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ORIGINAL RESEARCH

Serum levels of trace minerals and heavy metals in severe COPD patients with and without pulmonary hypertension

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Aim: The aim of the current study was to assess the serum levels of trace minerals/heavy metals in COPD patients with and without pulmonary hypertension (PH) and to investigate their correlations to demographic, clinical, and biochemical variables.

Materials and methods: This cross-sectional study was performed in Van Yuzuncu Yil University Medical Faculty between April 2013 and July 2013. Cases were allocated into three groups: Group 1 consisted of severe COPD patients; Group 2 was made up of COPD patients with PH; and healthy controls constituted Group 3. Demographic, radiological, and biochemical variables, as well as the serum levels of trace minerals and heavy metals, were noted and compared in these three groups.

Results: COPD patients were older and had higher rates of smoking habit, diabetes mellitus, and hypertension compared to the control group. Carotid intima-media thickness was increased bilaterally, and serum levels of Co, Cu, and Fe were higher in COPD patients. Left carotid intima-media thickness was increased, and serum levels of Cd, Co, and Fe were found to be higher in COPD cases with PH compared to COPD patients without PH.

Conclusion: Our results show that serum levels of trace minerals and heavy metals may be altered in COPD and PH.

Keywords: pulmonary hypertension, chronic obstructive pulmonary disease

Introduction

COPD is mainly characterized by airflow limitation. The pathophysiology has been linked with chronic inflammation of the airways.[1] Oxygen availability is crucial for maintenance of cellular function, and decreased availability of oxygen in COPD alters the entire metabolism of trace minerals. Trace minerals play an important role in the metabolism, starting from the lowest level of intracellular life extending to the functional activity of the largest organs.[2,3] Pulmonary hypertension (PH) is characterized by the intimal and medial proliferation of the small pulmonary arteries, which in turn leads to the development of secondary fibrotic and plexiform vascular lesions. Increased pulmonary vascular resistance and pulmonary arterial pressures in PH constitute a life-threatening situation owing to the increase in afterload leading to right ventricular failure.[4] COPD may constitute an important risk factor for cardiovascular morbidities, including systemic hypertension/PH and ischemic heart disease.[4]

COPD and atherosclerosis may occur due to similar risk factors and have a significant cause of morbidity and mortality. Persistent low-grade systemic inflammation in COPD and atherosclerotic disease has been reported as a possible factor in both pathologies. Carotid intima-media thickness (CIMT), which shows an inverse

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proportion with the percentage forced expiratory volume in 1 second (FEV_1%), is a noninvasive method that can be used in determining the risk of COPD.[5]

Immunological and inflammatory changes may influence the distribution of trace minerals in the body.[6–8] Many trace elements play important roles in activating or inhibiting enzymatic reactions, by competing with other elements and metalloproteins for the binding sites, by affecting the permeability of cell membranes, and by other mechanisms. They play important roles in the oxidant/antioxidant balance. As such, trace elements are thought to be involved directly or indirectly in the pathogenesis of several diseases.[6] There are only limited data on the serum levels of trace minerals and heavy metals in COPD patients, and only elderly (>70 years old) COPD patients or those under mechanical ventilation have been included in previous studies.[9,10] To the best of our knowledge, such an extensive study on both trace minerals and heavy metals involving a comparison between COPD patients with and without PH has not been carried out yet in the medical literature. The aim of this study was to assess the serum levels of trace minerals, heavy metals, as well as inflammatory and biochemical markers, in COPD patients with and without PH and in controls. Moreover, we investigated whether there is a correlation between serum levels of trace minerals/heavy metals and the demographic, biochemical, and cardiovascular indexes.

Materials and methods

Study design

This study has been performed after the approval of Van Yuzuncu Yil University Institutional Review Board and in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki. All participants gave written and informed consent.

Patients with severe COPD admitted to the Department of Chest Diseases of the Faculty of Medicine, Van Yuzuncu Yil University, between April 2013 and July 2013 constituted the study group. The study group consisted of patients who neither had an attack nor underwent hospitalization over the past 6 months. COPD patients included in the study were divided into two groups with respect to occurrence of PH.

The control group consisted of healthy subjects. The inclusion criteria for the control group were healthy status and normal lung function tests. The controls were not allowed to have any pulmonary or nonpulmonary disease that could influence the study, complaints of chronic cough and/or dyspnea, or atopic complaints.

None of the participants had been on any medications regularly, nor were any of them using any drugs containing trace minerals or vitamins. Subjects with occupational exposure to heavy metals were also excluded from this study.

Serum samples were obtained from the patients before any therapeutic intervention was carried out. Complete blood count assessments, as well as measurements of serum levels of C-reactive protein (CRP), D-dimer, blood lipids, Cd, Co, Cu, Fe, Mg, Pb, and Zn, were made. Echocardiography and carotid ultrasonography were performed in all cases.

Collection of blood samples

Fasting blood samples (5 mL) were taken in Vacutainers containing heparin as anticoagulant, and these venous samples were immediately stored at 4°C. In order to separate the serum samples from the blood cells, centrifugation was performed at 3,000 rpm for 10 minutes. These samples, which were prepared for measurement of trace element levels and heavy metals, were maintained at −80°C until further processing.

Serum levels of trace elements and heavy metals

Serum levels of trace minerals (Cu, Fe, Mg, Mn, and Zn) and heavy metals (Cd, Co, and Pb) were measured by atomic absorption spectrophotometry. A UNICAM-929 spectrophotometer (Unicam Ltd, York Street, Cambridge, UK) was used for this purpose. Values were expressed in micrograms per deciliter (μg/dL).

Evaluation of CIMT

Intima-media thickness (IMT) of the common carotid artery (CCA) was measured using ultrasonography by a single radiologist, who was blinded to the patient data and who had a 5-year experience in these radiological evaluations. All patients were evaluated by high-resolution ultrasonography (Aplio Ultrasound System; Toshiba Medical Systems, Tokyo, Japan), with a sectorial probe of 7.5 MHz with axial and lateral resolution of 0.15 mm. Patients were assessed in the supine position. IMT measurements were carried out at the level of the proximal part of the bulbus anteriorly on both sides for CCA, with the transducer head placed perpendicular to the vessels.

Assessment of ejection fraction (EF)

Transthoracic echocardiography was performed on all patients, and Philips HD 11 XE echocardiography device (Philips Medical Systems, Bothell, WA, USA) was used

for this purpose. Diameters of the cardiac chambers were measured by M-mode ultrasonography. EF was calculated by the modified Simpson method.

Cardiac catheterization

A 7F balloon-tipped pulmonary artery catheter (Edwards Lifesciences, Irvina, CA, USA) was passed through an introducer sheath located in the right internal jugular or a brachial vein for measurement of the right atrial pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure (PCWP). Cardiac output was measured using thermodilution, with measurements taken in triplicate. Pulmonary vascular resistance (PVR) was calculated as (mean pulmonary artery pressure – mean PCWP/cardiac output). Measurements were indexed to body surface area, as appropriate. PH was diagnosed by right heart catheterization and defined as "mean pulmonary artery pressure >25 mmHg and PCWP ≤15 mmHg", in accordance with PH guidelines.[4]

Statistical analysis

SPSS software version 21.0 for Windows was used for statistical analysis. The normal distribution of the quantitative data was tested via Kolmogorov–Smirnov test. Quantitative variables were expressed as either mean ± SD or median–interquartile range. A 95% CI was accepted, and level of statistical significance was determined as a p-value <0.05. Independent-samples t-test and Mann–Whitney U-test were used to compare independent groups. The comparison of quantitative variables in >2 groups was performed using analysis of variance (ANOVA) test. Correlation of variables was made by Pearson correlation and Spearman's rho tests. Categorical variables were compared with Pearson chi-square and Fisher's exact tests.

Results

The whole study group consisted of 49 females (51%) and 47 males (49%), allocated into three groups. The number of cases in Group 1 (COPD patients), Group 2 (COPD patients with PH), and Group 3 (healthy controls) was 38, 22, and 36, respectively. A comparison of COPD patients and controls with respect to descriptive, radiological, and biochemical variables is displayed in Table 1. It turned out that COPD patients were older and had higher rates of smoking habit and diabetes mellitus. Comparison of COPD patients and controls revealed that CIMT was thicker bilaterally in COPD patients.

Regarding trace minerals, serum levels of Co, Cu, and Fe were higher in the COPD group. Left CIMT was increased in COPD cases with PH, and serum levels of Cd, Co, and Fe also were found to be higher in this group. Comparison of COPD patients and controls in terms of diabetes mellitus, hypertension, and history of smoking is displayed in Table 2.

Correlation of demographic, biochemical, and radiological variables with serum levels of trace minerals and heavy metals is shown in Table 3.

Discussion

In this study, we attempted to demonstrate whether serum levels of trace minerals (Cu, Fe, Mg, Mn, Zn, and Co) and heavy metals (Pb and Cd) are altered in severe COPD patients with and without PH. We compared COPD cases (with and without PH) to healthy controls in terms of demographic, biochemical, and radiological variables. We observed that CIMT was bilaterally thicker in COPD patients and serum levels of Co, Cu, and Fe were increased significantly in the COPD group. Further comparison of COPD patients with and without PH showed that left CIMT was increased, and the serum levels of Cd, Co, and Fe were found to be higher in COPD cases with PH.

Insufficient oxygen in chronic hypoxia (such as in COPD) brings about a shortage of the main electron acceptor in the respiratory chain and causes decreased ATP.[9] This shortage has adverse effects on vital functions, including many enzymatic systems that are activated by minerals or trace elements.[10] Many trace elements play important roles in the activation or inhibition of enzymatic reactions or the oxidant/antioxidant balance.[3] Hence, trace elements may be involved either directly or indirectly in the pathogenesis of several diseases, including COPD.[11] It has been shown that trace mineral supplementation may aid in the reduction of the period spent on mechanical ventilation.[11]

From another point of view, eating problems may be frequently seen in COPD patients, and the dietary intake of micronutrients might be less than the recommended dose.[12] Vitamin D, Ca, and folate intakes are usually low in older COPD patients, and support prophylaxis can be useful in these patients.[13]

We did not come across any difference between the COPD and control groups in terms of levels of hemoglobin, CRP, D-dimer, and blood lipids. These substances are rather unlikely to serve as markers for the inflammatory process in our COPD series.

Cu, Zn, and Mn have protective effects against the increased free reactive oxygen species via Cu-Zn superoxide dismutases and Mn superoxide dismutase.[7,14,15] Cu and Fe are involved in many aspects of energy metabolism and

Table 1 Comparison among COPD patients (with and without PH) and controls in terms of radiological and biochemical variables

Variables	Group 1 (n=38) (COPD)	Group 2 (n=22) (COPD with PH)	Group 3 (n=36) (control)	*p*-value
Age (years)	60.6±8.66	59.6±8.17	46.9±10.23	*p*1vs2=0.65; *p*1vs3=0.04; *p*2vs3=0.03
Female/male	18/20	9/13	19/17	*p*1vs2=0.24; *p*1vs3=0.26; *p*2vs3=0.27
CIMT**				
Right	0.59±0.22	0.81±0.27	0.41±0.18	*p*1vs2=0.66; *p*1vs3=0.027; *p*2vs3=0.011
Left	0.71±0.25	0.79±0.19	0.39±0.31	*p*1vs2=0.005; *p*1vs3=0.028; *p*2vs3=0.017
EF**	59.8±4.7	61.1±3.8	62.5±7.5	*p*1vs2=0.82; *p*1vs3=0.82; *p*2vs3=0.81
O_2 saturation (%)	85.5±7.8	86.5±8.2	97.5±6.6	*p*1vs2=0.08; *p*1vs3=0.03; *p*2vs3=0.04
Hemoglobin (g/dL)*	15.0±2.2	15.8±2.2	14.8±1.6	*p*1vs2=0.22; *p*1vs3=0.27; *p*2vs3=0.26
Hematocrit (mg/dL)*	45.9±7.0	48.2±6.7	44.7±5.0	*p*1vs2=0.23; *p*1vs3=0.13; *p*2vs3=0.18
Triglycerides (mg/dL)**	123.5±85.6	116.1±74.2	88.5±68.4	*p*1vs2=0.51; *p*1vs3=0.03; *p*2vs3=0.04
HDL (mg/dL)**	36.5±38.8	41.1±32.4	46.0±39.2	*p*1vs2=0.09; *p*1vs3=0.08; *p*2vs3=0.06
LDL (mg/dL)	104.7±37.6	93.2±32.4	109.9±32.9	*p*1vs2=0.06; *p*1vs3=0.07; *p*2vs3=0.05
Cholesterol (mg/dL)*	171.0±52.0	155.0±32.7	178.0±41.2	*p*1vs2=0.20; *p*1vs3=0.22; *p*2vs3=0.18
CRP (mg/dL)**	0.89±2.61	1.09±2.14	0.42±2.54	*p*1vs2=0.42; *p*1vs3=0.22; *p*2vs3=0.12
D-dimer (μg/dL)**	145.0±111.0	162.5±204.0	137.00±72.00	*p*1vs2=0.76; *p*1vs3=0.54; *p*2vs3=0.34
Cd (μg/dL)**	0.003±0.001	0.02±0.005	0.002±0.001	*p*1vs2=0.001; *p*1vs3=0.12; *p*2vs3=0.001
Co (μg/dL)**	0.255±0.112	0.064±0.085	0.012±0.098	*p*1vs2=0.030; *p*1vs3=0.033; *p*2vs3=0.042
Cu (μg/dL)*	0.6±0.2	0.7±0.2	0.5±0.2	*p*1vs2=0.07; *p*1vs3=0.02; *p*2vs3=0.01
Fe (μg/dL)**	0.4±0.3	0.2±0.4	0.2±0.2	*p*1vs2=0.035; *p*1vs3=0.032; *p*2vs3=0.074
Mg (μg/dL)**	11.7±3.3	11.2±3.1	9.4±4.2	*p*1vs2=0.56; *p*1vs3=0.71; *p*2vs3=0.68
Mn (μg/dL)**	0.01±0.02	0.02±0.01	0.01±0.02	*p*1vs2=0.38; *p*1vs3=1.00; *p*2vs3=0.38
Pb (μg/dL)**	0.1±0.1	0.0±0.1	0.2±0.1	*p*1vs2=0.08; *p*1vs3=0.076; *p*2vs3=0.061
Zn (μg/dL)**	1.2±0.6	0.9±0.6	1.0±0.3	*p*1vs2=0.55; *p*1vs3=0.54; *p*2vs3=0.61

Notes: *Parametric method (variables with normal distribution); **nonparametric method (variables that do not display a normal distribution). Data presented as mean ± SD, unless otherwise stated.

Abbreviations: PH, pulmonary hypertension; CIMT, carotid intima-media thickness; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; EF, ejection fraction.

are important in the synthesis of hemoglobin, myoglobin, and cytochromes. Zn, Cu, and Mn are antioxidants that serve as cofactors for several enzymes that preserve the integrity of DNA.[6] Fe is an important cofactor of oxygen transport, and decreased bioavailability of Fe in the brain can result in alterations of neurotransmitter production and cognitive functions.[15,16] Chronic hypoxia in COPD and PH may cause changes in Fe metabolism.

Table 2 COPD (both with and without PH) patients and controls in terms of diabetes mellitus, hypertension, and history of smoking

Factors	Group I (n=38) (COPD), n (%)	Group 2 (n=22) (COPD with PH), n (%)	Group 3 (n=36) (control), n (%)
Diabetes mellitus			
–	30 (78.9)	21 (95.5)	36 (100)
+	8 (21.1)	1 (4.5)	0
Hypertension			
–	31 (81.6)	21 (95.5)	0
+	7 (18.4)	1 (4.5)	36 (100)
History of smoking			
–	14 (36.8)	7 (31.8)	28 (77.8)
+	24 (63.2)	15 (68.2)	8 (22.2)

Abbreviation: PH, pulmonary hypertension.

Mg functions in protein synthesis and is linked with bone metabolism.[14] Mn is a cofactor of enzymes that detoxify superoxide free radicals.[16] The trace element Co is an integral part of vitamin B_{12}, which is necessary for folate and fatty acid metabolism.[17] Cd and Pb are among the most dangerous occupational and environmental toxins, which amplify oxidative stress and inflammatory process.[17]

Impacts of trace minerals and heavy metals are closely associated with each other. For example, high intakes of Cd, Cu, and Zn can interfere with availability and storage of Fe. Low dietary Fe enhances the absorption of not only dietary Fe but also Cd, Co, Mn, Pb, and Zn.[6,17,18] Zn supplements have been shown to cause anemia secondary to decreased absorption of Cu. Moreover, not everything is known about the role of drugs and coexisting illnesses on the development of trace mineral deficiency.[17,18]

In our study, serum levels of many trace minerals seem to be correlated with each other, indicating a complex cascade of interactions between these substances. Therefore, disturbance in the metabolism of one mineral may affect the others, and a pathophysiological process may be elicited. Disturbance of trace mineral metabolism, attributable to inflammation and reactive oxygen species, can induce alterations in absorption and circulating levels of trace minerals.[6,8] Increased intestinal absorption or release during tissue damage may also contribute to the increased serum levels of trace minerals.[14,18] If the balance is disturbed, excessive trace minerals may induce oxidative stress, which could in turn lead to a vicious circle that reinforces chronic inflammation. Elimination of heavy metals may not be achieved due to oxidative stress and inflammation, and this may explain the high levels of Cd in the setting of COPD and PH. The predictive capacity of serum levels of heavy metals as markers of disease status and oxidative stress needs to be elucidated.

Table 3 Correlation of demographic, biochemical, and radiological variables with serum levels of trace minerals and heavy metals in our series

Variables	Correlate	*R*	*p*-value
Age (years)	EF	–0.301	0.019
	O_2 saturation	–0.286	0.027
	Pb	–0.336	0.011
Right CIMT (mm)	L-CIMT	0.639	<0.001
	Smoking	0.454	0.004
Left CIMT (mm)	D-dimer	0.291	0.025
	Smoking	0.453	0.004
Hemoglobin (g/dL)	Hematocrit	0.94	<0.001
Hematocrit (mg/dL)	Mg	–0.266	0.04
LDL (mg/dL)	Triglycerides	0.345	0.007
	Cholesterol	0.758	<0.001
	Cu	–0.267	0.039
Triglycerides (mg/dL)	Cholesterol	0.529	<0.001
	O_2 saturation	0.257	0.047
Cholesterol (mg/dL)	EF	0.274	0.034
CRP (mg/dL)	EF	–0.427	0.063
	O_2 saturation	–0.289	0.025
EF (%)	O_2 saturation	0.290	0.025
O_2 saturation (%)	Mn	–0.272	0.049
Co (µg/dL)	Fe	0.426	0.001
	Mg	0.283	0.029
	Mn	0.524	<0.001
	Pb	0.820	<0.001
	Zn	0.343	<0.001
Cu (µg/dL)	Mg	0.580	<0.001
	Zn	0.343	0.007
Fe (µg/dL)	Mg	0.277	0.040
	Mn	0.445	0.001
	Pb	0.485	<0.001
	Zn	0.449	0.001
Mg (µg/dL)	Mn	0.484	<0.001
	Pb	0.427	0.001
	Zn	0.630	<0.001
Mn (µg/dL)	Pb	0.641	<0.001
	Zn	0.557	<0.001
Pb (µg/dL)	Zn	0.616	<0.001

Abbreviations: EF, ejection fraction; CIMT, carotid intima-media thickness; L-CIMT, Left CIMT; LDL, low-density lipoprotein; CRP, C-reactive protein.

We observed that CIMT was increased in COPD patients, and we suggest that carotid ultrasonography can serve as a safe and practical diagnostic tool for early detection of cardiovascular morbidities accompanying COPD. In terms of heavy metals, serum Cd levels were found to be increased in COPD patients with PH. History of environmental and occupational exposure to heavy metals must be carefully and routinely questioned.

Potential of serum trace minerals to be used as markers of metabolic state and cardiovascular morbidity in COPD needs

to be elucidated. Unveiling the complicated relationship between clinical and biochemical variables is a challenge. The contribution of the unique nature of the metabolic and inflammatory profiles of every patient is another aspect that must be kept in mind.

The main limitation of the current study is the relatively small sample size of the study group. Secondly, it must also be remembered that peripheral levels of trace elements and heavy metals may not always indicate the tissue levels of the same. Finally, there was a significant difference between groups with respect to the age, presence of comorbidities, and the history of smoking, and this might have affected the results. The age of control individuals was considerably lower than that of COPD patients, which may explain the higher values observed for some of the minerals. Therefore, the latter can alternatively be related to the duration and intensity of their potential environmental (contamination), toxic (eg, tobacco), or food–water exposures. To alert the patients about such exposures is not sufficient to discard them since they can be generalized in a given ecosystem.

Conclusion

Our results showed that serum levels of trace minerals and heavy metals may be altered in patients with COPD and PH. Chronic hypoxia, as a trigger of serum abnormalities, would require another design, including COPD patients without hypoxemia or patients with other causes of this gas exchange abnormality.

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Disclosure

The authors report no conflicts of interest in this work.

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