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Selenium and mercury concentrations in biological samples from patients with COVID-19

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) is a systemic disease affecting multiple organs. Furthermore, viral infection depletes several trace elements and promotes complex biochemical reactions in the body. Smoking has been linked to the incidence of COVID-19 and associated mortality, and it may impact clinical effects, viral and bacterial conversion, and treatment outcomes.

Objectives: To study the relationship between severe acute respiratory syndrome coronavirus type 2 and the elemental concentrations of selenium (Se) and mercury (Hg) in biological samples from smokers and nonsmokers infected with the virus and in healthy individuals.

Method: We evaluated changes in the concentrations of essential (Se) and toxic (Hg) elements in biological samples (blood, nasal fluid, saliva, sputum, serum, and scalp hair) collected from male smokers and nonsmokers (aged 29–59 years) infected with COVID-19 and from healthy men in the same age group. The patients lived in different cities in Sindh Province, Pakistan. The Se and Hg concentrations were determined using atomic absorption spectrophotometry.

Results: Se concentrations in all types of biological samples from smokers and nonsmokers with COVID-19 were lower than those of healthy smokers and nonsmokers. Hg concentrations were elevated in both smokers and nonsmokers with COVID-19.

Conclusions: In the current study, persons infected with COVID-19 had higher concentrations of toxic Hg, which could cause physiological disorders, and low concentrations of essential Se, which can also cause weakness. COVID-19 infection showed positive correlations with levels of mercury and selenium. Thus, additional clinical and experimental investigations are essential.

1. Introduction

COVID-19 is a rapidly spreading disease that first appeared in December 2019 and has already killed hundreds of thousands of people around the world [1]. The virus that causes this condition is SARS-CoV-2, which is highly contagious and spreads predominantly through respiratory droplets [2]. Coronaviruses are a group of viruses that can infect both humans and animals [2]. Trace elements are nutritional components that are only necessary at trace levels but play critical roles in the immune system. In the context of viral infections, they mostly serve as catalysts in enzymatic reactions. A powerful association has been discovered between an imbalance in trace element levels (for example, iron, zinc, copper, selenium and magnesium) [3,4].

Selenium is a trace element present in selenocysteine, and its primary biological role is mediated by the selenium protein that contains 21 amino acids. The main source of selenium in the human body is dietary selenium from animals and plants, while the soil selenium content and soil selenium bioavailability for crops are the key factors that determine the amount of selenium in the human diet [4–8]. Selenium, found as selenocysteine in the catalytic centers of several selenoproteins, is an

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essential nutrient for mammalian redox biology [9,10]. For the synthesis of selenocysteine, which is integrated into selenoproteins, an appropriate amount of the amino acid serine is essential [11]. A selenium deficiency in the diet can affect both the immune response and the pathogenesis of a virus [12–14]. A viral genome can be altered by Selenium (Se) depletion, causing a typically benign or mildly pathogenic virus to become extremely virulent in the deficient, oxidatively stressed hosts [15]. Even hosts with regular nutrition can be damaged by the emerging pathogenic strain once viral changes occur [15]. After entering the host, a viral genome can be changed from a generally slightly pathogenic virus to a highly virulent agent due to nutritional selenium insufficiency and elevated oxidative stress [16]. It has been suggested that Se deficiency may play a significant role in the development of SARS-CoV [17]. The role of selenium as a key cofactor in a group of enzymes that, in conjunction with vitamin E, serve to limit the generation of reactive oxygen species, explains its possible protective effect (ROS). Excessive ROS can cause oxidative alterations in both invading bacteria and host cells [18].

Furthermore, selenium deficiency has been associated with a higher incidence of viral genome mutations in RNA viruses such as HIV, Ebola, Coxsackievirus Hantavirus, Influenza virus, and SARS-CoV [19–21]. Trace element imbalances combined with the structure of enzymes facilitate these processes (for antioxidant protection). Copper, zinc and manganese are essential for superoxide dismutase activity, and the presence of active selenium in the active center structure specifies the specific action of antioxidant enzymes (glutathion peroxide and glutathion transferase) [21]. There are some reasons to believe that adequate inhibition of oncological pathology deterioration after surgical involvement and/or radiotherapy (which causes the generation of free radicals) should be achieved by taking into account the content and quantity of toxic elements (cadium, lead, nickel, arsenic and mercury) [19]. Toxic elements increase the risk of hypertension [22–26]. Mercury causes hypertension by inhibiting catecholamine-0-methyltransferase, which increases levels of epinephrine, norepinephrine, and dopamine in the blood and urine. This has the effect of increasing blood pressure. This link is well understood; according to Houston (2011), "Mercury toxicity should be evaluated in every patient with hypertension." Heavy metals have also been associated with diabetes and obesity [27–31]. However, COVID-19 fatalities seem to be associated with the immune system initiating a "cytokine storm," in which abnormally high quantities of cytokines are generated, initiating a cascade of events that might result in death. Mercury increases proinflammatory cytokines that affect the cytokine response. Interleukin-1 (IL-1) cytokines, which are the same cytokines that mercury elevates, are the center of COVID-19-induced cytokine storms [32–34].

Selenium counteracts some of the negative effects of mercury (Hg) in tissues by generating a less toxic seleno-mercury complex [35]. Mercury-related cardiovascular illness and cerebrovascular accidents are reduced when people consume more Se [35]. Glutathione peroxidase, which detoxifies hydrogen peroxide and lipoperoxides, has selenium at its active core [4]. Mercury influences the distribution and retention of other heavy metals by acting as a catalyst for lipid peroxidation [36]. Mercury promotes free radical generation, inactivates antioxidant defenses, and forms seleno-mercury complexes with thiol-containing compounds [37] and Se, reducing Se availability for glutathione peroxidase activity [38].

Toxic elements (TEs) such as aluminum (Al), arsenic (As), cadmium (Cd), mercury (Hg), nickel (Ni), and lead (Pb) have been hypothesized as causal agents of cigarette smoke-induced physiological illnesses, and smoking is a major source of exposure to these TEs [39]. In fact, a study found that significant symptoms (intense desires to smoke, anxiety, or failed attempts to quit smoking) occurred in young people within weeks or days of starting to smoke [40]. Tobacco usage is the leading cause of disease and mortality around the world. Tobacco-related morbidity results from repeated inhalation exposure to a variety of toxic constituents in cigarette smoke, which are created by pyrosynthesis or released during combustion. Tobacco smoke is carcinogenic, poisonous, genotoxic, and mutagenic [41].

In this study, we determined the concentrations of selenium and toxic (mercury) elements in scalp hair, blood, serum, nasal fluid, sputum, and saliva samples from smokers and nonsmoking COVID-19 patients age range 29–59 years. Although the associations between essential trace and toxic element exposure and COVID-19 patients are not well understood before that, that is why this sort of study is necessary to elucidate the precise mechanism of metal-induced carcinogenesis.

2. Materials and methods

2.1. Approval from the ethical committee

Before sample collection, the protocol of the study was approved (Approval Number: DIR/665/2021) by the ethical committee of NCEAC, University of Sindh, Jamshoro, Pakistan.

2.2. Study design and pretreatment

We tried our best to obtain biological samples from COVID-19 patients. However, due to the global lockdown, we had to collect biological samples from our families, friends, and other family members (N = 115) who were affected by COVID-19 disorders. Entire patients who were admitted to different hospitals. These samples were obtained between October 2021 and January 2022.

For information purposes, an oral session was held at the patients' home, during which the contributors/subjects were informed about the necessity and pattern. Before collecting samples, patients received a written consent form to fill out and sign. Another form was filled out with information on patients' lifestyles, dietary habits, and employment histories. All COVID-19 patients were between the ages of 29–59 years. COVID-19 participants were separated into two groups for the comparison study: smokers and nonsmokers. Seventy-one smokers and 87 nonsmokers were among the 158 healthy referents. Among the COVID-19 patients, 63 were nonsmokers and 52 were smokers, for a total of 115 patients (Table 1). COVID-19 patients are categorized into three basic types based on the following clinical symptoms, according to the seventh edition of the COVID-19 diagnostic and treatment plan. (1) mild conditions: fever, respiratory or digestive symptoms, and chest computed tomography (CT)-diagnosed pneumonia; (2) severe conditions: shortness of breath and respiratory rate of 30 times per minute, oxygen saturation of 93 % at rest, or chest CT imaging showing progression of the lesion of more than 50 % within 24–48 h; (3) critical conditions: respiratory failure and need for mechanical ventilation, shock, or chest CT imaging showing progression of the lesion of more than 50 % in 24–48 h.

All of these participants were subjected to an RNA detection test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). PCR was used to perform this test on a nasopharyngeal/oropharyngeal swab (qualitative) (Table 2). Following a clinical examination by a specialist to confirm the existence of COVID-19, samples were collected in the presence of a specialist doctor at several hospitals around Sindh, Pakistan, after COVID-19 patients signed a permission form. Initially, we collected biochemical data from each participant (Table 2).

Healthy referents were chosen according to the following criteria: Have no fever and/or any respiratory tract-related disease/infection at the time of selection; and ii) no known history of tuberculosis in the previous 2 years from the time of sample collection. Healthy individuals were chosen using a probability sampling procedure. Prior to the collection of biological samples, all 158 healthy referents were examined by a specialist doctor.

All 115 COVID-19 patients underwent acid-fast bacilli sputum tests, as well as radiographic, clinical, and microscopic tests, which revealed abnormalities related to pulmonary TB in their samples. Patients with

Table 1
Demographic information of smoker and non-smoker referents and Covid-19 patients.

Characteristics	Non-Smokers				Smokers			
	Referents	Covid-19 patients (n = 63)			Referents	Covid-19 patients (n = 52)		
		Mild (n = 49)	Severe (n = 12)	Critical (n = 02)		Mild (n = 46)	Severe (n = 04)	Critical (n = 02)
Signs and Symptoms								
Fever	No any disorder	49	12	02	No any disorder	59	04	02
Cough		47	11	02		57	03	02
Chest distress		48	11	02		54	04	02
Myalgia or fatigue		39	08	02		26	03	02
Anorexia		27	07	02		33	04	02
Diarrhea		25	06	02		19	04	02
Dyspnea		00	00	02		00	00	00
Palpitation		19	00	02		16	00	02
Chest pain		22	07	02		17	03	02

Table 2
Anthropometric information of smoker and non-smoker referents and COVID 19 Patients.

Important biochemical tests	Non-Smokers		Smokers	
	Referents	COVID 19 Patients	Referents	COVID 19 Patients
BMI (kg/m^2)	20.8 ± 4.36 19.5–22.1	17.4 ± 3.76 16.2–18.6	19.6 ± 3.16 18.5–20.7	16.2 ± 2.68 15.4–17.0
Hemoglobin [g/dl]	14.3 ± 0.69 13.9–14.7	12.6 ± 0.53 12.2–12.9	13.2 ± 0.95 12.7–13.6	11.3 ± 0.72 10.8–11.7
Leucocyte [$10^3/\mu l$]	7350 ± 450 7160–7560	8690 ± 820 8270–9100	7630 ± 640 7310–7950	9050 ± 890 8650–9470
ESR (mm/hour)	11.0 ± 1.01 10.7–11.3	49.0 ± 6.61 46.8–51.2	14.0 ± 1.49 13.5–14.5	57.0 ± 9.0 54.3–59.7
CRP (mg/l)	0.75 ± 0.09 0.722–0.777	5.60 ± 0.40 5.47–5.73	2.805 ± 0.310 2.698–2.911	16.8 ± 0.86 16.54–17.06
Serum cholesterol [mg/dl]	152 ± 14.6 144–165	127 ± 9.65 122–132	146 ± 9.90 141–152	115 ± 8.77 111–119
HDL [mg/dl]	47.5 ± 2.65 46.0–49.0	33.5 ± 1.50 32.0–35.0	46.0 ± 2.00 45.0–47.0	31.0 ± 2.00 30.0–32.0
LDL [mg/dl]	125 ± 9.80 120–129	95.0 ± 5.90 92.2–97.8	138 ± 7.15 134–142	102 ± 8.15 98.2–106
Triglyceride [mg/dl]	109 ± 6.85 98.6–105	62.5 ± 7.95 59.0–65.0	105 ± 6.60 102–109	59.0 ± 8.55 55.9–64.0
Total Albumin [g/l]	43.5 ± 5.35 40.9–46.0	34.9 ± 3.85 33.0–37.2	42.0 ± 2.95 40.3–43.6	31.9 ± 3.19 30.0–33.4
Total protein [g/l]	33.6 ± 3.05 32.1–35.2	21.5 ± 1.06 20.9–22.1	32.5 ± 1.85 31.6–33.4	19.2 ± 0.99 18.7–19.8

COVID-19 had to be at least 25 years old and have a smear-positive case of pulmonary tuberculosis. Excluded patients were retreated for tuberculosis, had multidrug-resistant tuberculosis, and/or had extrapulmonary tuberculosis.

2.3. Ethical consideration

Before sample collection, multiple hospitals in Sindh, Pakistan, gave their ethical approval. The Declaration of Helsinki code was rigorously followed when collecting consent from volunteers and/or their legal guardians. To protect the identities of the volunteers, secret IDs were used to code them. All COVID-19 patient data were kept private, and only the project's principal investigator and a physician specialist had access to it.

2.4. Sample collection

2.4.1. Blood sample collection

In the current study, all enrolled volunteers agreed to participate and donate blood samples. Blood samples were collected from each subject after fasting overnight using a BD (Oxford, UK) Vacutainer (trace element tube with potassium-EDTA) with folded aluminum foil to prevent blood samples from being exposed to sunlight. According to standard protocols, these blood samples were drawn with a sterile 10 cc syringe (Supplier BD, Pakistan) using a winged infusion set. Blood samples were centrifuged at 3000 rpm for 15 min to separate serum from blood cells. Hemolytic sera were discarded. After centrifugation, the obtained samples were stored at −20 °C for future analysis.

2.4.2. Saliva sample collection

Each volunteer received a sterile plastic container without any elemental or bacterial contamination. Each volunteer's secret identity number was preprinted on the sterile container. To reach the 5 mL mark, each volunteer was instructed to collect saliva in their oral cavity and spit into a plastic container. This method of collecting saliva is known as direct collection.

2.4.3. Sputum sample collection

Male nurses were instructed to direct each volunteer patient and referent to fill a sterile plastic container with 5 mL of blood, which was provided to each participant with a prelabeled confidential identifier number. All of the samples were subsequently transported to Jamshoro's National Centre of Excellence in Analytical Chemistry (NCEAC) for analysis in a sealed container to avoid exposure to direct sunlight. The use of nebulized 4.5 % hypertonic saline to elicit sputum collection was used in healthy volunteers.

2.4.4. Nasal fluid sample collection

Nasal fluids were collected using a small plastic spoon with dimensions of 10 mm × 5 mm x 2 mm. After collecting 1.5 mL of material into Eppendorf tubes, each sample was centrifuged.

2.4.5. Scalp hair sample collection

Hair samples weighing approximately 0.5 g (0.5–2.0 cm long) were collected from each ill and healthy subject using stainless steel scissors. Before and after cutting the hair of each participant, the scissors were carefully washed and sanitized with an alcohol swab. Hair samples from the back of the head were taken and stored in sterile plastic bags. Following the collection of samples, each participant's bag was labeled with a confidential identification number. These plastic bags were affixed to each volunteer's questionnaire and kept for digestion and further examination.

2.5. Apparatus

A double beam Perkin-Elmer atomic absorption spectrometer model Analyst 700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400 (PerkinElmer) and an autosampler AS-800 was used to analyze Hg and Se (PerkinElmer). The MHS-15 chemical vapor generation device was used to determine the amount of mercury in samples and standard solutions (PerkinElmer). An atomic absorption spectrometer was used to calculate integrated absorbance signals, which were used throughout.

The hollow cathode Hg and Se lamps were operated according to the manufacturer's recommendations (Table 3). The biological samples were digested in a Pel (PMO23, Tokyo, Japan) household microwave oven with a maximum heating power of 900 W. For the production and storage of solutions, acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used.

2.6. Reagents and glassware

The ultrapure water used in the procedures was provided by Milli-Q USA. The company E. Merck, which makes analytical chemicals such as hydrogen peroxide and nitric acid, was used. All samples were verified for metal contamination before being used. Fluka Kamica 1000 ppm Hg and Se standard solutions (Buchs, Switzerland) were used. HNO3 (0.2 mol/L) was used to serially dilute the standard (stock) solutions. The solutions produced were kept at 4 °C in plastic bottles for future research. Certified reference materials (CRMs) of human hair BCR 397 (Brussels, Belgium) and Clinchek®, Control lypholized blood (Germany Munich, Recipe) were purchased to achieve a sensitive and selective technique. The plastic items and the apparatus were soaked for 24 h in 2 mol/L HNO3 and then washed and rinsed with Milli-Q water.

2.7. Microwave-assisted acid digestion method

With the use of a microwave oven-based digestion procedure, duplicates of each sample were generated for elemental analysis. Six replication samples of the certificated reference materials (serum and blood) were prepared. For each sample, 200 mg of hair and 0.2 mL of nasal fluid, serum, sputum, and blood were combined with 1 mL of the newly made H_2O_2 – HNO_3 mixture (1:2, v/v) in a PTFE flask. This combination flask was placed in an MW oven for 3 min before being exposed to 950 MW microwaves until complete sample digestion was achieved. The digested samples were allowed to cool to room temperature before being diluted with Milli-Q water to a final volume of 10 mL. ETAAS was used to assess the samples for Se and Hg. For the preparation of blank samples, the same process was used.

2.8. Data and statistical analyses

Data statistical analysis was performed using Excel X state and Minitab software. Certified reference materials from Hair BCR 397 and Clin check blood were used to test the accuracy of the analytical method. The approach was determined to be the most reliable compared to certified values of Hg and Se values, with a recovery rate of 95.7–98.4 % (Table 4). The linear ranges for Hg and Se from the quantification limit (LOQ) were 5.0 and 50 g/L, respectively, with correlation coefficients (r^2) of 0.999 and 0.999 for Hg and Se, respectively. The limit of detection (LOD) and quantification (LOQ) were calculated by LOD $= 3 \times s/m$ and LOQ $= 10$ s/m, respectively, where s is the standard deviation of ten blank observations and m is the slope of the calibration graph. The LODs of Hg and Se were 0.145 and 0.057 μg/L, respectively. Triplicate samples, reagent blanks, procedure blanks, and CRM samples were all analyzed to ensure that the process worked.

Table 3
Measurement conditions for electro thermal atomization and MHS 15 system in AAS 700.

Parameters	Selenium	Mercury
Lamp current (mA)	12.5	6.0
Wave length (nm)	196.0	253.7
Slit width (nm)	0.2	0.2
Drying Temp (°C)/ramp hold (s)	140/15/5	140/15/5
Ashing Temp (°C)/ramp hold (s)	1100/10/20	1100/10/20
Atomization Temp (°C)/ramp hold (s)	2100/0/5.0	2200/0/5.0
Cleaning Temp (°C)/ramp hold (s)	2600/1/3	2600/1/3
Chemical Modifier	$Mg(NO_3)_2$	$Pd(NO_3)_2$

Sample volume (10 μl), Cuvette = Cup, Carrier gas = (200 mL/min), Background correction (D2 Lamp) used for both elements.

Table 4
Determination of Hg, and Se in certified samples by microwave digestion method (N = 6).

Elements	Certified values	MWD Mean ± SD	(%) Recovery	Paired t-test[a] $t^{Experimental}$
	Certified sample of whole blood (μg /L)			
Se	**118 ± 23.7**	**116.2 ± 3.47 (3.07)**[b]	**98.4**	**0.249**
Hg	3.5 ± 1.0	3.35 ± 0.27 (8.71)	95.71	0.241
	Certified sample of human hair (μg /g)			
Se	**2.05 ± 0.04**	**1.98 ± 0.12 (6.05)**	**96.5**	0.0212
Hg	**12.7 ± 0.879**	12.2 ± 0.27 (1.70)	96.0	0.155

$t_{Critical}$ at 95 % confidence limit= 2.57 % Recovery was calculated according to: ([MWD])/([Certified Value]) x $100^{(}$ [0] [b] Values in side this bracket are RSD.
[a] Paired t-test between certified values vs. found values, degree of freedom (n−1) = 5.

3. Results

COVID-19 patients had lower serum levels of cholesterol, HDL, LDL, triglycerides, total albumin, total protein and hemoglobin than the referents; however, COVID-19 patients had higher levels of leukocytes, ESR, and CRP than the referents (Table 2). Table 5 shows the mean amounts of selenium and mercury in biological samples (serum, scalp hair, saliva, blood, nasal fluid and sputum). The findings show changes in selenium and mercury concentrations in biological samples (saliva, blood, nasal fluid, and serum) from male smokers and nonsmokers in COVID-19 patients. The selenium concentration in scalp hair, blood, serum, sputum, saliva, and nasal fluid samples from male nonsmoking and smoker patients with COVID-19 was found to be lower in the 95 % confidence interval {[CI 0.98–1.06] μg/g, [120–130] μg/L, [CI 50.2–56.5] μg/L, [2.79–3.09] μg/L, [CI 1.85–2.04] μg/L and [2.18–2.49] μg/L} and {[CI 0.85–0.92] μg/g, [97.9–105] μg/L, [CI 43.7–48.0] μg/L, [CI 1.70–1.99] μg/L, [CI 1.30–1.45] μg/L, and [CI 1.64–1.82] μg/L}, respectively, compared to Se levels in scalp hair, blood, serum, sputum, saliva and nasal fluid samples of male nonsmoker and smoker referents {[CI 1.80–2.12] μg/g, [CI 2.25–2.39] μg/L, [CI 99.6–110] μg/l, [(CI: 6.39–6.68] μg/L, [CI: 3.60–3.85] μg/L, and [CI: 4.69–4.98] μg/L)} and {([CI: 1.59–1.80] μg/g, [CI: 203–215] μg/L, [CI: 81.2–89.3] μg/L, [CI: 4.13–4.55] μg/L, [CI: 3.20–3.39] μg/L, and [CI: 3.68–4.05] μg/L}, respectively (Table 5). Hg concentrations in biological samples from male smokers and nonsmoking patients with COVID-19 were significantly higher at 95 % C.I. {[CI: 1.79–2.00] μg/g, [CI: 1.60–1.89] μg/L, [CI: 0.84–0.92] μg/L, [CI: 1.09–1.20] μg/L, [CI: 0.82–0.87] μg/L and [CI: 0.91–0.99] μg/L} and {[CI 2.03–2.34] μg/g, [CI: 1.80–2.15] μg/L, [CI: 0.85–0.97] μg/L, [CI: 1.21–1.40] μg/L, [CI: 0.95–1.00] μg/L and [CI: 0.99–1.12] μg/L} compared with those in healthy male nonsmokers and smokers ($p < 0.01$). An independent Student's t test was used to compare the mean values of COVID-19 smokers and nonsmokers and reference participants.

4. Discussion

The first corona case in Pakistan was reported on February 26, 2020. COVID-19 is currently a global pandemic. The objective of this study was to determine the levels of essential trace (Se) and toxic elements (Hg) in biological samples from patients with COVID-19 and controls in metropolitan Sindh, Pakistan (Hyderabad), with smoking and nonsmoking habits. Although it is uncertain whether the decrease in serum HDL cholesterol in COVID-19 infection follows the same path as HIV-1 infection, HDL particles and cholesterol have been linked to virus

Table 5
Elemental concentrations in biological samples of referent and COVID-19 patients.

Referents/ Patients	Adduct	Biological samples	Selenium	Mercury
Referents	Non smokers	Scalp hairs (μg/ g)	1.95 ± 0.32	1.03 ± 0.15
	Smokers		1.70 ± 0.24	1.19 ± 0.12
COVID 19 Patients	Non smokers		0.98 ± 0.13	1.89 ± 0.20
	Smokers		0.85 ± 0.11	2.20 ± 0.35
Referents	Non smokers	Blood (μg/ L)	232 ± 15.9	0.99 ± 0.09
	Smokers		209 ± 12.0	1.15 ± 0.14
COVID 19 Patients	Non smokers		125 ± 9.97	1.75 ± 0.30
	Smokers		102 ± 8.95	1.99 ± 0.37
Referents	Non smokers	Serum (μg/ L)	105 ± 10.4	0.59 ± 0.08
	Smokers		85.6 ± 8.05	0.67 ± 0.06
COVID 19 Patients	Non smokers		53.0 ± 6.09	0.87 ± 0.08
	Smokers		45.2 ± 5.19	0.93 ± 0.11
Referents	Non smokers	Sputum (μg/ L)	6.52 ± 0.32	0.57 ± 0.09
	Smokers		4.39 ± 0.52	0.68 ± 0.13
COVID 19 Patients	Non smokers		2.95 ± 0.29	1.15 ± 0.09
	Smokers		1.85 ± 0.31	1.32 ± 0.20
Referents	Non smokers	Saliva (μg/ L)	3.72 ± 0.25	0.45 ± 0.06
	Smokers		3.29 ± 0.19	0.53 ± 0.09
COVID 19 Patients	Non smokers		1.94 ± 0.18	0.85 ± 0.05
	Smokers		1.37 ± 0.15	0.97 ± 0.07
Referents	Non smokers	Nasal Fluid (μg/ L)	4.82 ± 0.36	0.51 ± 0.05
	Smokers		3.89 ± 0.27	0.57 ± 0.08
COVID 19 Patients	Non smokers		2.35 ± 0.33	0.95 ± 0.09
	Smokers		1.75 ± 0.20	1.05 ± 0.12

infection. In vitro investigations revealed that cholesterol in lipid rafts is essential for SARS-CoV replication at the early stage and during the binding stage of SARS-CoV entry into host cells [42,43]. Furthermore, HDL is an anti-inflammatory lipoprotein in general [44]. Inflammation, on the other hand, it has been linked to structural changes in HDL particles and the accumulation of serum amyloid A (SAA) from the acute phase protein within the HDL protein moiety [45]. Furthermore, minor diarrhea during the onset of the disease could cause a drop in serum albumin levels [45]. A low level of selenium was reported in biological samples from COVID-19 patients compared to reference subjects. The selenium deficit appears to be widespread in COVID-19, as evidenced by a study in South Korea [46], which reported a significant rate of selenium deficiency based on blood selenium measurements. Selenium insufficiency was associated with higher mortality in COVID-19 patients in one of the first studies of its kind [47]. Nutrient shortage is fairly common in hospitalized patients and selenium deficiency can be quite common in the severe type of COVID-19. Inadequate selenium consumption affects a high percentage of the global population in numerous nations, and this can have a significant impact on COVID-19 infection and its consequences. A study from China found that selenium levels were linked to COVID-19 cure rates [48]. Selenium deficiency is very prevalent in severely extremely sick individuals [49,50]. Low selenium status is also more common among patients with COVID-19 with severe illness [47,51]. Supplementation with selenium improves immunological function and reduces oxidative stress, inflammation, and virus pathogenicity [52–54]. Supplementation with selenium and selenium-containing compounds may also reduce the capacity of SARS-CoV-2 to infect humans [51]. Selenium supplementation has been found to improve the immunological functions of elderly individuals. The addition of selenium to the diet increased total T cells, particularly CD4+ T cells, as well as the percentage of NK cells, leading to an increase in NK-cell cytotoxicity [50]. Furthermore, selenium deficiency can be associated with a higher risk of death in severely ill individuals. High doses of selenium were observed to prevent mortality from septic shock in a clinical investigation [55]. In critically ill patients with COVID-19, respiratory problems are the most serious [56]. Lung selenoproteins can help alleviate these issues by acting as antioxidants and influencing multiple immune response pathways to reduce the impact of viral invasion and tissue injury [57,58]. Several studies have examined whether the addition of selenium to the diet of a patient can reduce the death rate and the result of pneumonia in ventilated patients, with contradictory results [59–61]. In a randomized controlled trial (RCT), selenium supplementation was observed in mechanically ventilated patients after sepsis to minimize the occurrence of ventilator-associated pneumonia [59]. Supplementation of parental selenium in critically ill patients with systemic inflammatory response syndrome reduced the incidence of ventilator-associated pneumonia and the severity of the disease in another clinical trial [60]. In critically ill patients, a large dose of selenium increased antioxidant status as evaluated by plasma GPx3, but did not reduce the risk of ventilator-associated pneumonia [61].

In general, selenium supplementation appears to be beneficial; therefore, ensuring adequate selenium intake, particularly among COVID-19 ICU patients, should be encouraged. While selenium supplementation is essential, it is also critical to adhere to the recommended amount and blood levels should be closely monitored to avoid toxicity. In this study, higher levels of Hg were reported in biological samples of patients with nonsmoking and smoking COVID-19 compared to reference subjects. Higher quantities of toxic elements can affect our respiratory system [46,57]. Metals or metalloids can form versatile compounds inside the cell, which can impact the cell's functioning [57]. Numerous technological advances have been brought to the tobacco industry in recent decades to improve the taste and design of cigarettes, resulting in the application of various aspects [62]. Tobacco plants are known to absorb harmful metals from soil, insecticides, and other sources [62]. The uptake of toxic elements (TEs) can be influenced by a variety of circumstances, including changes in soil pH, fertilizer use, rainfall, and metal concentrations in irrigation water sources [63]. Tobacco combustion is believed to have generated approximately 87 carcinogenic chemicals and TEs, to which a smoker is exposed when inhaling cigarette smoke [63]. Because each tobacco manufacturer has its own processing method, the amount of exposure to heavy metals may differ from one commercial tobacco product to the next. By increasing oxidative stress due to Zn insufficiency, an antioxidant element could cause pulmonary TB, atherosclerosis, and myocardial infarctions [64]. The lack of critical components can promote the digestion and accumulation of toxic elements, which can lead to tuberculosis, heart disease, and hypertension [64]. Although acute inhalation of mercury (Hg) vapor is known to cause respiratory impairment [65], research on the association between environmental Hg pollution and pulmonary function is limited. In participants from small-scale and artisanal gold mining locations, previous research found a substantial adverse relationship between hair Hg and respiratory function [66].

However, further data show mixed results, emphasizing the significance of future research on the link between prenatal and lifetime Hg exposure and asthma [67]. Children with recurrent wheezing have

higher levels of mercury and lead in their blood [68]. Exhaled nitric oxide levels were also associated with blood Hg levels, which is believed to be a sign of airway inflammation [69]. It is also worth noting that a high maternal Hg content was linked to an increased risk of lower respiratory infections among infants but not upper respiratory infections [70]. Through the development of oxidative stress and apoptosis, both methylmercury (CH_3Hg) [71] and $HgCl_2$ [72] have been proven to be harmful to A549 cells. The latter could be caused by Hg-induced changes in Bax, p53, and Bcl 2 expression [73]. In alveolar type II epithelial cells exposed to mercury, a similar impact was observed (Lu et al., 2010). Similarly, exposure to Hg was found to cause oxidative stress and the production of the heat shock protein 70 (Hsp 70) [74]. Endoplasmic reticulum stress (ER stress) could possibly be a contributing factor to Hg poisoning in the lungs [75]. It is also worth noting that inhalation of elemental mercury vapor greatly increased the expression of proinflammatory cytokines in lung tissue [76]. Although there is a lack of direct evidence that Hg affects airway epithelial permeability, exposure to Hg has been shown to change the expression of tight junction proteins in colonic epithelial cells [77].

Tobacco-related illness results from repeated inhalation exposure to a variety of toxic substances, including hazardous elements in cigarette smoke that are created by pyrosynthesis or released during combustion. According to the World Health Organization (WHO), a person dies from tobacco use every ten seconds around the world [78]. Tobacco plant uptake of hazardous elements is influenced by their concentration in the soil, soil amendments with sewage sludge, and soil pH [79]. Cigarettes manufactured from tobacco cultivated in different geographical regions or under different agricultural conditions are likely to contain variable quantities of heavy metals in the tobacco filler, resulting in different levels of heavy metals in the smoke [80,81]. Tobacco leaves naturally accumulate and concentrate relatively high levels of toxic heavy elements, making tobacco smoking an important source of exposure for smokers [80,81].

The chemical constitution of cigarette tobacco is heavily influenced by the country of origin and product type. One pack of cigarettes deposits 0–1.4 μg As, 2–4 μg Cd, 0.46–6.5 ng Hg, and 12–2 μg Pb into a smoker's lungs, while some of the smoke travels into the air and is inhaled by both smokers and nonsmokers [39]. This result was also consistent with another study indicating that smokers have much higher body loads of As, Cd, Hg, and Pb than nonsmokers [80]. In recent years, more attention has been paid to interactions between toxic elements (TEs) and bio-elements that are important for life in the organism. Bio-elements including zinc (Zn), copper (Cu), iron (Fe), selenium (Se), calcium (Ca), and TEs like Cd, Pb, and Hg are involved in these complicated relationships [82]. The basis of Hg toxicity is its negative impact on cell enzymatic systems, which is caused by the substitution of other essential elemental ions (mainly Se) in selenoproteins, as well as its extremely strong affinity for biological structures with SH groups, such as proteins, enzymes, and nucleic acids [83]. The importance of As, Cd, Hg, Pb, Ni, Cu, and Fe–Zn and Se interactions should be examined in light of worldwide TE exposure [84] and global deficiency of essential trace elements, primarily due to dietary considerations [85,86].

5. Conclusion

Our findings revealed that patients with COVID-19 smokers and non-smokers had higher concentrations of toxic Hg, and low concentrations of essential Se than those from healthy smokers and non-smokers, which might contribute to the recurrence of COVID-19 and other lung-related diseases. Here, we provide some recommendations for future research to determine the role and effect of underlying trace elements in patients with COVID-19.

CRediT authorship contribution statement

GQC, HIA, and AU: Formal analysis and revised the manuscript. FNT, HBK, NL, AG and GQM, collected the samples. GQC, HIA, FNT, ARC: Experiments. AU, HIA, GQC: wrote the original draft. AG, NU, and FNT, NL and ARC, RN modified the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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