growth hormone (GH), insulin-like growth factor (IGF)-I, free thyroxin (FT4), and thyrotropin (TSH) are compared between patients with and those without (A) general fatigue, (B) dysosmia/dysgeusia and (C) hair loss. We regarded ** *p < 0.01 and * p < 0.05 as statistically significant differences between the indicated groups.

**Fig. 4** Hormonal levels in long COVID patients related to severity of initial infection and serum anti-SARS-CoV-2 antibody. A) Levels of plasma ACTH and serum cortisol and the ratios and levels of serum FT4 and TSH are compared between the mild severity (Mild) and moderate/high severity (Mod/High) groups. B) ACTH/cortisol ratio and FT4/TSH ratio are compared among the groups based on the serum anti-SARS-CoV-2 antibody (Ab) titers at 100, 200 and 500 U/mL. We regarded * p < 0.05 as statistically significant differences between the indicated groups.
As for the long COVID status, Clarke et al. found in a prospective study that adrenal and thyroid functions were still preserved more than three months after the acute COVID-19 condition [26]. In their study, despite the fact that most of the patients continued to experience long-lasting fatigue, a significant alteration of adrenal and thyroid functions was not observed. Their study cohort had a normal response to an ACTH analog regardless of the severity of COVID-19, antibody titer, or dexamethasone administration [26]. On the other hand, the present study revealed that long COVID patients with dysosmia/dysgeusia had lower levels of serum cortisol without alteration of plasma ACTH levels, indicating the possibility of central impairment of the HPA axis. Since there have been no studies in which adrenal function was assessed in larger populations with long COVID, adrenal insufficiency should always be included as a differential diagnosis of long COVID.

Adult GH deficiency is also an important disorder that causes general fatigue [27] in patients who have hypothalamo-pituitary damage. We recently reported a middle-aged man who had been suffering from chronic fatigue syndrome (CFS) for five years and was finally diagnosed with combined ACTH and GH deficiency possibly caused by an autoimmune mechanism [28]. Given that his fatigue symptoms were improved by glucocorticoid and GH replacement therapy, it is possible that such combined endocrine deficiency represented the patient’s CFS conditions. This case indicated the importance for physicians to consider the possibility of deficiencies of ACTH and/or GH in patients who have persistent fatigue [28]. In the present study, hormonal evaluation in long COVID patients who were suffering from general fatigue showed that patients with general fatigue had lower levels of serum GH but not lower serum IGF-I SD levels. Since serum IGF-I SD levels were not significantly different in patients with and those without fatigue, the reduction of endogenous GH seems to be transient or still small for affecting the levels of serum IGF-I [18].

The impact of COVID-19 on thyroid function has remained uncertain [11]. In a postmortem study of COVID-19 patients, chronic inflammation in the thyroid gland with follicular epithelial cell disruption was occasionally detected [29]. Immune-mediated thyroid damage, in addition to direct infectious injury, can be postulated since SARS-CoV-2 enables activation of systemic immune responses. Several reports on manifestations of Hashimoto’s thyroiditis [30] and autoimmune hyperthyroidism [31] after COVID-19 supported the hypothesis of an etiological connection to autoimmune diseases triggered by COVID-19.

Given the clinical resemblance between long COVID and hypothyroidism, the thyroid axis may also be involved in the long COVID condition. In the present study, scores of SDS and FAS were positively correlated with serum levels of FT4, and patients suffering from general fatigue had higher levels of serum FT4. On the other hand, it has also been reported that levels of triiodothyronine and TSH were lower in acute severe COVID-19 patients [32], implying a condition of non-thyroidal illness (NTI). However, in a follow-up study for long COVID, abnormal data for thyroid function in acute COVID-19 were restored and incidental dysfunction was rarely seen [33]. In another cohort study on COVID-19 patients, most of the patients were euthyroid or their TSH and FT4 levels were lower than baseline, i.e., NTI, but most of those cases were found to be recovered to the euthyroid phase a few months later [34], though follow-up of long COVID symptoms was not performed in that study. In the present study, the level of serum TSH was significantly higher and the ratio of FT4/TSH was lower in the moderate/high severity group than in the mild severity group, indicating the possibility of “occult hypothyroid” status in severe conditions of initial infection. It was also shown that a relatively high titer of serum antibody (>500 U/mL) decreases the ratio of FT4/TSH, indicating the possibility of latent hypothyroid status. Although thyroid involvement is unlikely to be a critical cause of persistent fatigue in long COVID, the severity of initial infection and the presence of a high titer of the antibody may be involved in facilitation of occult hypothyroid.

There are several limitations of the present study. First, treatment during the acute phase of COVID-19 may influence the degrees of clinical manifestations and endocrinology parameters of long COVID patients. Especially, use of corticosteroid agents could have influenced our results. Next, because of the limited number of patients, all possible symptoms of long COVID might not have been included. As another limitation in this study, the timing for each blood sampling was delayed to late morning due to the restriction of patients’ visits for infection prevention. However, to minimize the effects of circadian rhythm on endocrine data related to the HPA axis, we evaluated the ratios of ACTH/cortisol that can, at least in part, indicate the relative responsiveness and functional acuity of cortisol secretion to ACTH stimulation. Also, we could not sufficiently stratify or adjust the disease severity and patients’ characteristics for estimating the association of each long COVID symptom with endocrinological imbalance. In this regard, we have earlier reported that, during three-month observation of post-COVID conditions in 65 long COVID patients [8], the number of residual symptoms of long COVID was significantly larger at 1 to 2 months after the first visit.
Severity of initial infection and anti-SARS-CoV-2 antibody titer should also be considered to evaluate endocrine data for long COVID patients. Multifaceted approaches for establishing methods for evaluation of the severity and prediction of prognosis and for establishing treatment strategies are needed for the management of long COVID.

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Author Contributions

N.S. and F.O. conceived and designed the study; N.S., Y.N., Y.S., Y.M., T.H., and Y.O. performed data collection; K.T., H.H., Y.N., and Y.H. analyzed the data; N.S., H.H., K.Y., and F.O. wrote the paper; and H.H., K.U., and H.K. revised the paper. All authors have read and agreed to publication of the final version of the manuscript.

Disclosure

The authors have nothing to disclose.

References


