

Role of certain trace minerals in oxidative stress, inflammation, CD4/CD8 lymphocyte ratios and lung function in asthmatic patients

Chih-Hung Guo¹, Po-Jen Liu^{1,2}, Simon Hsia¹, Chia-Ju Chuang¹ and Pei-Chung Chen¹

¹Institute of Biomedical Nutrition, Hung Kuang University, Taichung 433; ²Department of Otolaryngology, Cheng-Ching Hospital, Taichung 407, Taiwan, Republic of China

Corresponding author: Professor Pei-Chung Chen. Email: chenpc@sunrise.hk.edu.tw

Abstract

Background: Asthma is associated with increased inflammation, oxidative stress and abnormal immune system function. We determined the distributions of several essential trace minerals and assessed their relationships to factors that are associated with the pathophysiological status of patients with mild/moderate asthma.

Methods: We enrolled 25 asthmatic patients and 25 healthy subjects. We measured: blood trace minerals, zinc (Zn), copper (Cu) and selenium (Se); oxidative stress markers thiobarbituric acid reactive substances (TBARS); antioxidant enzyme activities; percentages of CD4 and CD8 lymphocyte subsets; high-sensitivity C-reactive protein (hs-CRP); and a lung function index (FEV1/FVC%).

Results: Compared with healthy subjects, asthmatics had lower concentrations of Zn and Se; higher Cu concentrations, and Cu/Zn and Cu/Se ratios; and lower antioxidant enzyme glutathione peroxidase (GPx), glutathione reductase (GR) and catalase activities. Significantly increased concentrations of hs-CRP, TBARS and CD4/CD8 lymphocyte ratios were also observed. Furthermore, plasma TBARS or hs-CRP concentrations were negatively associated with Se concentrations, but were positively associated with Cu/Se ratios. CD4/CD8 lymphocyte ratios were inversely correlated with Se, while it was positively correlated with Cu/Se ratio. FEV1/FVC% was also significantly correlated with Se concentrations, and Cu/Se and Cu/Zn ratios.

Conclusions: Abnormal distributions of these trace minerals may aggravate oxidative damage and inflammation, increased CD4/CD8 lymphocyte ratios and decreased lung function in asthma.

Ann Clin Biochem 2011; 48: 344–351. DOI: 10.1258/acb.2011.010266

Introduction

Asthma is a major public health problem of increasing concern worldwide. It is a chronic inflammatory disorder of the airways that is characterized by variable bronchial obstruction and hyper-responsiveness.¹ Infiltrations of the airway walls by mast cells, T-lymphocytes and eosinophils are involved in the inflammatory process, which correlates significantly with disease severity.^{1,2} It has been shown that an imbalance of Th1/Th2 helper cells and CD4⁺ Th2-type lymphocyte cytokine production are involved in the recruitment of inflammatory cells and hyper-responsiveness.³

Recently, evidence suggests that oxidative stress and inflammation play critical roles in both the initiation and development of asthma.^{4,5} Oxidative stress induces inflammatory response in the respiratory system, which causes tissue injury and immune-mediated damage.⁶ Reduced

glutathione (GSH) is an important protective, regulatory antioxidant.³ Decreased intracellular GSH can lower Th1 activity and increase Th2 activity.⁷ Among GSH-depleted peripheral T-cells, there is a markedly increased ratio of CD4/CD8 cells.⁸ In addition, different antioxidant enzymes, such as GSH peroxidase (GPx), GSH reductase (GR), superoxide dismutase (SOD) and catalase, with a wide range of activities are found in lung tissues and blood.^{9,10} Compared with healthy subjects, asthmatic children have been found to have significantly increased serum oxidative stress and decreased GPx activity.¹¹

Essential trace minerals such as zinc (Zn), copper (Cu) and selenium (Se) are known to play important roles in the maintenance of redox homeostasis, which are also required for immune system function. Alterations in these minerals' status may result in higher inflammatory responses and oxidative stress.¹² For example, disturbances of Zn homeostasis

can lead to a shift in the Th1/Th2 balance towards a Th2 response.¹³ In an animal model, an intracellular pool of Zn in airway epithelium was associated with inflammatory status.¹⁴ Also, Se stimulates a Th1 immune response against viral infections.¹⁵ There appears to be a lower serum concentration of Se in patients with asthma,¹⁶ although conflicting results have been reported.¹⁷ In addition, Cu deficiency and excess can induce oxidative stress that can lead to chronic inflammation.¹⁸ Elevated serum Cu may be a useful marker of inflammation and immune status.¹⁹ However, there is limited information regarding the distribution of plasma Cu in asthmatic patients.

Disturbances in plasma trace minerals' homeostasis can influence the states of oxidative stress, inflammation and immune responses. However, asthma may create disturbances in the homeostasis of trace minerals and how these are related to the pathogenesis of asthma has given conflicting results. Abnormalities in the homeostasis of one mineral affect the distribution patterns of other minerals,²⁰ which is one possible reason for conflicting results. In addition, possible correlations between these minerals with oxidative stress, inflammation, immune system response and lung function in asthmatic patients have not been examined.

In the present study, plasma trace minerals (Cu, Zn, Se concentrations, Cu/Zn and Cu/Se ratios), antioxidant enzyme activities (SOD, GPx, GR and catalase), inflammatory status by high-sensitivity C-reactive protein (hs-CRP), and immune cell CD4/CD8 ratios were compared between asthma patients and healthy controls. The relationships of trace minerals to lung function, oxidative stress, hs-CRP and CD4/CD8 lymphocyte ratios were also examined in asthmatic patients.

Methods

Patients

Between January and March 2007, 25 patients with mild/moderate asthma diagnosed according to the American Thoracic Society criteria at the otolaryngology unit of Cheng-Ching Hospital (Taichung, Taiwan) were enrolled. Asthma is determined by pulmonary function parameters, including forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), which is measured using a Jaeger MasterScreen (VIASYS Healthcare GmbH, Hoechberg, Germany).

None of the subjects was a smoker or had a respiratory tract infection or exacerbation of asthma. Subjects with heart disease, diabetes mellitus, liver disease, cancer and systemic inflammatory disorders, and who received immune suppressant drugs or supplementation with antioxidant vitamins/minerals were excluded. As a control, 25 volunteer healthy subjects of similar age and gender were studied. All subjects signed informed consent statements. The study protocol was approved by the ethics in human research committee of our hospital (Institution Review Board [IRB], #HP060007).

Biochemical analysis

Blood samples were drawn in the morning after an overnight fast of 12 h. Plasma concentrations of albumin,

haemoglobin and immunoglobulin E (IgE) were estimated using routine laboratory techniques.

Measurement of oxidative stress and hs-CRP

Lipid peroxidation was determined by assaying the formation of thiobarbituric acid reactive substances (TBARS). Plasma samples were mixed with 3% sodium dodecyl sulphate, 0.1 N HCl, 10% phosphotungstic acid and 0.7% thiobarbituric acid and then incubated at 95°C. The TBARS were extracted into *n*-butanol and the fluorescence of the *n*-butanol layer was measured.¹⁹

Plasma concentrations of hs-CRP were measured using the human CRP ELISA kit (E-80CRP; Immunology Consultants Laboratory, Inc, Newberg, OR, USA). The intra- and inter-assay coefficients of variation (CVs) were <3 and <4%, respectively. Briefly, 200 µL of combined enzyme-antibody conjugate and 3,3',5,5'-tetramethylbenzidine substrate was added to each well and was incubated in darkness at room temperature. After 10 min the reaction was stopped with the addition of stop solution. The absorbance at 450 nm was measured, and readings were interpolated into the standard curve.

Determination of trace minerals

The concentrations of Zn and Cu were measured with a flame atomic absorption spectrophotometer (932 plus; GBC, Melbourne, Australia) using an air-acetylene flame without background correction at 213.9 and 324.71 nm, respectively. Samples were digested in a H₂O₂/HNO₃ mixture in a START D microwave-assisted digestion system (Milestone Microwave Labstation ETHOS D), and the volume was increased with double-deionized water.

The accessory hydride formation system (HG 3000), from GBC, was used for determining Se concentrations. Samples were digested for a total of 6.5 h with an initial temperature of 60°C for 0.5 h, followed by increasing temperatures in 10°C increments and finally heated up to 200°C for 0.5 h in a mixture of 3.2 mL nitric acid (16 N) and 0.8 mL concentrated perchloric acid to convert all Se species to selenate.¹⁹

Measurement of enzyme activity

Erythrocyte SOD activity was determined with a RANSOD kit (Randox, San Diego, CA, USA); one unit was defined as the amount of enzymes necessary to produce 50% inhibition in the rate of *p*-iodonitro-tetrazolium reduction. Catalase activity was performed with a commercially available kit (IBL Immunobiological Laboratories, Hamburg, Germany); one unit was defined as the amount of enzymes that caused the formation of 1.0 nmol of formaldehyde per minute at 25°C.

GPx activity was measured with a kit from Cayman Chemical (cat #703102; Ann Arbor, MI, USA); the rate of decrease in absorbance at 340 nm is directly proportional to the GPx activity. GR activity was assessed by monitoring the oxidation of NADPH to NADP⁺ via addition of oxidized GSH; one unit was defined as the amount of enzyme that catalysed the reduction of 1 mmol of oxidized GSH.

Determination of immunological parameters

Peripheral blood T-lymphocytes of all subjects were stained with the following monoclonal antibodies, which conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE): CD3 FITC/CD4 PE/CD8 PE (eBioscience, San Diego, CA, USA). Briefly, 100 μ L of whole blood were incubated with 20 μ L of monoclonal antibody reagent for 15 min in the dark at room temperature. Following leukocyte fixation and erythrocyte lysis with the CyLyse lysing reagent kit (Partec GmbH, Münster, Germany), the percentages of lymphocyte subsets were measured using a Partec CyFlow ML flow cytometer (Partec GmbH).

Statistical analysis

Quantitative variables were expressed as mean (SD) or median (interquartile range). A two-tailed *P* value less than 0.05 was considered statistically significant. The Kruskal–Wallis test was applied to evaluate the distribution of variances. Data in normal distribution was analysed using Student's *t*-test; data in non-normal distribution was analysed by the Mann–Whitney test. In addition, Pearson's or Spearman's correlation coefficients were performed to identify correlations of blood variables.

Results

Clinical characteristics

No significant differences in age, sex, body mass index (BMI) or plasma albumin levels were found between asthmatic patients and controls (*P* > 0.05) (Table 1). Compared with healthy subjects, asthma patients' haemoglobin concentrations were higher. Also, asthmatic patients had significantly higher IgE concentrations. FEV1/FVC% is used for the diagnoses of obstructive and restrictive lung diseases. For the asthmatic patients, the mean FEV1/FVC% = 78.68 \pm 9.90.

The levels of plasma markers of oxidative stress, TBARS, were considerably increased in asthmatic patients compared with healthy subjects. We also found significantly higher hs-CRP concentrations in asthmatic patients.

Table 1 Characteristics of asthmatic patients and healthy subjects*

| | Patients (n = 25) | Controls (n = 25) |
|---|-----------------------------------|--------------------|
| Gender (M/F) | 8/17 | 9/16 |
| Age (y) | 35 (7) | 38 (10) |
| Body mass index, BMI (kg/m ²) | 22 (1) | 23 (2) |
| Haemoglobin (g/L) | 163 (47) [†] | 142 (25) |
| Albumin (g/dL) | 4.1 (0.1) | 4.3 (0.1) |
| FEV1/FVC% | 78.68 (9.90) | – |
| FVC (L) | 3.17 (0.83) | – |
| FEV1 (L) | 2.48 (0.64) | – |
| IgE (KU/L), median (IQR) | 90.70 (53.55–301.00) [†] | 26.30 (4.86–68.20) |
| hs-CRP (ng/mL) | 3.45 (2.92) [†] | 0.94 (0.83) |
| TBARS (nmol/L) | 5.08 (0.64) [†] | 3.30 (0.66) |

*Values are mean (SD) unless stated otherwise.

[†]*P* < 0.05 versus controls.

FVC, forced vital capacity; FEV1, forced expiratory volume in one second; hs-CRP, high-sensitivity C-reactive protein; TBARS, thiobarbituric acid-reactive substances; IQR, interquartile range

Trace mineral status, antioxidant enzymes and immunological variables

The plasma concentrations of Zn and Se were significantly reduced in asthmatic patients compared with healthy subjects. Asthmatic patients had significantly higher plasma Cu concentrations. In addition, markedly higher Cu/Zn and Cu/Se ratios were observed in asthmatic patients (Table 2).

Asthmatic patients had significant decreases in erythrocyte antioxidant enzyme GPx, GR and catalase activities. There was no significant difference in SOD activity between patients and healthy controls.

The asthmatic patients had higher CD4 and lower CD8 T-lymphocyte percentages compared with healthy subjects. The CD4/CD8 ratios for asthmatic patients were significantly higher than for controls.

Correlations between minerals and other parameters in asthmatics

For asthmatic patients, TBARS values showed statistically significant relationships to Zn (*r* = –0.450), Cu (*r* = 0.503) concentrations, and Cu/Zn ratio (*r* = 0.483) (data not shown) and Se (*r* = –0.446) concentration and Cu/Se ratio (*r* = 0.488) (Figure 1).

Significant correlations between hs-CRP concentrations and Se (*r* = –0.527) and Cu/Se ratio (*r* = 0.635) were also obtained (Figure 1). Plasma Zn, Cu and Cu/Zn ratio were not significantly related to hs-CRP levels. The hs-CRP levels were also strongly associated with TBARS (*r* = 0.415) (data not shown).

Table 2 Plasma levels of trace minerals and antioxidant enzymes, and immune function in asthmatic patients and healthy subjects*

| | Patients (n = 25) | Controls (n = 25) |
|------------------------------------|----------------------------------|-------------------|
| Trace minerals | | |
| Zn (ug/mL) | 0.68 (0.09) [†] | 0.83 (0.14) |
| Cu (ug/mL) | 1.10 (0.28) [†] | 0.76 (0.17) |
| Se (ug/mL) | 0.057 (0.024) [†] | 0.116 (0.022) |
| Cu/Zn ratio, median (IQR) | 1.51 (1.18–1.95) [†] | 0.89 (0.79–1.12) |
| Cu/Se ratio, median (IQR) | 16.18 (12.82–31.86) [†] | 6.59 (5.40–7.96) |
| Antioxidant enzymes | | |
| SOD (U/mg haemoglobin) | 3.44 (0.87) | 3.27 (0.84) |
| Catalase (nmol/min/mg haemoglobin) | 16.27 (2.75) [†] | 26.06 (5.14) |
| GPx (nmol/min/mg haemoglobin) | 28.20 (9.33) [†] | 48.87 (20.01) |
| GR (nmol/min/mg haemoglobin) | 1.83 (0.74) [†] | 6.94 (2.29) |
| Immune markers | | |
| CD3 (%) | 66.47 (5.22) | 68.08 (5.35) |
| CD4 (%) | 47.28 (3.86) [†] | 42.05 (4.16) |
| CD8 (%) | 19.27 (3.60) [†] | 21.40 (3.17) |
| CD4/CD8 ratio, median (IQR) | 2.41 (2.16–2.77) [†] | 1.99 (1.68–2.31) |

*Values are mean (SD) unless stated otherwise.

[†]*P* < 0.05 versus controls.

Zn, zinc; Cu, copper; Se, selenium; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; IQR, interquartile range

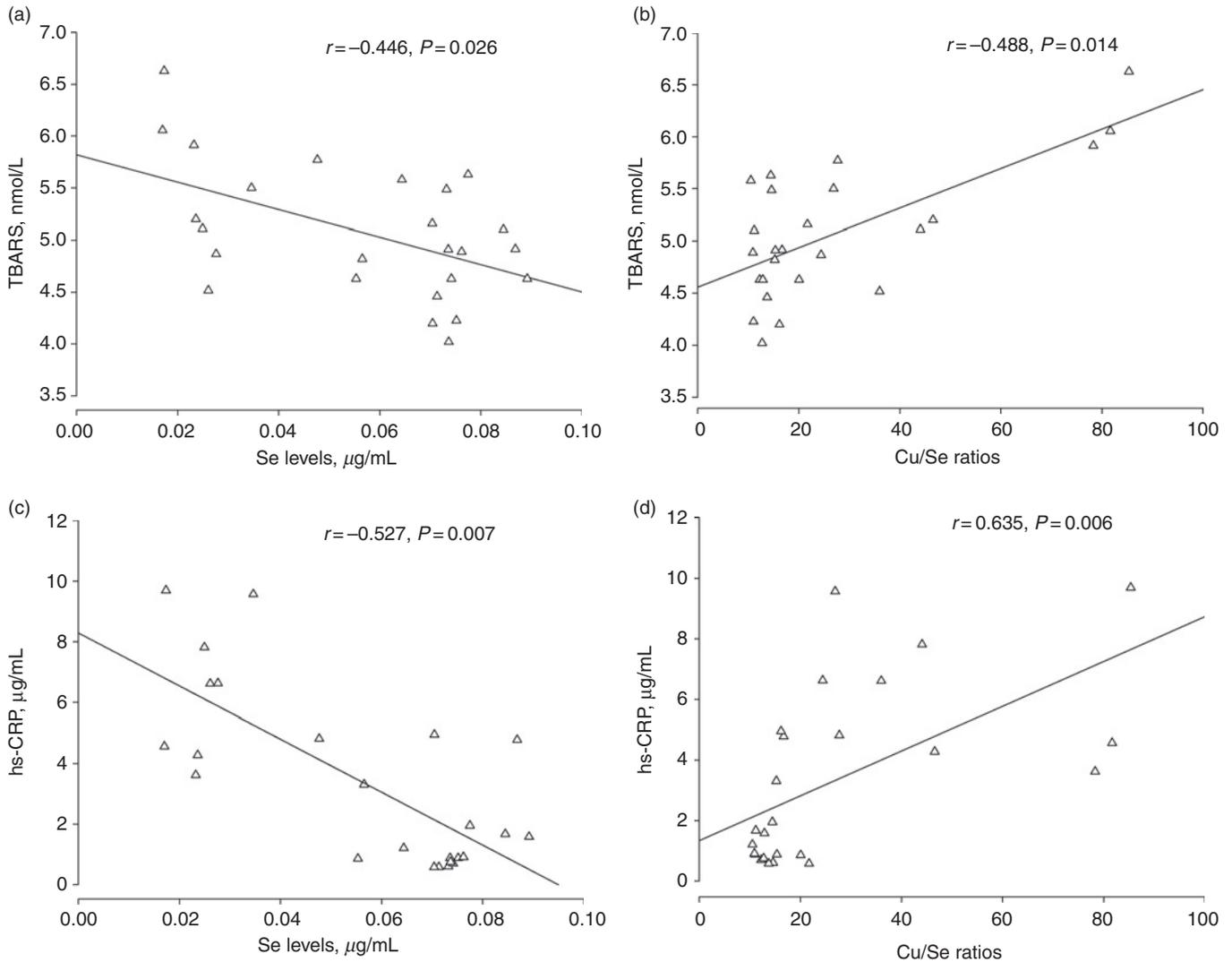


Figure 1 Correlations of TBARS or hs-CRP with trace mineral levels Se (a and c), and Cu/Se ratios (b and d) in asthmatic patients. TBARS, thiobarbituric acid-reactive substances; hs-CRP, high-sensitivity C-reactive protein

The pulmonary function indicator FEV1/FVC% was positively correlated with plasma Se concentrations ($r = 0.504$). There were significant inverse correlations between FEV1/FVC% and Cu/Zn ($r = -0.412$) and Cu/Se ratios ($r = -0.417$). There was also an inverse relationship between FEV1/FVC% and TBARS ($r = -0.397$) (Figure 2).

Se concentrations were negatively correlated with CD4/CD8 lymphocyte ratios. Plasma Cu/Se ratios were significantly positively related to CD4/CD8 lymphocyte ratios (Figure 3). In addition, CD4/CD8 ratios were related to plasma levels of TBARS ($r = 0.298$, $P = 0.150$), hs-CRP ($r = 0.319$, $P = 0.119$) and FEV1/FVC% ($r = -0.211$, $P = 0.300$), although this was not statistically significant (data not shown).

Discussion

In the present study, patients with mild-to-moderate asthma had abnormal levels of three trace minerals (Cu, Zn and Se), higher TBARS values (indicator of oxidative stress), lower levels of antioxidant enzymes, increased hs-CRP levels,

and higher CD4/CD8 lymphocyte ratios compared with controls. In addition, in these asthma patients, we found that disturbances of these trace minerals were associated with altered lung function, oxidative stress, inflammation status and immune response.

Relative to healthy controls, our asthma patients had significantly higher levels of haemoglobin, which may have been due to a compensatory need to carry more oxygen. The aetiology of asthma is dependent on numerous risk factors, including alterations in oxidant-antioxidant balances.⁴ Essential trace minerals, such as Cu, Zn and Se, are key co-factors for regulating certain antioxidant enzyme expressions. We found that asthma patients had significantly lower plasma Zn and Se concentrations, and higher Cu concentrations compared with healthy controls.

Suboptimal Zn status is typically associated with many inflammatory diseases.²¹ Increased expression of intracellular metallothioneins following oxidative stress or inflammation can sequester plasma Zn.²² Increased Zn uptake may be needed to counteract the loss of Zn in the airways and to meet an increased demand for Zn-dependent

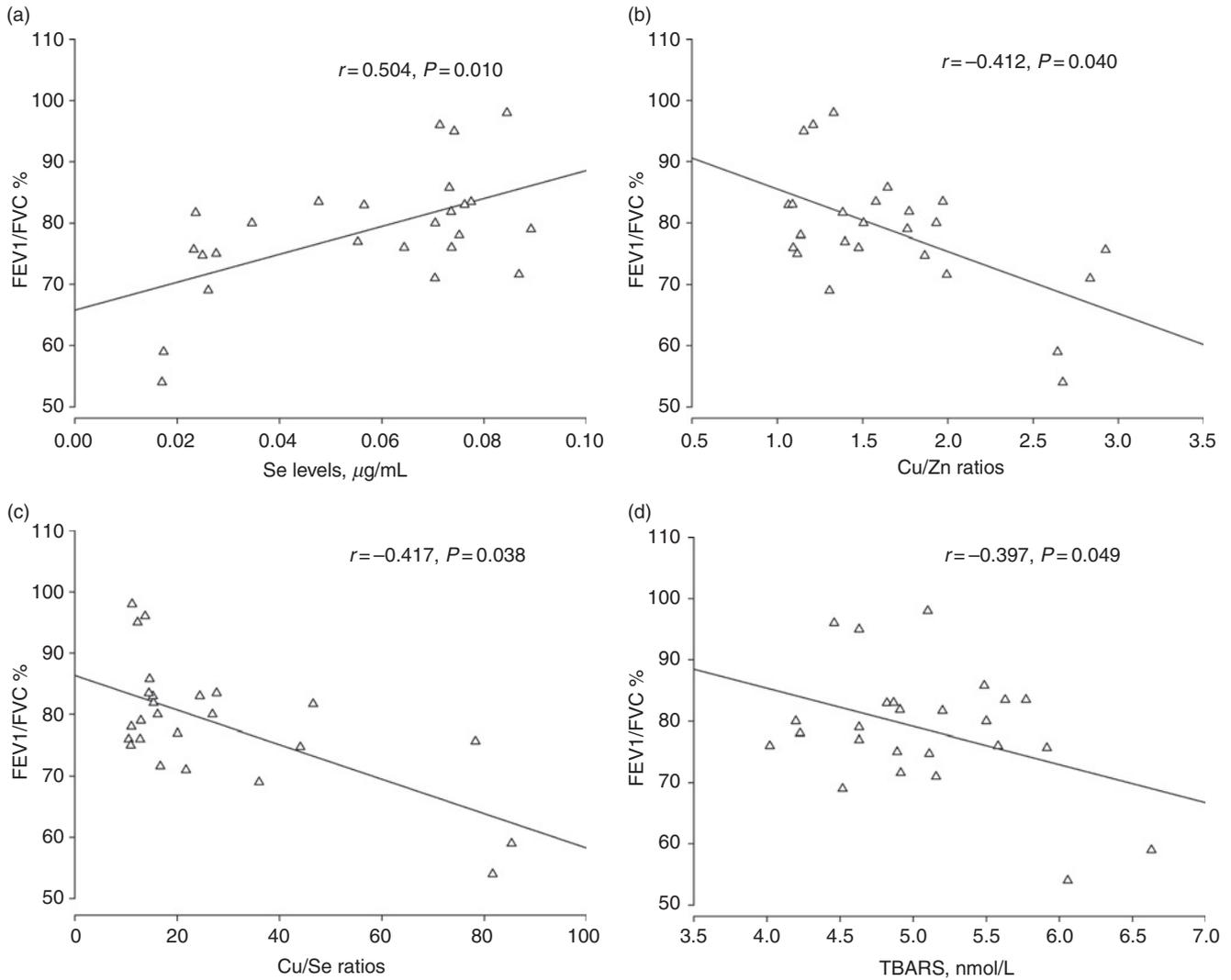


Figure 2 Associations between lung function FEV1/FVC% and blood variables Se (a), Cu/Zn ratio (b), Cu/Se ratios (c) or TBARS (d) in asthmatic patients. FVC, forced vital capacity; FEV1, forced expiratory volume in one second; TBARS, thiobarbituric acid-reactive substances

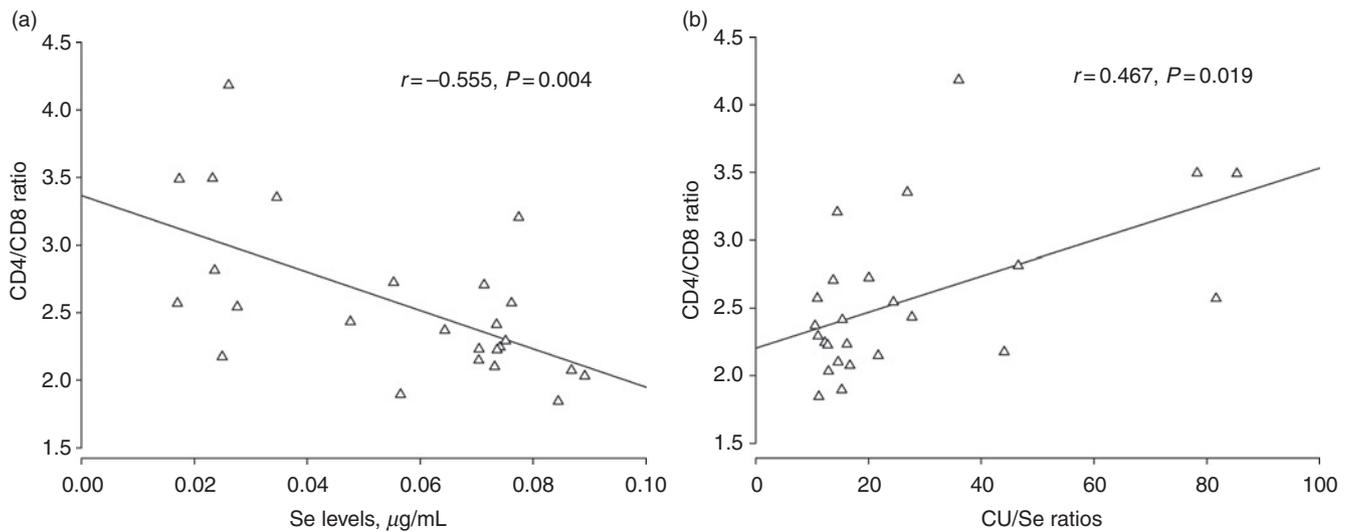


Figure 3 Relationships between immune index CD4/CD8 ratio and Se levels (a), and Cu/Se ratios (b) in asthmatic patients

proteins.²³ Up-regulation of Zn transporter genes is found in the inflammatory cells and can also reduce the levels of plasma Zn.²¹ Moreover, increased plasma Cu concentrations may be explained by the release of Cu during inflammatory tissue damage,¹⁹ and that high plasma Cu significantly inhibits the influx of Zn across small intestinal brush border membranes.²⁴

Plasma Zn concentrations were not found to be significantly different between patients with childhood asthma and controls.²⁵ However, hair and serum concentrations of Zn were markedly lower in wheezy infants than in healthy controls, suggesting that Zn deficiency may alter the risk of wheezing in early childhood.²⁶ Maternal intake of Zn during pregnancy is also negatively associated with wheezing illnesses in their childhood.²⁷

Se is also a potent antioxidant and acts as an anti-inflammatory agent. Increased inflammation may decrease the absorption of Se resulting in low Se status.²⁸ Decreased Se intake and serum levels in patients with asthma have been observed;²⁹ although, this finding was not confirmed by Jacobson *et al.*¹⁷ In clinical intervention studies, opposite results on the clinical benefits to asthma patients after Se supplementation have also been observed.³⁰ These results suggest that the distributions of trace minerals and the pathogenesis of asthma may not be simply dependent on a single mineral.

Our results showed that, relative to healthy controls, asthmatic patients had significantly higher products of oxidation, TBARS and lower levels of antioxidant enzymes. We also found higher concentrations of plasma hs-CRP, a marker of inflammation, in these asthma patients.

Increases in oxidative stress can initiate and augment inflammation, and play an important role in inflammatory diseases, including asthma.³¹ Decreased antioxidant enzyme GR activity was found to be significantly associated with asthma symptoms.³¹ Also, antioxidant GSH status affects the early asthmatic reaction and airway hyper-responsiveness.³² Supplements with Se could significantly reduce oxidative stress and increase the antioxidant enzyme activity of GPx in asthmatic patients.¹¹

As expected, we found that the plasma concentrations of Cu, Zn and Se, and the ratios of Cu/Zn and Cu/Se were significantly correlated with TBARS in asthmatic patients. Increased serum Cu concentrations and Cu/Zn ratios are associated with oxidative stress in some diseases.¹⁹ The Cu/Zn or Cu/Se ratios may therefore be a clinical predictor of oxidative stress and inflammatory status.^{19,33}

Recent studies have found that serum hs-CRP concentrations were significantly higher in patients with mild-to-moderate asthma than in healthy controls. Increased serum hs-CRP levels are associated with airflow obstruction and airway inflammation, and may serve as a marker of airway inflammation and asthma severity.³⁴ Associations between increased levels of serum hs-CRP with a high frequency of airway hyper-responsiveness and lowered FEV1 or FEV1/FVC% (lung function indices) were also found.³⁵ Thus, hs-CRP may be related to asthma exacerbations and allergic inflammation.

Recent studies have also indicated that the phosphatidylinositol 3-kinase (PI3K) pathway plays an important role in

the expression and activation of inflammatory mediators, inflammatory cell recruitment, immune cell function and airway remodelling.³⁶ Cu may activate the PI3K pathway, stimulate interleukin (IL)-6 production, and enhance the expression of intercellular adhesion molecule-1.^{37,38} In contrast, Se may reduce inflammation via its antioxidant action and by modulating the expressions of selenoprotein genes.¹⁵ Se status is significantly associated with suppressed IL-6 levels in disabled older subjects.²⁹ Se supplementation stimulated GPx enzyme activity and reduced NF- κ B activation in HIV-infected patients.³⁹ Our results also indicated significant associations between hs-CRP, TBARS, Se and the Cu/Se ratio in asthma patients. This suggests that plasma Se concentrations and Cu/Se ratios may accurately reflect both oxidative stress and the degree of inflammation severity in patients with asthma.

Our results indicated significant associations between peripheral lymphocyte CD4/CD8 ratios with Se concentrations, and Cu/Se ratios in asthmatic patients. Increased CD4/CD8 ratios in the peripheral blood, bronchoalveolar lavage lymphocytes, and lung biopsies of asthma patients have been observed, and the CD4/CD8 ratio was negatively correlated with lung function.⁴⁰ In addition, activated CD4 T-cells increase and produce type 2 cytokines, such as IL-6 and tumour necrosis factor (TNF- α), which are involved in the recruitment of inflammatory cells and hyper-responsiveness.⁴¹ Oxidative stress may alter the Th1/Th2 immune response and result in the activation of pro-inflammatory genes.⁴² A trend for a positive correlation between the CD4/CD8 lymphocyte ratio and TBARS was observed in our study. These results suggest that increases in oxidative stress induced by an abnormal status of Se and Cu, resulting in higher CD4/CD8 ratios, can alter the balance between Th1/Th2 responses; however, we did not evaluate the production of Th1 and Th2 cytokines.

We found a significant negative correlation between oxidative stress and lung function (FEV1/FVC%) in these asthmatic patients. In addition, significant associations between FEV1/FVC% with plasma Se concentrations, and Cu/Zn and Cu/Se ratios were also found in asthma patients. Indeed, inflammation driven by increased oxidative stress has been implicated in the pathogenesis of asthma. Evidence indicates that inflammatory status is negatively associated with FEV1/FVC% in asthma patients.⁴³

An inverse association between TBARS with pulmonary function and a positive association between the antioxidant enzyme activity of GPx with pulmonary function have been observed.⁴⁴ A negative correlation between serum Se concentrations and attacks of wheezing has also been reported,¹⁵ along with a positive relationship between serum Cu/Zn ratio and wheezing in respiratory diseases.⁴⁵ In our asthmatic patients, the plasma Se concentration, but not Cu or Zn, was associated with parameters of lung function, although the Cu/Zn and Cu/Se ratios were correlated with lung function. It appears that an inter-dependency among these trace minerals (Cu, Zn and Se) is a factor that may be important for the pathogenesis and severity of asthma.

The present investigation found that patients with mild to moderate asthma had significant disturbances in trace

mineral (Cu, Zn and Se) homeostasis, and that this was presumably related to their oxidative stress status. A disruption of the Se status and the Cu/Se ratio may be responsible for inflammatory responses and increased CD4:CD8 lymphocyte ratios and, thus, decreased lung function in asthmatics. Therefore, Se or Cu/Se ratio may be a potentially important marker for mild to moderate asthma. Additional large-scale studies will be needed to clarify the exact roles of these trace minerals, Cu in particular, to evaluate conventional antioxidant therapeutic strategies for asthmatic patients.

DECLARATIONS

Competing interests: The authors do not have any conflicts of interest to declare.

Funding: Research supported in part by a grant from Cheng-Ching Hospital, Taichung, Taiwan (H94-021-01).

Ethical approval: The study protocol was approved by the Ethics in Human Research Committee of Cheng-Ching Hospital (IRB, #HP060007).

Guarantor: P-CC.

Contributors: C-HG was responsible for the development of intellectual content, interpretation of the results, and drafting of the paper. P-JL and P-CC were involved in protocol development, gaining ethical approval, recruitment and screening of subjects, and data analysis. SH was responsible for the study design and interpretation of the results. C-JC was responsible for haematological measurements, and sample analyses.

REFERENCES

- Sugita M, Kuribayashi K, Nakagomi T, Miyata S, Matsuyama T, Kitada O. Ilergic bronchial asthma: airway inflammation and hyper-responsiveness. *Intern Med* 2003;**42**:636–43
- Postma DS, Kerstjens HA. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;**158**:S187–92
- Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003;**8**:223–46
- Bakkeheim E, Mowinckel P, Carlsen KH, Burney P, Lødrup Carlsen KC. Altered oxidative state in schoolchildren with asthma and allergic rhinitis. *Pediatr Allergy Immunol* 2011;**22**:178–85
- Kalhan R, Tran BT, Colangelo LA, et al. Systemic inflammation in young adults is associated with abnormal lung function in middle age. *PLoS One* 2010;**5**:e11431
- Shvedova AA, Kisin ER, Murray AR, et al. Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57BL/6 mice. *Toxicol Appl Pharmacol* 2007;**221**:339–48
- Koike Y, Hisada T, Utsugi M, et al. Glutathione redox regulates airway hyper-responsiveness and airway inflammation in mice. *Am J Respir Cell Mol Biol* 2007;**37**:322–9
- Groen H, Pater JM, Nieuwenhuis P, Rozing J. Elevated intracellular glutathione levels in CD4+ T cells of BB rats. *Transplant Proc* 1997;**29**:1679–80
- Comhair SA, Erzurum SC. Redox control of asthma: molecular mechanisms and therapeutic opportunities. *Antioxid Redox Signal* 2010;**12**:93–124
- Hassan AM. Selenium status in patients with aspirin-induced asthma. *Ann Clin Biochem* 2008;**45**(Pt 5):508–12
- Perišić T, Srećković M, Matic G. An imbalance in antioxidant enzymes and stress proteins in childhood asthma. *Clin Biochem* 2007;**40**:1168–71
- Nair HB, Sung B, Yadav VR, Kannappan R, Chaturvedi MM, Aggarwal BB. Delivery of antiinflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. *Biochem Pharmacol* 2010;**80**:1833–43
- Sprietsma JE. Modern diets and diseases: NO-zinc balance. Under Th1, zinc and nitrogen monoxide (NO) collectively protect against viruses, AIDS, autoimmunity, diabetes, allergies, asthma, infectious diseases, atherosclerosis and cancer. *Med Hypotheses* 1999;**53**:6–16
- Murgia C, Lang CJ, Truong-Tran AQ, et al. Zinc and its specific transporters as potential targets in airway disease. *Curr Drug Targets* 2006;**7**:607–27
- Kocabaş CN, Adalioglu G, Coşkun T, Tuncer A, Sekerel BE. The relationship between serum selenium levels and frequent wheeze in children. *Turk J Pediatr* 2006;**48**:308–12
- Omland Ø, Deguchi Y, Sigsgaard T, Hansen JC. Selenium serum and urine is associated to mild asthma and atopy. The SUS study. *J Trace Elem Med Biol* 2002;**16**:123–7
- Jacobson GA, Yee KC, Ng CH. Elevated plasma glutathione peroxidase concentration in acute severe asthma: comparison with plasma glutathione peroxidase activity, selenium and malondialdehyde. *Scand J Clin Lab Invest* 2007;**67**:423–30
- Spee B, Mandigers PJJ, Arends B, et al. Differential expression of copper-associated and oxidative stress related proteins in a new variant of copper toxicosis in Doberman pinschers. *Comp Hepatol* 2005;**4**:3 doi: 10.1186/1476-5926-4-3
- Guo CH, Wang CL, Chen PC, Yang TC. Linkage of some trace elements, peritoneal blood lymphocytes, inflammation, and oxidative stress in patients undergoing either hemodialysis or peritoneal dialysis. *Perit Dial Int* 2010 [Epub ahead of print]
- Ahmed SS, Santosh W. Metallomic profiling and linkage map analysis of early Parkinson's disease: a new insight to aluminum marker for the possible diagnosis. *PLoS One* 2010;**5**:e11252
- Devirgiliis C, Zalewski PD, Perozzi G, Murgia C. Zinc fluxes and zinc transporter genes in chronic diseases. *Mutat Res* 2007;**622**:84–93
- Liuzzi JP, Lichten LA, Rivera S, et al. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypo-zincemia of the acute-phase response. *Proc Natl Acad Sci USA* 2005;**102**:6843–8
- Lang C, Murgia C, Leong M, et al. Anti-inflammatory effects of zinc and alterations in zinc transporter mRNA in mouse models of allergic inflammation. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L577–84
- Condomina J, Zornoza-Sabina T, Granero L, Polache A. Kinetics of zinc transport in vitro in rat small intestine and colon: interaction with copper. *Eur J Pharm Sci* 2002;**16**:289–95
- Kocyigit A, Armutcu F, Gurel A, Ermis B. Alterations in plasma essential trace elements selenium, manganese, zinc, copper, and iron concentrations and the possible role of these elements on oxidative status in patients with childhood asthma. *Biol Trace Elem Res* 2004;**97**:31–41
- Willers SM, Devereux G, Craig LC, et al. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 2007;**62**:773–9
- Tahan F, Karakukcu C. Zinc status in infantile wheezing. *Pediatr Pulmonol* 2006;**41**:630–4
- Walston J, Xue Q, Semba RD, et al. Serum antioxidants, inflammation, and total mortality in older women. *Am J Epidemiol* 2006;**163**:18–26
- Rubin RN, Navon L, Cassano PA. Relationship of serum antioxidants to asthma prevalence in youth. *Am J Respir Crit Care Med* 2004;**169**:393–8
- Hasselmark L, Malmgren R, Zetterstrom O, Unge G. Selenium supplementation in intrinsic asthma. *Allergy* 1993;**48**:30–6
- Malling TH, Sigsgaard T, Andersen HR, et al. Differences in associations between markers of antioxidative defense and asthma are sex specific. *Genet Med* 2010;**7**:115–24
- Kloek J, Mortaz E, van Ark I, Lilly CM, Nijkamp FP, Folkerts G. Glutathione prevents the early asthmatic reaction and airway hyperresponsiveness in guinea pigs. *J Physiol Pharmacol* 2010;**61**:67–72
- Ciftci TU, Ciftci B, Yis O, Guney Y, Bilgihan A, Ogretsenoy M. Changes in serum selenium, copper, zinc levels and Cu/Zn ratio in patients with pulmonary tuberculosis during therapy. *Biol Trace Elem Res* 2003;**95**:65–71
- Sävykoski T, Harju T, Paldanius M, et al. Chlamydia pneumoniae infection and inflammation in adults with asthma. *Respiration* 2004;**71**:20–5
- Kony S, Zureik M, Driss F, Neukirch C, Leynaert B, Neukirch F. Association of bronchial hyperresponsiveness and lung function with

- C-reactive protein (CRP): a population based study. *Thorax* 2004;**59**:892–6
- 36 Ito K, Caramori G, Adcock IM. Therapeutic potential of phosphate-dylinositol 3-kinase inhibitors in inflammatory respiratory disease. *J Pharmacol Exper Therap* 2007;**321**:1–8
- 37 Kennedy T, Ghio AJ, Reed W, *et al.* Copper-dependent inflammation and nuclear factor-kappaB activation by particulate air pollution. *Am J Respir Cell Mol Biol* 1998;**19**:366–78
- 38 Ostrakhovitch EA, Lordnejad MR, Schliess F, Sies H, Klotz LO. Copper ions strongly activate the phosphoinositide-3-Kinase/Akt pathway independent of the generation of reactive oxygen species. *Arch Biochem Biophys* 2002;**397**:232–9
- 39 Kupka R, Msamanga GI, Spiegelman D, *et al.* Selenium status is associated with accelerated HIV disease progression among HIV-1-infected pregnant women in Tanzania. *J Nutr* 2004;**134**:2556–60
- 40 Lee SY, Kim SJ, Kwon SS, *et al.* Distribution and cytokine production of CD4 and CD8 T-lymphocyte subsets in patients with acute asthma attacks. *Ann Allergy Asthma Immunol* 2001;**86**:659–64
- 41 Venkayya R, Lam M, Willkom M, Grünig G, Corry DB, Erle DJ. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyper-responsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol* 2002;**26**:202–8
- 42 Dozor AJ. The role of oxidative stress in the pathogenesis and treatment of asthma. *Ann N Y Acad Sci* 2010;**1203**:133–7
- 43 Fujita M, Ueki S, Ito W, *et al.* C-reactive protein levels in the serum of asthmatic patients. *Ann Allergy Asthma Immunol* 2007;**99**:48–53
- 44 Ochs-Balcom HM, Grant BJ, Muti P, *et al.* Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr* 2006;**60**:991–9
- 45 Schwartz J, Weiss ST. Dietary factors and their relation to respiratory symptoms. The Second National Health and Nutrition Examination Survey. *Am J Epidemiol* 1990;**132**:67–76

(Accepted 14 February 2011)

Copyright of Annals of Clinical Biochemistry is the property of Royal Society of Medicine Press Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.