Title: Thyroid Function Analysis in COVID-19: A Retrospective Study from a Single Center

Running Head: Thyroid Analysis in COVID-19 Pneumonia

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Abstract

Background and Objective
Coronavirus disease 2019 (COVID-19) is an on-going epidemic with a multitude of long-ranging effects on the physiological balance of the human body. It can cause several effects on thyroid functions as well. We aimed to assess the lasting sequelae of COVID-19 on thyroid hormone and the clinical course of the disease as a result.

Methods
Out of 76 patients, 48 patients of COVID-19 positive and 28 patients of COVID-19 negative polymerase chain reaction (PCR) were assessed for thyroid functions, IL-6, and Procalcitonin between moderate, severe, and critical pneumonia.

Results
Seventy-five percent of patients with COVID-19 had thyroid abnormalities and higher IL-6 levels (76.10 ± 82.35 vs. 6.99 ± 3.99, 95% CI 52.18-100.01, P-value <0.01). Logistic regression analysis suggested TT3 (P-value 0.01), IL-6 (P-value <0.01), and Procalcitonin (P-value 0.03) as independent risk factors for COVID-19. ROC curve demonstrated IL-6 as the most sensitive marker (P-value <0.01), and TT3, and Procalcitonin as the predictor for COVID-19 disease.

Conclusion
This pilot study from Pakistan demonstrates that changes in serum TSH and TT3 levels may be important manifestations of the courses of COVID-19 pneumonia.

Keywords: COVID-19, Thyroid diseases, Thyroid-Stimulating Hormone, Triiodothyronine
Introduction

The on-going pandemic of coronavirus disease 2019 (COVID-19) has been found to have multiple long-ranging effects on the normal physiological balance of the human body. Complex and severe effects on human organ systems are being identified rapidly, including the respiratory, gastric, circulatory, immune, renal, hepatic, cardiac, and hematological systems. Literature on COVID-19 affecting human thyroid function is increasing gradually and the understanding of thyroid dysfunction and its mechanism is growing. The expression of ACE2 combined with transmembrane protease serine 2 (TMPRESS2) is the key cellular complex for the virus to infect the human cells and interestingly both expression levels are present in the thyroid gland, even more than the lungs.

COVID-19 initiates an immune response over-activity leading to release of pro-inflammatory cytokines, particularly interleukin-6 (IL-6), which leads to overt thyroid dysfunction by disruption of desiodases and thyroid transport proteins. Levels of T3 is inversely proportional to IL-6 with a modest decrease of TSH and T4. This abnormality is described as sick euthyroid syndrome. During the course of COVID-19 pneumonia, thyrotoxicosis may be caused secondary to graves thyroiditis or subacute inflammatory thyroiditis. It can be complicated by arrhythmias or thromboembolic episodes. Hence, abnormalities of thyroid dysfunction are important to evaluate in COVID-19.

We conducted a retrospective, cross-sectional study of COVID-19 patients in our hospital in Rawalpindi, Pakistan, compared with a matched cohort of non-COVID-19 patients. Our objectives were to determine if COVID-19 caused acute and subacute thyroid abnormalities, as determined by a diagnostic assessment of thyroid function, and whether thyroid dysfunction leads to a more severe clinical course of COVID-19 in a South Asian population.
Materials and Methods

This retrospective study was approved by the ethical committee at our institute following the World Medical Association Declaration of Helsinki. Patients were considered for inclusion with positive high resolution computed tomography (HRCT) for SARS-CoV-2.

The medical records of all the patients admitted at our institute from April 2020 to July 2020 with pneumonia were retrospectively examined. Patients with a history of thyroid disease, without assessment and follow-up of thyroid function and IL-6/procalcitonin, pregnancy, and mild COVID-19 infections were excluded from the study. A total of 76 patients were enrolled in this study. Forty-eight with COVID-19 and 28 with non-COVID-19 pneumonia fulfilled the criteria for this study. They were included in this study after written informed consent from the patients. Attrition bias was taken into account. All the tests were taken on initial blood sampling (Day 1) before steroid treatment, IL-6 receptor antagonists, and remdesivir and after three months’ (Day 90) follow-up so that no confounding factors produced a bias in this study. This group was screened for underlying thyroid disease or any other medical history that can affect thyroid function.

All the cases were labeled as moderate, severe, or critical based on the clinical symptoms, laboratory tests, and chest computed tomography (CT) scans. Moderate and severe cases were classified as having pneumonia manifestations seen on imaging, respiratory rate of ≥ 30 breaths/min, and oxygen saturation ≤ 90% at rest. Critical cases were labeled as those requiring mechanical ventilation due to respiratory failure, other organ failures that require intensive care unit, and the presence of shock.

All patients included in the study had their thyroid functions, including thyroid-stimulating hormone (TSH), total triiodothyronine (TT3), serum total thyroxine (TT4),
procalcitonin, and IL-6 levels done via electrochemiluminescent immunoassay (ECLIA) in the Elecsys® 2010 immunoassay system. All the parameters were analyzed and compared between the COVID-19 and the control group.

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY). Quantitative data were presented as mean ± standard deviation (SD) and qualitative data in frequency and percentages. Both groups were compared using the students’ t-test and Chi-square when feasible. Stem and leaf plots were calculated to see the distribution of continuous variables. A receiver operating characteristic (ROC) curve was calculated for thyroid function tests and the acute phase reactants to find out sensitivity and specificity of the lab parameters in COVID-19 pneumonia. Confidence interval (CI) were presented for the lab parameters in all groups. A P-value of less than 0.05 was considered significant.
Results

During the course of our study, a total of 76 patients were enrolled. Forty-eight with confirmed COVID-19 and 28 patients with non-COVID-19 pneumonia with their thyroid profiles done were also included as controls. Patient demographics and characteristics of pneumonia in both the groups were evenly matched. They are differentiated in Table 1.

Table 1. Demographics and severity of pneumonia

| Demographics | COVID (n=48) | Non-COVID (n=28) | P-value |
|--------------|-------------|-----------------|---------|
| Age (Mean ± SD) | 51 ± 19.30 | 64.79 ± 11.44 | 0.68 |
| Male (%) | 31(64.6%) | 15(53.6%) | 0.34 |
| Severity | | | |
| Moderate | 22(45.8%) | 8(28.6%) | 0.13 |
| Severe | 9(18.8%) | 10(35.7%) | 0.09 |
| Critical | 17(35.4%) | 10(35.7%) | 0.97 |

Our examination of the thyroid function analysis suggested that 36 (75%) patients with COVID-19 pneumonia had abnormal thyroid functions while 24 (85.7%) patients were detected with non-COVID-19 pneumonia. IL-6 was significantly higher in COVID-19 group as compared with non-COVID-19 group (76.10 ± 82.35 vs. 6.99 ± 3.99, 95% CI 52.18-100.01, P-value <0.01).
At follow-up, there was a downtrend in IL-6 levels in both groups (14.16 ± 16.58 vs. 4.88 ± 2.66, \( P \)-value <0.01). Procalcitonin levels were statistically significant between both the groups (\( P \)-value <0.01). IL-6 was associated with abnormal TSH, TT3, and TT4 levels (\( P \)-value <0.01). Table 2 differentiates thyroid profile and acute phase reactants in both COVID-19 and non-COVID-19 group.

Table 2. Comparison of thyroid functions and acute phase reactants in both groups

| Tests         | COVID (n=48) | 95% CI | Non-COVID (n=28) | 95% CI | \( P \)-value |
|---------------|--------------|--------|------------------|--------|--------------|
| IL6 (pg/mL)   | 76.10 ± 82.35 | 14.16 ± 16.58 | 52.18-100.01 | 6.99 ± 3.99 | 4.88 ± 2.66 | 5.44-8.54 | <0.01 |
| Procalcitonin (mcg/L) | 0.36 ± 0.52 | 0.14 ± 0.32 | 0.21-0.51 | 0.06 ± 0.05 | 0.05 ± 0.08 | 0.01-0.11 | <0.01 |
| TSH (mIU/L)   | 1.48 ± 2.47 | 1.11 ± 1.15 | 0.76-2.20 | 1.73 ± 2.06 | 1.65 ± 1.73 | 0.93-2.53 | <0.01 |
| TT3 (ng/dL)   | 74.62 ± 33.71 | 89.98 ± 35.15 | 64.83-84.42 | 100.21 ± 45.46 | 101.86 ± 40.90 | 82.59-117.84 | <0.01 |
Comparison of TSH and TT3 shows significantly lower mean values in severe COVID-19 infection as compared with critical patients (0.32 ± 0.22 vs. 2.32 ± 3.44, P-value <0.01) (67.44 ± 34.51 vs. 77.12 ± 48.22, P-value <0.01). A comparison of lab parameters among different classes of pneumonia is shown in Table 3.

**Table 3.** Comparison of lab parameters between severity of disease. *P-value <0.05

| Tests     | Moderate | Severe | Critical |
|-----------|----------|--------|----------|
|           | Covid (n=22) | Non-covid (n=8) | Covid (n=9) | Non-covid (n=10) | Covid (n=17) | Non-covid (n=10) |
| TSH       | 1.31 ± 1.87* | 1.28 ± 0.93 | 0.32 ± 0.22 | 2.34 ± 2.61 | 2.32 ± 3.44* | 1.49 ± 2.14* |
| Follow-up | 1.13 ± 1.01* | 1.68 ± 1.09 | 0.34 ± 0.14 | 1.27 ± 0.76 | 1.50 ± 1.44* | 2 ± 2.68* |
| TT3       | 75.64 ± 17.03* | 111 ± 42.93* | 67.44 ± 34.51 | 95.50 ± 51.08* | 77.12 ± 48.22* | 96.30 ± 44.86* |
| Follow-up | 86.14 ± 117.38 | 92.44 ± 88.70 | 93.65 ± 102.60 |
Logistic regression was performed to determine how levels of thyroid functions and phase reactants affect the risk of COVID-19 pneumonia. The model suggested TT3 (P-value < 0.01), IL-6 (P-value < 0.01), and Procalcitonin (P-value 0.03) as independent risk factors for COVID-19. None of the patients received thyroid hormone replacement therapy, and the levels of all the thyroid hormones returned to normal after recovery.

The stem and leaf plot was generated to demonstrate the distribution of the lab parameters. It is shown in Figure 1. The area under the ROC (AUC) for IL-6 and Procalcitonin was 0.941 (95% CI 0.89-0.98, P-value < 0.01) and 0.745 (95% CI 0.00-0.63, P-value < 0.01), respectively. TT3 was a predictor of COVID-19 pneumonia with AUC 0.283 (95% CI 0.15-0.40, P-value < 0.01). Figure 1 suggests that thyroid function tests are not good predictors for progression to severe COVID-19 infection. The ROC curve indicated IL-6 to be inversely proportional to TT3.

**Figure 1. ROC curve for lab parameters**

Differentiation of sensitivity and specificity is shown in **Table 4** and Multivariate analysis in **Table 5**.
### Table 4. Sensitivity and specificity in predicting COVID-19

| Test          | Area under the curve | Sensitivity | Specificity | P-value |
|---------------|----------------------|-------------|-------------|---------|
| IL6           | 0.941                | 0.979       | 0.357       | <0.01   |
| Procalcitonin | 0.745                | 0.667       | 0.357       | <0.01   |
| TSH           | 0.378                | 0.438       | 0.679       | 0.07    |
| TT3           | 0.283                | 0.375       | 0.786       | <0.01   |
| TT4           | 0.542                | 0.479       | 0.393       | 0.54    |

### Table 5. Multivariate analysis of lab parameters in moderate, severe, and critical pneumonia

#### Moderate Pneumonia

| Lab Parameters | B    | SE  | Wald | OR, 95% CI       | P-value |
|----------------|------|-----|------|-------------------|---------|
| IL-6           | 0.02 | .01 | 3.49 | 1.67, 10.46-25.00 | 0.06    |
| TT3            | 0.00 | .00 | 0.64 | 0.12, 73.81-96.32 | 0.42    |
| TT4            | -0.24| .12 | 4.20 | 23.81, 6.23-8.10  | 0.04    |
| TSH            | 0.14 | .14 | 0.91 | 0.67, 0.69-1.92   | 0.33    |
| Procalcitonin  | 2.73 | 1.73| 2.49 | 0.15, 0.28-0.12   | 0.11    |

#### Severe Pneumonia

| IL-6 | 0.00 | 0.00 | 1.53 | 0.32, 16.32-25.00 | 0.21 |

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|                 |     |     |     |     |
|----------------|-----|-----|-----|-----|
| **TT3**        | 0.00| 0.00| 0.18| 48.60|
| **TT4**        | -0.01| 0.10| 0.01| 0.66|
| **TSH**        | 0.06| 0.13| 0.21| 0.90|
| **Procalcitonin** | -0.27| 0.68| 0.15| 0.64|
| **Critical Pneumonia** |     |     |     |     |
| **IL-6**       | -0.02| 0.00| 7.80| 0.00|
| **TT3**        | -0.00| 0.00| 1.01| 0.01|
| **TT4**        | 0.29| 0.13| 5.29| 0.02|
| **TSH**        | -0.17| 0.12| 1.78| 0.18|
| **Procalcitonin** | -1.17| 0.77| 2.26| 0.13|

**Discussion**
The results of our study showed that TSH was raised in moderately and critically affected patients of COVID-19 as compared to non-COVID-19 patients. Additionally, TT3 levels were raised significantly more at follow-up in COVID-19 patients than non-COVID-19 patients with little to no discernable effect on TT4 levels. Our findings are in contrast to a similar retrospective study from China, which found lower TSH levels in more severely affected patients of COVID-19 and a study from Italy in which COVID-19 patients were found to have thyrotoxicosis after a confirmatory diagnosis of COVID-19.\(^5,6\) Although all thyroid function tests returned close to baseline at follow-up in both the Chinese and Italian studies, our study sample showed an outlier in having raised TT3 levels across all three levels of severity of COVID-19. This is of great concern, particularly when paired with the lasting damage to cardiac and respiratory systems which has been documented with regards to COVID-19.\(^7\) Cardiac abnormalities secondary to thyroid abnormalities may worsen when regarded in the context of post-infectious sequelae.

The thyroid gland is a major organ in maintaining many long term functions of the body, in particular, cardiovascular, respiratory, and catabolic tasks, which is why many lower-income countries have implemented salt iodination as a way to counter the consequences of iodine-deficient thyroid disorders.\(^8,9\) As an emerging disease, COVID-19 has taken a devastating toll in lower-middle-income countries, with seemingly no end in sight to the rising death toll which, at the time of this writing, stands to cross more than 1, 000, 000.\(^10\) As the pandemic rages on, more and more insights are emerging into not just the multi-systemic involvement of the virus, but its impact on disrupting the endocrine system-- with more long-term effects-- can also no longer be overlooked, particularly in the context of countries already struggling with prevalent endocrine diseases.\(^2\)
In Pakistan, the prevalence of hyperthyroidism and subclinical hyperthyroidism is 5.1% and 5.8%, respectively, and hypothyroidism is at 4.1%. Even though hyperthyroid conditions are relatively rare in Pakistan.\textsuperscript{4} Our prevalence seems to be well above the prevalence of both disorders in Europe (0.7%) and the United States (0.5%), while in our closest neighbor, India, subclinical and overt hyperthyroidism were present in 1.6% and 1.3%, respectively and hypothyroidism is comparable at 3.4%.\textsuperscript{9,11}

Unlike other countries where COVID-19 has decimated population bases, Pakistan seems to have emerged relatively unscathed from the pandemic.\textsuperscript{12} However, given the thyroid abnormalities which have emerged in our study sample, it would seem that the long-term effects of COVID-19, particularly with regards to follow-up of critically ill patients, may be overlooked especially for elderly patients, in which the symptoms of excessive TT3 in circulation—body aches, confusion, increased heart rate—may simply be dismissed as recovery from a viral infection. Concurrently, a thyroid storm with raised inflammatory markers also has overlapping signs and symptoms with the cytokine storm characteristic of COVID-19 worsening in critically ill patients.\textsuperscript{8,13,14,15} Indeed, precedent for the effect of a previous coronavirus affecting the thyroid was established by post-mortem studies of thyroid samples from patients infected with SARS during the 2002 outbreak.\textsuperscript{16} This immunogenic and hormonal overlap of a novel disease may further complicate the management and recovery of COVID-19, as corticosteroids used in the treatment of severe COVID-19 may inadvertently cause auto-immune damage to the thyroid gland.\textsuperscript{3,16}

Our study was limited to a small pool of consenting patients and less duration of follow-up. One other shortcoming was that free T3, free T4, and other pituitary hormones were not assessed at admission and follow-up due to the retrospective nature of the study. Our institute
being a tertiary care, it received a higher than moderately severe COVID-19 cases. Hence, patients with mild COVID-19 were not assessed for thyroid functions.
Conclusion

To the best of our knowledge, this is the first study regarding thyroid disease in Pakistan. This retrospective study provides solid evidence of the high risk of altered thyroid function after COVID-19 pneumonia. We believe our study represents an important direction of exploration of the true cost of the disease, where the overt crises of COVID-19 may mask an emerging crisis of long-term endocrine dysfunctions. Clinical vigilance must be practiced by physicians when monitoring for recovery from COVID-19 for signs and symptoms of thyroid hormone disruptions, particularly in patients of South Asian descent. Further prospective studies will clarify the clinical and prognostic relevance of abnormal behavior of the thyroid gland with COVID-19.
Conflict of Interest

None to declare

Source of funding:

None

Ethical considerations

All patients were enrolled for this study after an informed written consent. Ethical review board of Foundation University gave approval regarding usage of the patient data (FFH/37/DCA/2020), in accordance with the ethical standards of the Declaration of Helsinki.

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None
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