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LETTER TO THE EDITOR

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Derangements of biochemical markers and thyroid function analysis among COVID-19-positive patients: A developing country single-center experience

1 | INTRODUCTION

Coronavirus disease-19 (COVID-19) was initially reported in the Hubei Province of China in December 2019 and eventually declared a Public Health Emergency of International Concern by the World Health Organization (WHO) on January 30, 2020. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has affected nearly 153 million and resulted in greater than 3.21 million deaths as of May 4, 2021. The virus poses a major threat to us today, making COVID-19 one of the deadliest pandemics in history.[1]

The novel virus has a predilection for multiorgan involvement in addition to the respiratory manifestations due to the widespread presence of angiotensin-converting enzyme 2 (ACE-2) receptors. The virus also affects the endocrine system due to the close interplay between immunological and endocrine responses at multiple levels.[2] The thyroid gland expresses the ACE-2 receptor, which is necessary for the virus to dock and enter the cell. It also has a possible role in viral replication inside the cell. The activation of inflammatory mediators and immune-mediated glandular damage via the formation of antibodies or cell-mediated damage to the thyroid gland resulting in subacute thyroiditis has also been reported in COVID-19-positive patients.[2] Furthermore, thyroid hormone dysfunction has also been linked to increased mortality in critically ill patients with acute respiratory distress syndrome, which is a leading complication in COVID-19.[3] Although there are postulates contemplating hypothalamic–pituitary–adrenal axis disruption or pituitary dysfunction as a sequela to the SARS-CoV-2 infection, the role of the thyroid hormone is critical in assessing the outcome of critically ill individuals. Hence, we aimed to explore derangements of biochemical markers and thyroid function tests among patients suffering from COVID-19 infection.

2 | METHOD

A retrospective study was conducted at our center in which patients infected with COVID-19 were evaluated for thyroid hormones. The diagnosis of COVID-19 was reached via either nasopharyngeal or oropharyngeal swab for polymerase chain reaction. The diagnostic kit utilized the principle of real-time fluorescence (RT-PCR), USA-WA1/2020 stock concentration 2.8E + 05 TCID50/ml, with a lower detection limit of 0.003 TCID50/ml. Fifty-four patients with no previous history of thyroid disease were considered and categorized into severity groups based on CDC criteria for disease severity and prognosis; which included mild-moderate (mild respiratory symptoms and fever, on an average 5–6 days after infection), severe disease (dyspnea, respiratory frequency ≥30/min, blood oxygen saturation ≤93%, and/or lung infiltrates >50% of the lung field within 24–48 h) and critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure). All patients had laboratory-confirmed COVID-19, age more than 18 years without a history of thyroid disease, and their thyroid function tests were taken during the hospital stay. The thyroid hormones were measured usually within the first 24 h of admission. A total of 3–5 cc of clotted blood or serum was taken for thyroid hormone analysis via Elecsys® E411 Assay (Roche Diagnostics) utilizing chemiluminescence immunoassay. The pediatric population and pregnant females were excluded from the analysis. The reference values of our laboratory for thyroid hormones were as follows: free serum T3: 1.9–5.1 pg/ml; free serum T4: 0.9–1.7 ng/dl; and serum TSH: 0.4–4.2 uIU/ml for 21–54 years age group, 0.5–8.9 uIU/ml for 55–87 years age group.

The study was conducted according to the criteria set by the declaration of Helsinki, and ethical approval was waived by the institutional review board due to the retrospective nature of the study. Data were analyzed by using the statistical package for social sciences (SPSS version 25.0). Data were presented as either mean and standard deviation for quantitative and frequency, relative percentages for categorical variables. The student's *t*-test was applied for quantitative measurements, while Pearson's correlation coefficients were applied for linear relationships. The receiver operating characteristic curve was used to obtain optimum cut-off for laboratory parameters predicting disease severity. The values were reported as the area under the curve (AUC), standard error (*SE*), sensitivity, specificity, positive predictive value, and negative predictive value. Kaplan–Meier survival curve was deployed for those parameters and factors compared with log-rank (Mantel–Cox) test.

3 | RESULTS

Our analysis of 54 COVID-19 patients had a mean age of 53.29 ± 15.59 years, with the majority of them being females (61%). There was a major difference in serum TSH observed among severe/critical COVID-19 patients ($p < 0.001$). As a result, inferential statistics were used to assess the relationship between serum TSH and disease incidence and other biochemical markers (Table 1). T3 and T4 levels in the blood were not found to be linked to disease severity. Low serum TSH (<0.996 uIU/ml) was found to be statistically significant with disease severity (mean: 1.14 ± 0.35, $p < 0.001$). Cox proportional hazard model showed a significantly higher risk of disease severity with low TSH levels (hazard ratio: 3.303 [95% confidence interval, 1.124–9.705]; $p = 0.03$). Thyroid hormones were also shown to have a longitudinal relationship with other baseline hematological, biochemical, and inflammatory biomarkers (Table 2). T3 levels ($r = 0.717$, $p < 0.05$), urea ($r = 0.462$, $p < 0.05$), creatinine ($r = 0.592$, $p < 0.001$), and lymphocytes ($r = 0.480$, $p < 0.001$) were directly correlated with serum TSH levels. A higher neutrophil count was correlated with a lower TSH ($r = -0.403$, $p < 0.05$). T4 levels ($r = -0.719$, $p < 0.05$), platelets ($r = -0.710$, $p < 0.05$), and monocytes ($r = -0.770$, $p < 0.05$) were found to be negatively correlated with serum T3, whereas TLC ($r = 0.722$, $p < 0.05$), mean corpuscular volume (MCV; $r = 1.00$, $p < 0.001$), C-reactive protein ($r = 0.838$, $p < 0.001$), and lactate dehydrogenase ($r = 0.662$, $p < 0.05$) levels showed a positive correlation with the same. Furthermore, platelet count ($r = 0.793$, $p < 0.001$), MCV ($r = -0.935$, $p < 0.001$), serum bicarbonate ($r = 0.693$, $p < 0.05$), and ferritin ($r = 0.524$, $p < 0.05$) levels were associated with correlated T4.

The receiver operating characteristic analysis, at a TSH cut-off value of less than 0.996 uIU/ml exhibited a sensitivity of 85.7% and specificity of 72.7% for indicating disease severity (AUC = 0.779, $p = 0.001$) as shown in Figure 1. Figure 2 illustrates the Kaplan–Meier survival curve with respect to TSH levels. Patients with low TSH levels (<0.996 uIU/ml) demonstrated significantly low survival time while the population with sufficient TSH (>0.996 uIU/ml) had a higher cumulative survival proportion (χ^2: 12.650, $p < 0.001$).

4 | DISCUSSION

Our patients who appear to have the euthyroid sick syndrome exhibited severe COVID-19 manifestations; the specific clinical relevance of a low TSH is unclear. However, a euthyroid sick syndrome in severe COVID-19 patients was previously discussed in a few studies.[4,5] The vast majority of hospitalized patients with critical illness are known to undergo transient thyroid hormone dysregulations,[6,7] which are termed as nonthyroidal illness syndrome or thyroid allostasis in critical illness, tumors, uremia, and starvation (TACITUS).[8] The probable molecular mechanisms responsible are oxidative stress causing inflammatory mediators release leading to upregulation of thyroid hormones and feedback mechanisms.[9] There were a few limitations of our study, a single-center analysis with limited sample size, and no study groups were defined on the basis of thyroid status. An approach of comparing severity among those having normal thyroid function versus those with deranged

TABLE 1 Baseline data and major outcomes of the study population ($n = 54$)

					p Value
Mean age (in years)	53.29 ± 15.59				
Gender	Males: 21 (38.8%)		Females: 33 (61.1%)		–
Disease severity	Moderate: 24 (44.4%)	Severe: 20 (37.0%)	Critical: 10 (18.5%)		–
Mode of respiration	Ventilator: 12 (22.2%)		Oxygen by mask: 14 (25.9%)		–
	BiPAP: 18 (33.3%)		High flow nasal canula: 6 (11.1%)		
Survival	Recovered: 30 (55.5%)		Death: 24 (44.4%)		–
Days of hospital stay	9.33 ± 7.80				–
Mean difference in disease severity (TSH)	Moderate: 1.72 ± 0.45		Severe/critical: 1.14 ± 0.35		<0.001*
Mean difference in disease severity (T3)	Moderate: 1.86 ± 1.06		Severe/critical: 1.06 ± 0.69		0.158*
Mean difference in disease severity (T4)	Moderate: 1.80 ± 1.46		Severe/critical: 2.63 ± 3.34		0.445*
Predictive analysis of TSH (based on ROC) cut-off: 0.996 uIU/ml	AUC: 0.779	95% CI: 0.639–0.919	Sensitivity: 85.7%	Specificity: 72.7%	0.001
	SE: 0.072		PPV: 80.0%	NPV: 80.0%	
Cox regression for disease severity at TSH < 0.996 uIU/ml	HR: 3.303	95% CI: 1.124–9.705	Wald: 4.723	*B*: 1.195	0.030
			SE: 0.550	*df*: 1	

Abbreviations: AUC, area under the curve; *B*, coefficients; CI, confidence interval; *df*, degree of freedom; HR, hazard ratio; IQR, interquartile range; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; *SE*, standard error; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine.

*p-Value calculated by independent Student's t-test.

TABLE 2 Correlation of descriptive biochemical markers in COVID-19-infected patients with thyroid profile ($n = 54$)

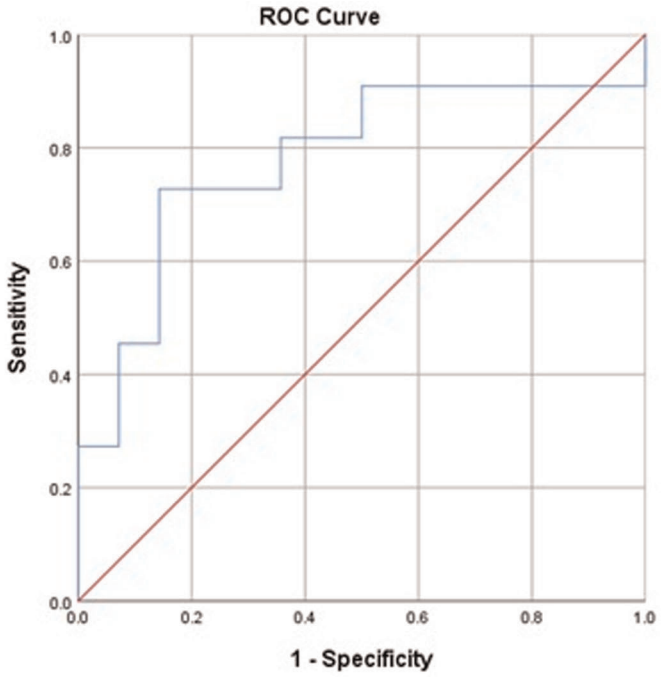
Laboratory investigation	Mean ± *SD*	Median (IQR)	Range	95% Confidence interval	Correlation with TSH	Correlation with T3	Correlation with T4
TSH (uIU/ml)	3.55 ± 13.01	0.61 (0.43–1.24)	0.13–66.47	−0.14–7.25	–	$r = 0.717^{*}$	$r = -0.228$
Free T3 (pg/ml)	1.46 ± 0.95	1.46 (0.59–2.03)	0.27–2.96	0.85–2.06	$r = 0.717^{*}$	–	$r = -0.719^{*}$
Free T4 (ng/dl)	2.18 ± 2.46	1.35 (0.83–1.60)	0.47–8.94	1.09–3.28	$r = -0.228$	$r = -0.719^{*}$	–
Hemoglobin (g/dl)	11.56 ± 2.44	11.24 (9.90–13.50)	7.70–16.83	10.82–12.30	$r = 0.297$	$r = 0.016$	$r = -0.215$
MCV (fl)	83.40 ± 9.17	82.00 (78.00–89.00)	63.00–98.00	79.33–87.46	$r = 0.198$	$r = 1.000^{**}$	$r = -0.935^{**}$
Platelets ($\times 10^{9}$/L)	221.80 ± 125.06	205.00 (142.00–249.25)	56.00–683.00	186.25–257.34	$r = -0.052$	$r = -0.710^{*}$	$r = 0.793^{**}$
TLC ($\times 10^{9}$/L)	12.16 ± 6.73	11.53 (7.10–13.70)	2.80–28.32	10.32–14.00	$r = -0.156$	$r = 0.722^{*}$	$r = 0.480$
Neutrophils (%)	77.85 ± 11.17	80.00 (68.00–87.00)	55.00–94.00	74.80–80.90	$r = -0.403^{*}$	$r = 0.403$	$r = 0.116$
Lymphocytes (%)	15.85 ± 10.05	15.00 (7.00–25.00)	3.00–40.00	13.10 ± 18.59	$r = 0.480^{**}$	$r = -0.241$	$r = -0.088$
Monocytes (%)	5.45 ± 2.65	5.00 (4.00–6.00)	2.00–11.00	4.64–6.26	$r = -0.046$	$r = -0.770^{*}$	$r = -0.098$
Urea (mg/dl)	67.88 ± 57.73	45.65 (23.00–83.89)	10.36–210.69	51.80–83.97	$r = 0.462^{*}$	$r = 0.159$	$r = -0.104$
Creatinine (mg/dl)	1.94 ± 1.71	1.30 (0.72–2.21)	0.52–7.40	1.46–2.41	$r = 0.592^{**}$	$r = 0.520$	$r = -0.177$
Sodium (mEq/L)	139.23 ± 11.12	139.00 (134.00–142.25)	113.00–173.00	135.77–142.70	$r = -0.139$	$r = -0.036$	$r = -0.243$
Potassium (mEq/L)	4.09 ± 0.75	4.10 (3.47–4.60)	2.60–5.64	3.86–4.32	$r = 0.188$	$r = 0.069$	$r = 0.285$
Chloride (mEq/L)	104.42 ± 11.10	105.00 (101.75–107.25)	72.00–133.00	100.96–107.88	$r = -0.232$	$r = 0.029$	$r = -0.446$
Bicarbonate (mEq/L)	20.33 ± 3.74	19.00 (18.07–23.00)	13.00–29.00	19.16–21.50	$r = 0.021$	$r = -0.426$	$r = 0.693^{*}$
CRP (mg/L)	108.02 ± 101.51	109.90 (10.30–151.10)	1.00–322.00	80.31–135.73	$r = -0.223$	$r = 0.838^{**}$	$r = -0.246$
LDH (U/L)	426.45 ± 146.86	403.00 (319.75–541.50)	230.00–763.00	383.81–469.10	$r = -0.140$	$r = 0.662^{*}$	$r = 0.060$
Ferritin (ng/ml)	779.34 ± 757.94	528.00 (275.57–1160.50)	29.84–3255.00	563.94–994.75	$r = -0.095$	$r = -0.382$	$r = 0.524^{*}$
D-dimer (mcg/ml)	8.86 ± 14.96	2.83 (1.70–9.78)	0.34–67.47	4.31–13.41	$r = -0.134$	$r = 0.025$	$r = 0.338$
Procalcitonin (ng/ml)	5.57 ± 16.84	0.28 (0.07–1.06)	0.01–77.80	0.44–10.69	$r = -0.076$	$r = -0.365$	$r = 0.314$

Note: Pearson's correlation used to compute p Values.

Abbreviations: COVID-19, coronavirus disease-19; CRP, C-reactive protein; IQR, interquartile range; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; *r*, correlation coefficient; *SD*, standard deviation; TLC, total leukocyte count; TSH, thyroid-stimulating hormone; T3, triiodothyronine; T4, thyroxine.

*Significant $p < 0.05$.

**Significant $p \leq 0.001$.



Area Under the Curve

Test Result Variable(s): TSH

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.779	0.072	0.001	0.639	0.919

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

FIGURE 1 Receiver operating characteristic curve for TSH showing an AUC of 0.779 for disease severity obtaining estimated cut-off level at 0.996 uIU/ml. AUC, area under the curve; ROC, receiver operating characteristic; TSH, thyroid-stimulating hormone

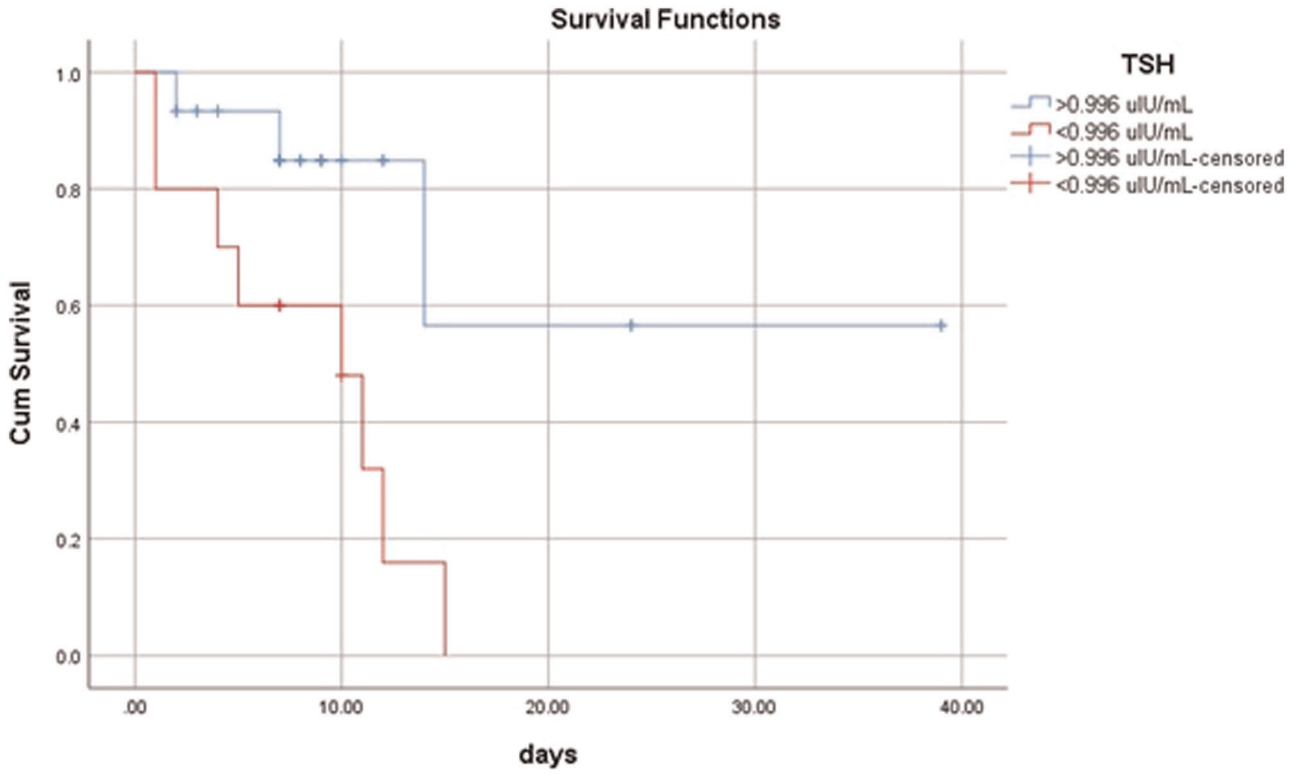
profiles with a larger sample size would have given significant results. In addition, the thyroid hormones were tested while most patients were receiving glucocorticoids for their COVID-19 disease course, hence their effect on thyroid hormone levels cannot be ruled out. Other possible confounders are at least a part of the subjects might have received iodinated radiocontrast agents, which may have a profound effect on thyroid function. The majority of patients admitted in our center were moderate to severe or critical according to the COVID-19 classification of disease severity, hence patients with mild COVID-19 lacked thyroid function data, which could be analyzed. Nonetheless, the strength of the study included a cut-off estimation of TSH decline predicting disease severity and correlation of thyroid profile with other laboratory and inflammatory markers which was not previously emphasized in the literature. Thus, changes in serum TSH and thyroid hormone levels may be important manifestations of the course of COVID-19. In conclusion, we discovered a difference in TSH levels in patients with severe COVID-19 infection. A possible hurdle for this study is the small sample size. Although some studies report a mild thyroid illness following COVID-19 infection,[10] this association further warrants investigation. Studies need to be carried out on a larger scale to evaluate the association between thyroid markers and COVID-19.

5 | CONCLUSION

It is wise on the part of the healthcare providers to adopt a cautious approach when treating COVID-19 patients with altered thyroid levels as they can predict disease severity and correlate with biomarkers.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.



Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	12.650	1	<0.001

Test of equality of survival distributions for the different levels of TSH.

FIGURE 2 Kaplan–Meier curve of survival function with TSH < 0.996 uIU/ml giving a statistically significant difference of $p < 0.001$. TSH, thyroid-stimulating hormone

ETHICS STATEMENT

Ethical approval was waived in this study from the institutional review board, and consent to participate was not required due to the retrospective nature of data collection.

AUTHOR CONTRIBUTIONS

Muhammad S. Asghar: conceptualization (lead), data curation (lead), formal analysis (lead), methodology (lead), project administration (lead), resources (equal), software (equal), supervision (equal), writing-review and editing (equal); **Farah Yasmin**: conceptualization (equal), data curation (equal), investigation (equal), methodology (equal), project administration (equal), resources (equal), software (equal), validation (equal), writing-original draft (equal); **Kartik Dapke**: funding acquisition (equal), investigation (equal), resources (equal), validation (equal), visualization (equal), writing-review & editing (equal); **Rachana Phadke**: data curation (equal), formal analysis (equal), project administration (supporting), validation (supporting), visualization (supporting), writing-original draft (supporting); **Muhammad D. B. Zafar**: conceptualization (supporting); funding acquisition (supporting), resources (equal), validation (equal), writing-review and editing (supporting); **Syed Muhammad Ismail Shah**: data curation (lead), investigation (supporting), resources (supporting), software (equal).

DATA AVAILABILITY STATEMENT

Any datasets will be made available upon reasonable request from the corresponding author.

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