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Assessment of 7 trace elements in serum of patients with nontuberculous mycobacterial lung disease

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ABSTRACT

Nontuberculous mycobacterial (NTM) lung diseases are an emerging cause of pulmonary infection, becoming more common in the clinical setting as incidence of NTM lung diseases steadily increases worldwide. Trace elements are essential micronutrients and are known to play many important roles in infectious diseases. We investigated the concentrations of trace elements in patients with NTM lung disease and compared these values to patients with pulmonary tuberculosis and healthy controls. A case-control study was conducted to evaluate the serum trace element concentrations in 95 patients with NTM lung disease, 97 patients with pulmonary tuberculosis, and 99 healthy control subjects. The serum concentrations of 7 trace elements (cobalt, copper, chromium, manganese, molybdenum, selenium, and zinc) were measured using inductively coupled plasma-mass spectrometry. We also analyzed demographic data, clinical outcomes, and other biochemical parameters. The median serum concentrations of copper and molybdenum were higher in patients with NTM lung disease (109 vs. 91 μg/dL, $p < 0.001$ and 1.70 vs. 0.96 μg/L, $p < 0.001$). In contrast, the median serum concentrations of selenium and zinc were significantly lower in patients with NTM lung disease than in healthy controls (105 vs. 115 μg/L, $p < 0.001$ and 94 vs. 102 μg/dL, $p < 0.001$). Compared to patients with pulmonary tuberculosis, the serum concentrations of molybdenum and zinc were higher in patients with NTM lung disease, while cobalt and copper concentrations were lower ($p < 0.001$). Correlations among trace element concentrations were observed (copper and zinc, $r = -0.367$; cobalt and molybdenum, $r = -0.360$; selenium and zinc, $r = 0.335$; and manganese and zinc, $r = 0.327$, respectively). None of the 7 trace elements were associated with treatment outcomes. Patients with NTM lung disease showed different serum trace element concentrations. Our study indicates that altered trace element status is associated with mycobacterial disease. Further study investigating the clinical significance of individual trace elements and their association with nutritional status in patients with NTM lung disease would be required.

1. Introduction

Nontuberculous mycobacteria (NTM) are a diverse group of mycobacterial species excluding *Mycobacterium tuberculosis* complex and *M. leprae* that are widespread in the environment and readily isolated from soil and water [1]. Over 170 different species of NTM have been identified to date, and the most common clinical manifestation of NTM disease is lung disease [2,3]. The incidence of NTM diseases has steadily increased worldwide since the 1950s, and NTM lung diseases are an emerging cause of pulmonary infection becoming more common in clinical settings [1,4]. The incidence and prevalence of NTM lung disease are increasing also in South Korea [5,6].

Nutritional status is considered one of the most important determinants of resistance to infection and of general well-being.

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Malnutrition is associated with impaired immune function [7–9]. Unlike tuberculosis (TB), NTM are ubiquitous in the environment, and therefore isolation of these organisms does not necessarily equate with disease [3,10]. However, if the host is an immunosuppressed or nutritional deficiency, NTM lung disease can occur as a result of NTM infection. Thus, micronutrients related to immunity are thought to play an important role in NTM infection.

Trace elements are essential micronutrients that are known to play several important roles in human health. These elements mediate vital biochemical reactions by acting as cofactors for many enzymes, as well as act as centers for stabilizing the structures of enzymes and proteins [11]. Selenium (Se) has an important function in maintaining immune processes and thus may have a critical role in clearance of mycobacteria; it also plays a role as a co-factor for metalloenzymes such as glutathione peroxidase [12]. Also, Se is a potent nutritional antioxidant that carries out biological effects through its incorporation into selenoproteins [13]. The intake of Se-vitamin E supplements has the potential to decrease reactive oxygen species and increase antioxidant activities in patients with TB [14]. Zinc (Zn) is an essential component of the pathogen-eliminating signal transduction pathways and is involved in modulation of the proinflammatory response. Zn is also involved in controlling oxidative stress and regulating inflammatory cytokines and plays an important function during an immune response [15]. Zn accumulated in the *M. tuberculosis* phagosome is likely to be exploited by macrophages to destroy intracellular pathogens [16,17]. Zn and copper (Cu) also play a role in the catalytic components of numerous enzymes and as structural components of other proteins important for immune reactions [11,18,19]. Although Cu is an essential element, an excessive amount of copper can inhibit the growth of *M. tuberculosis* [20,21]. Manganese (Mn) plays an essential role in many cellular processes including lipid, protein, and carbohydrate metabolism [22]. Since invading microbes utilize Mn to resist the effects of host-mediated oxidative stress, this metal plays an important role in adapting pathogenic bacteria to the human host [23].

Measuring nutritional indicators, such as trace element concentrations, is helpful to assess the nutritional status of patients [18,24]. Although changes in concentrations of trace elements have been reported in patients with pulmonary TB [25–27], there has been no similar study in patients with NTM lung disease. Therefore, we measured the serum concentrations of seven trace elements including cobalt (Co), Cu, Chromium (Cr), Mn, Molybdenum (Mo), Se, and Zn. We investigated for the first time the concentrations of trace elements in patients with NTM lung disease and compared these values to patients with pulmonary TB and healthy controls. In addition, we also assessed their association with clinical outcomes and laboratory variables in patients with NTM lung disease.

2. Materials and methods

2.1. Study populations

Patients with NTM lung disease and TB were recruited consecutively between February 2011 and January 2016 at Samsung Medical Center (Seoul, Korea). All patients with NTM lung disease or pulmonary TB met the American Thoracic Society diagnostic criteria [28,29]. Diagnosis was based on culture positivity from at least two separate expectorated sputum samples and culture positivity on bronchial wash or bronchoalveolar lavage fluids. Patients were bacteriologically confirmed as NTM or TB using the AdvanSure TB/NTM real-time PCR kit (LG Life Science, seoul, Korea) [30]. We used the AdvanSure Mycobacteria GenoBlot assay (LG Life Science, seoul, Korea) to identify NTM species [31]. The exclusion criteria were as follows: (*a*) patients with cancers; (*b*) patients who test positive for human immunodeficiency virus; (*c*) patients with hepatic or renal impairment (total bilirubin > 2.5 mg/dl, AST or ALT > 3 times the upper limits of reference range, alkaline phosphatase > 5 times the upper limits of reference range, serum creatinine > 1.8 mg/dl); (*d*) patients with uncontrolled bleeding disorders; (*e*) patients with life-threatening disease; (f) patients with concurrent NTM infection and pulmonary TB. Thus, 95 patients with NTM lung disease and 97 patients with pulmonary TB were enrolled in this study. 99 healthy subjects who visited a health promotion center for regular health checkups with no prior diagnosis of NTM or TB history and currently without clinical signs of illness were selected as a control group. Demographic and clinical information were obtained through electronic medical records. Because racial and ethnic differences are associated with body mass index (BMI), we have defined a BMI of less than 18.5 kg/m^2 as underweight status in accordance with the WHO guidelines for Asian populations in this study [32].

Treatment outcome of NTM lung disease was assessed at 12 months after the antibiotic treatment initiation. Treatment success was defined as culture conversion with three consecutive negative sputum cultures and maintenance of negative culture status until the end of treatment [33].

This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center, Seoul, Korea (IRB No: SMC 2008-09-016) and followed the Declaration of Helsinki. The subjects provided written consent for their participation in the study.

2.2. Determination of trace elements in serum

Blood samples from patients with NTM were collected during the first visit, before the start of treatment. Serum was separated from whole blood and aliquots were stored at 70℃ until analysis. National Institute of Standards and Technology (NIST)-traceable elemental standards were used for preparation of five point calibration. Serum samples (200 μl) were diluted 10-fold with a solution consisting of 1.5% (w/v) 1-butanol, 0.05% (w/v) EDTA, 0.05% (w/v) triton X-100, and 0.14% (w/v) NH_4OH. The samples and internal standard solution (containing Germanium for Se, Indium for Mo and Mn, Rhodium for Cu and Zn, and Scandium for Cr and Co) were vortex-mixed for 10 s before inductively coupled plasma-mass spectrometry (ICP-MS) analysis. The quality control materials used were ClinChek controls levels 1 and 2 (Recipe Chemicals, Munich, Germany). The blanks, standards, and control materials were prepared in the same manner as the samples. The serum concentrations of seven trace elements were measured with a quadrupole ICP-MS (7900x ICP-MS system, Agilent Technologies, Santa Clara, CA, USA) as previously described [34]. Instrumental parameters and measurement conditions are shown in Supplementary Table 1. Both intra-assay and inter-assay precisions of all assays showed good repeatability, with all the coefficients of variation below 10%. The accuracy was verified using the Proficiency Testing/Quality Management program of the United States College of American Pathologists survey, twice a year.

2.3. Biochemical assessment

C-reactive protein (CRP), total protein, albumin, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured to assess biochemical status. Serum biochemical markers were measured simultaneously with the serum trace element concentrations by a Roche cobas 8000 c702 analyzer (Roche Diagnostics Corp., Indianapolis, IN, USA) as per manufacturer's instructions.

2.4. Statistical analysis

Continuous variables were presented as median and interquartile range (IQR) for variables. The assessment of normality was conducted by the Shapiro-Wilk test. *P* values of less than 0.05 were regarded as statistically significant. We conducted one-way analysis of variance (ANOVA) with Bonferroni's post hoc test to evaluate the significance of differences in trace element status and biochemical results among

patients with NTM lung disease, patients with pulmonary TB, and healthy controls. The analysis of covariance (ANCOVA) was performed to control for potentially confounding variables, such as age and sex. We also conducted subgroup analysis to detect sex differences. Student's *t*-test and the Wilcoxon Mann-Whitney test were used for continuous variables to assess the significance of differences in trace element status and biochemical measurements of patients with NTM lung disease versus patients with pulmonary TB, patients with NTM lung disease versus healthy controls, patients with pulmonary TB versus healthy controls and treatment success group versus treatment failure group. Spearman's correlations were used to assess the relationships between trace element status, demographic data, and biochemical measurements. We performed stepwise multiple regression analysis for assessment of the associations between trace elements and laboratory markers after adjustment for confounders. These analyses have been performed using IBM SPSS software v24.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline study population characteristics

Among the 95 patients with NTM lung disease, thirty-six (37.9%) patients were sputum smear-positive, and 19 (20.0%) patients had a cavitary lesion on their high resolution computed tomography (HRCT) scans. The most frequently isolated NTM species were *M. avium* complex (70.5%), followed by *M. abscessus* complex (15.0%). Mixed infection between *M. avium* complex and *M. abscessus* complex cases were observed in 10 (10.5%) patients. *M. fortuitum, M. kansasii, M. chelonae, M. shinjukuense* were each observed in one case. The baseline characteristics of the patients with NTM lung disease, patients with pulmonary TB, and healthy controls are summarized in Table 1. The age and sex distributions of the three groups were significantly different. Significant differences in BMI were observed between patients with NTM lung disease and both patients with pulmonary TB and healthy controls. ANCOVA adjusted for age and sex also showed a significant difference in BMI between patients with NTM lung disease and healthy controls ($p < 0.05$). The significant difference in BMI observed in patients with NTM disease and patients with pulmonary TB disappears after adjusting for age and sex.

The median concentrations of total protein and CRP were higher in both patients with NTM lung disease and patients with pulmonary TB than in healthy controls ($p < 0.05$). On the other hand, the total cholesterol concentrations were significantly lower in both patients with NTM lung disease and patients with pulmonary TB than in healthy controls ($p < 0.05$). There was no significant difference in AST and ALT concentrations among the three groups.

3.2. Trace element concentrations

The serum trace element concentrations are summarized in Table 2 and Fig. 1. The median serum concentrations of Cu and Mo were higher in patients with NTM lung disease than in healthy controls ($p < 0.05$). Cu levels were 20% higher and Mo levels were 77% higher in patients with NTM lung disease compared to healthy controls. In contrast, the median serum concentrations of Se and Zn were significantly lower in patients with NTM lung disease than in healthy controls ($p < 0.05$). Se levels were 9% lower and Zn levels were 8% lower in patients with NTM lung disease compared to healthy controls. In the subgroup analysis matched by sex, the results were the same as the overall results.

Compared to patients with pulmonary TB, the serum concentrations of Mo, and Zn were higher in patients with NTM lung disease, while Co and Cu concentrations were lower ($p < 0.05$). The median serum Cr and Mn concentrations were not significantly different among the three groups.

3.3. Factors associated with trace element concentrations of the study population

Positive correlations between some of the trace elements (Se and Zn, $r = 0.335$, $p < 0.05$; Mn and Zn, $r = 0.327$, $p < 0.05$ and Co and Cu, $r = 0.262$, $p < 0.05$) were observed. Negative correlations between Cu and Zn ($r = -0.367$, $p < 0.05$); Co and Mo, $r = -0.360$, $p < 0.05$ and Mo and Se ($r = -0.243$, $p < 0.05$) were observed. The correlations between the concentrations of trace elements and demographic data or other biochemical measurements are shown in Table 3 and Supplementary Fig. S1. Cu showed positive correlations with total protein, CRP, and ALP concentrations. Albumin and total cholesterol showed positive correlations with Se and Zn. Cu and CRP showed the strongest correlation in the study population. As a result of multiple regression analysis entering variables by a stepwise method, Cu showed correlations with CRP ($\beta = 0.413$, $p < 0.05$), ALP ($\beta = 0.173$, $p < 0.05$),

Table 1
Baseline characteristics of study population.

	Patients with NTM (n = 95)	Patients with TB (n = 97)	Healthy controls (n = 99)	p-value[a]	p-value[b] (NTM vs. Controls)	p-value[b] (NTM vs. TB)	p-value[b] (TB vs. Controls)
Demographic characteristics							
Age, years, median (IQR)	58 (53–67)	45 (36–58)	54 (41–65)	< 0.001	< 0.001	< 0.001	0.003
Female, number (%)	76 (80)	37 (38)	49 (49)	< 0.001	< 0.001	< 0.001	0.110
BMI, kg/m^2	20.9 (19.1–22.3)	22.0 (20.0–24.3)	22.7 (20.5–24.8)	< 0.001	< 0.001	0.001	0.085
BMI $< 18.5\,kg/m^2$, number (%)	12 (12.6)	9 (9.3)	6 (6.1)				
BMI $\geq 18.5\,kg/m^2$, number (%)	83 (87.4)	88 (90.7)	93 (93.9)				
Serum chemistry results, median (IQR)							
Total protein (g/dL)	7.4 (7.0–7.7)	7.5 (7.1–7.8)	7.1 (6.8–7.4)	< 0.001	< 0.001	0.356	< 0.001
Albumin (g/dL)	4.5 (4.3–4.6)	4.4 (4.3–4.6)	4.5 (4.3–4.7)	0.162	0.192	0.742	0.132
Albumin/globulin ratio	1.5 (1.4–1.7)	1.5 (1.3–1.7)	1.7 (1.6–1.9)	< 0.001	< 0.001	0.602	< 0.001
CRP (mg/dL)	0.07 (0.04–0.23)	0.15 (0.04–0.53)	0.03 (0.03–0.07)	0.001	< 0.001	0.078	< 0.001
Total cholesterol (mg/dL)	178 (162–207)	170 (147–192)	197 (177–216)	< 0.001	0.009	0.002	< 0.001
AST (U/L)	21 (18–25)	22 (18–26)	22 (18–27)	0.174	0.331	0.134	0.655
ALT (U/L)	15 (11–22)	16 (13–24)	19 (14–25)	0.084	< 0.001	0.107	0.031
ALP (U/L)	63 (52–74)	72 (58–84)	59 (51–67)	< 0.001	0.033	0.021	< 0.001

NTM: nontuberculous mycobacteria; TB: tuberculosis; IQR: interquartile range; BMI: body mass index; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.
Results are presented as medians (interquartile range).
[a] p-values from the ANOVA test.
[b] p-values from the Wilcoxon Mann-Whitney test.

Table 2
Serum concentrations of trace elements in the study population.

	Patients with NTM (n = 95)	Patients with TB (n = 97)	Healthy controls (n = 99)	p-value[a]	p-value[b] (NTM vs. Controls)	p-value[b] (NTM vs. TB)	p-value[b] (TB vs. Controls)
Cobalt (μg/L)	0.24 (0.20–0.35)	0.54 (0.22–0.83)	0.23 (0.19–0.27)	< 0.001	0.006	< 0.001	< 0.001
Copper (μg/dL)	109 (97–134)	129 (111–153)	91 (82–102)	< 0.001	< 0.001	< 0.001	< 0.001
Chromium (μg/L)	0.23 (0.19–0.27)	0.23 (0.18–0.27)	0.23 (0.19–0.28)	0.404	0.677	0.314	0.572
Manganese (μg/L)	0.90 (0.81–1.07)	0.93 (0.71–1.31)	0.92 (0.80–1.58)	0.002	0.069	0.516	0.096
Molybdenum (μg/L)	1.70 (1.23–2.30)	0.67 (0.23–1.22)	0.96 (0.80–1.23)	< 0.001	< 0.001	< 0.001	< 0.001
Selenium (μg/L)	105 (95–116)	108 (99–119)	115 (105–123)	0.003	< 0.001	0.193	0.001
Zinc (μg/dL)	94 (84–107)	84 (75–93)	102 (92–116)	< 0.001	< 0.001	< 0.001	< 0.001

NTM: nontuberculous mycobacteria; TB: tuberculosis.
Results are presented as medians (interquartile range).
[a] p-values from the ANOVA test.
[b] p-values from the Wilcoxon Mann-Whitney test.

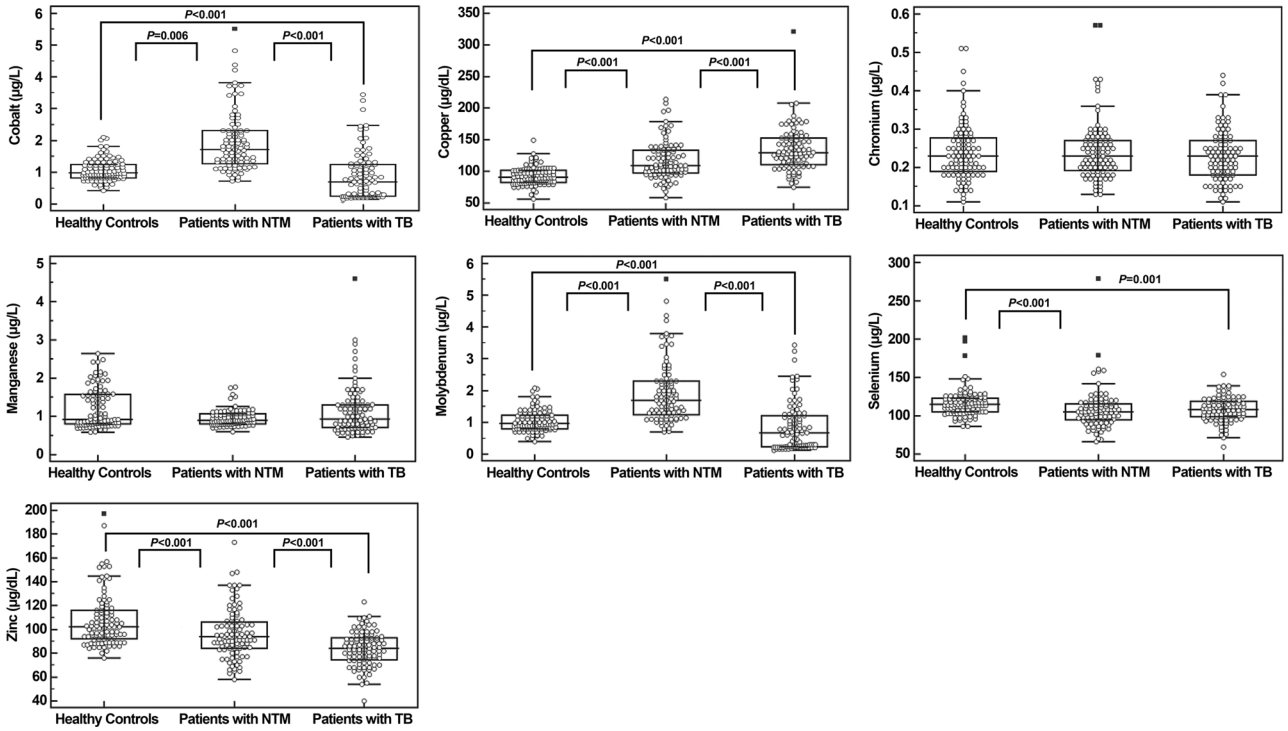

Fig. 1. Concentrations of trace elements among healthy controls, patients with nontuberculous mycobacterial (NTM) lung disease, and patients with pulmonary tuberculosis (TB).

total protein (β = 0.306, p < 0.05), and albumin (β =-0.193, p < 0.05). Se showed correlations with albumin (β = 0.241, p < 0.05), ALP (β =-0.143, p < 0.05), total cholesterol (β = 0.125, p < 0.05), and ALT (β = 0.122, p < 0.05). Zn showed correlations with CRP (β =-0.235, p < 0.05), and total cholesterol (β = 0.184, p < 0.05).

3.4. Associations between trace element concentrations and treatment outcome of NTM lung disease

Forty-nine patients with NTM lung disease did not receive antibiotic treatment and 46 patients underwent antibiotic treatment and regular sputum culture tests. Among 46 patients, 17 (37%) failed to achieve

Table 3
Correlations between trace element concentrations, basal characteristics, and other biochemical measurements of the study population.

	Age	BMI	Total protein	Albumin	CRP	Total cholesterol	AST	ALT	ALP
Cobalt	−0.241[a]	−0.121[a]	0.143[a]	0.024	0.011	−0.081	−0.012	−0.063	0.004
Copper	0.017	−0.217[a]	0.400[a]	−0.130[a]	0.598[a]	−0.179[a]	−0.081	−0.284[a]	0.413[a]
Chromium	0.147[a]	−0.014	0.038	−0.018	0.001	0.043	−0.009	−0.002	−0.004
Manganese	0.106	0.051	−0.025	−0.050	−0.107	0.099	0.135[a]	0.179[a]	−0.015
Molybdenum	0.461[a]	−0.143[a]	−0.029	−0.141[a]	0.045	0.001	0.018	−0.091	0.092
Selenium	−0.106	0.200[a]	0.059	0.317[a]	−0.160[a]	0.268[a]	0.067	0.211[a]	−0.194[a]
Zinc	0.085	0.091	−0.033	0.244[a]	−0.295[a]	0.260[a]	0.110	0.209[a]	−0.183[a]

BMI: body mass index; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.
Results are presented as Spearman's correlation coefficients.
[a] p-value < 0.05.

Table 4
Trace element concentrations and treatment outcomes in NTM patients after 12 months.

	Success (N = 29)	Failure (N = 17)	p-value
Demographic characteristics			
Age, years, median (IQR)	57 (52–69)	58 (55–67)	0.523
BMI, kg/m^2	21.3 (19.2–22.4)	19.5 (17.5–21.4)	0.090
Serum chemistry results, median (IQR)			
Total protein (g/dL)	7.4 (7.0–7.7)	7.4 (7.1–7.8)	0.567
Albumin (g/dL)	4.5 (4.2–4.6)	4.3 (4.2–4.6)	0.739
CRP (mg/dL)	0.09 (0.05–0.24)	0.13 (0.05–1.60)	0.268
Total cholesterol (mg/dL)	175 (159–219)	177 (169–203)	0.633
AST (U/L)	23 (18–24)	18 (15–24)	0.163
ALT (U/L)	14 (12–20)	11 (8–21)	0.045
ALP (U/L)	66 (48–92)	69 56–80)	0.829
ESR (mm)	28 (12–44)	45 (25–88)	0.110
Trace elements concentrations, median (IQR)			
Cobalt (μg/L)	0.24 (0.22–0.44)	0.24(0.19–0.29)	0.316
Copper (μg/dL)	116 (100–142)	135 (120–175)	0.109
Chromium (μg/L)	0.25 (0.20–0.26)	0.22 (0.17–0.29)	0.665
Manganese (μg/L)	0.91 (0.83–1.04)	0.85 (0.76–1.09)	0.524
Molybdenum (μg/L)	1.77 (1.11–2.75)	1.75 (1.35–2.17)	0.964
Selenium (μg/L)	105 (95–117)	100 (89–109)	0.168
Zinc (μg/dL)	96 (82–107)	95 (72–118)	0.425

IQR: interquartile range; BMI: body mass index; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; ESR: erythrocyte sedimentation rate.
Results are presented as medians (interquartile range) with p-values from the Wilcoxon Mann-Whitney test.

sputum culture conversion by 12 months of treatment. However, none of the trace elements, demographic variables, or biochemical indicators showed significant differences between treatment success group and treatment failure group, as shown in Table 4.

4. Discussion

There have been no previous studies on NTM and nutritional indicators, and this is the first comprehensive comparative analysis of the statuses of trace elements in patients with NTM lung disease compared with both healthy controls and patients with pulmonary TB. We also investigated potential associations between clinical data, laboratory results, and trace element status in patients with NTM lung disease.

Trace element deficiencies and infectious diseases often coexist and exhibit complex interactions [9,35]. In this study, serum Se and Zn concentrations were significantly lowers in patients with NTM lung disease than in healthy controls. Se and Zn are the most widely studied trace elements in the context of infection including TB. In a previous study, Zn was found at reduced concentrations at localized sites of infection [22]. Although there were no previous studies measuring trace elements in NTM patients, lower concentrations of Se and Zn have been reported in patients with TB than in controls in previous studies [25]. Se and Zn deficiency could make individuals vulnerable to oxidative stress and consequently increase susceptibility to NTM infection. Serum Cu and Mo concentrations were significantly higher in patients with NTM lung disease than in controls. The association of elevated serum Cu with elevated CRP may reflect a nonspecific increase in the serum concentration of the Cu-binding protein ceruloplasmin during the acute-phase response to infection [26,36]. Unlike other trace elements, there is little information regarding the effects of Mo on immune development and function. However, Mo is an essential microelement for organismal survival, and there are several Mo enzymes in mycobacteria that exert several important physiological functions, such as dormancy regulation, metabolism of energy sources, and nitrogen source [37]. Serum Mn and Cr concentrations were not significantly different between patients with NTM lung disease and healthy controls. During bacterial infection, Mn concentrations in the liver and kidney are decreased, suggesting the existence of additional mechanisms that starve microbes during inflammation [38]. Repression of Mn uptake is essential for survival of *M. tuberculosis* [39]. In contrast, Mn uptake was found to be indispensable for replication of *M. tuberculosis* in macrophages in a previous study [39]. On the other hand, our knowledge about the role of Cr in NTM is incomplete and there has been little study about the mechanisms of Cr and their relationships with mycobacteria. Future study is required to evaluate the roles of trace elements and its clinical implications in NTM infection.

In this study, there were more elderly patients and women in the group with NTM lung disease than in the control groups, which is consistent with previous studies showing that older women appear to be more susceptible to NTM lung disease [10,40–43]. Since it was impossible to control the nature of the NTM lung disease that is more common in women, we conducted further statistical analyses for adjusting age or sex and there was no significant difference in the results of statistical analysis. BMI of patients with NTM lung disease was lower than that of healthy controls, consistent with previous studies [42,44–46]. The hypothesis that abnormal expression of adipokines, sex hormones, and/or TGF-β is responsible for increased susceptibility to NTM lung disease in slender, older women was proposed in a previous study [42].

Total cholesterol was lower in patients with NTM lung disease, in agreement with previous study [46]. The cause of the low total cholesterol concentrations is most likely multifactorial, involving both decreased synthesis and enhanced catabolism [47]. In correlation analysis between trace elements and nutritional status-associated parameters, CRP concentration showed the strongest correlation with Cu concentration, and a negative correlation with Zn concentration. Our results are consistent with previous studies reporting that changes in CRP concentration are associated with decreased serum Zn concentrations and increased serum Cu concentrations [26,36,48–52].

Serum Se and Zn concentrations were significantly lower in patients with TB than in controls, while the Co and Cu concentrations were higher. Our results are consistent with previous studies reporting altered concentrations of trace elements in patients with pulmonary TB compared with controls [7,25–27,53–55]. Trace element concentrations in patients with NTM lung disease were intermediate between healthy controls and patients with pulmonary TB, as shown in Fig. 1. Previous studies have shown that the nutritional status of patients is related to the severity of various diseases [56–58]. The association between poor nutrition and disease severity could be due to several factors, such as impaired immune response. NTM are generally considered less virulent than *M. tuberculosis*, and these results were probably due to the difference of virulence between NTM and *M. tuberculosis* [59].

Although higher serum Se and Zn and lower Cu concentrations were found in the treatment success group, analysis of trace elements and treatment outcome did not yield significant results. Future studies with more treatment outcome groups are needed to clarify this issue.

This study did have some limitations. Since older and more slender women are more susceptible to NTM infection, there were different baseline characteristics among patients with NTM lung disease, patients with pulmonary TB, and healthy controls. Although we adjusted the data for age or sex, these characteristics of patients should also be considered. This study was a case-control study at a single referral medical center and may not represent the epidemiology in South Korea as a whole. In addition, little data were available on dietary supplementation of trace elements. The concentrations of serum trace elements measured at a single point in time may not reflect long-term trace element status. To clarify the significance of trace elements in NTM lung disease, future prospective researches with a large general population or a patient cohort addressing these problems are needed.

In summary, we compared the statuses of multiple trace elements in patients with NTM lung disease versus healthy controls and patients with pulmonary TB for the first time, and as a result, the concentrations

of trace elements showed in patients with lung disease were different. Our study indicates that altered trace element status is associated with mycobacterial disease. Therefore, further study investigating the clinical significance of individual trace elements and their association with nutritional status in patients with NTM lung disease would be required.

Conflicts of interest

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Appendix A. Supplementary data

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