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The immunomodulatory role of zinc in asthmatic patients

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ABSTRACT

Background: Zinc deficiency may play an important role in the development of atopic asthma.
The aim of the work: To assess serum zinc levels in adult atopic, non-atopic asthmatic patients, and in healthy controls and to investigate its modulatory effect on production of interferon gamma (IFN-γ) and interleukin-10 (IL-10) by peripheral blood mononuclear cells (PBMCs) in vitro.
Methods: Sixty asthmatics and 30 apparently healthy volunteers were included in this study. All patients were subjected to history taking, clinical examination, pulmonary function tests, skin prick test (SPT), serum zinc assessment by a colorimetric method as well as serum total IgE measurement by Enzyme-linked immunosorbent assay (ELISA). PBMCs were activated in vitro in the presence and absence of zinc, and then cell culture supernatants were analyzed for IFN-γ and IL-10 by ELISA.
Results: Serum zinc levels were significantly lower in atopic asthmatics than non-atopic asthmatics and healthy controls. In atopic asthmatics, highly significant correlations were found between zinc levels and total Ig E levels as well as FEV1. In culture, zinc triggers IFN-γ and inhibits IL-10 production by PBMCs, in atopic asthmatics. In non atopic asthmatics and healthy controls, IFN-γ and IL-10 were slightly affected by zinc supplementation in culture.
Conclusion: Serum zinc levels affect asthma phenotypes. Atopic asthmatics might benefit from zinc supplements.

1. Introduction

Atopic asthma is characterized by excessive T helper 2 (Th2)-like immunity to allergens in the bronchial mucosa, circulating specific immunoglobulin E (IgE) antibodies [1], positive skin prick tests (SPT) to common aeroallergens and airway hyperresponsiveness [2]. Shifting of the inflammatory response towards an increased Th2 cytokine profile is considered to be a crucial step in asthma pathogenesis [1].

Th2 cells produce interleukin (IL)-4 and Il-13 that induce IgE production [3] as well as IL-5 and IL-9 which promotes eosinophilic inflammation in the airways of asthmatics [4,5], while interferon-γ (IFN-γ) from Th1 cells downregulates IgE synthesis [6]. Interleukin-10(IL-10) is an anti-inflammatory cytokine that inhibits Th1 and Th2 cells and IgE synthesis , as well as shortening eosinophil survival [7]. Zinc (Zn) is an essential trace element for a well-operating immune system [8]. It influences the function of nearly > 300 enzymes [9] and is involved in apoptosis, signal transduction [10], cell growth and proliferation [11], and DNA synthesis [12]. Additionally, Zn can modulate allergic immunereaction by several mechanisms including improvement of Th1/Th2 balance [13], suppression of Th17 cell development [14], increasing the effect of antioxidant defense [15] and induction of Tregs [16]. Moreover, Zn inhibits mast cell degranulation and thereby decreases histamine production [17].

Allergic diseases are often associated with low serum zinc levels [18], therefore studies target to zinc-induced modulation.

Our first aim is to assess serum levels of zinc in adult atopic and non-atopic asthmatic patients, and in healthy controls. The second aim is to investigate the modulatory effect of zinc on IFN-γ and IL-10 production by peripheral blood mononuclear cells (PBMCs) in vitro.

2. Materials and methods

2.1. Subjects and sample collection

Prior to initiation, an informed consent was taken from the study participants after explaining the aim and procedures of the study and ensuring the confidentiality of the data. The study was carried out after the approval of Ain Shams University Ethics Committee.

This cross-sectional study was conducted at Ain Shams University Hospital during the period from April 2015 to December 2016 and included 30 atopic asthmatic patients and 30 non-atopic asthmatic patients.

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Asthmatic patients were recruited from the Allergy and Clinical Immunology outpatient clinic. Asthma diagnosis was established according to GINA guidelines [19]. Positive skin prick test (SPT) to at least one of the common environmental allergens is diagnosed as atopy. The study also included 30 apparently healthy individuals as a control group. All controls had no history of asthma or other allergic diseases and had negative SPT reactions. Exclusion criteria included infections, pregnancy, hypoalbuminemia, current or past immunotherapy, presence of other allergic diseases or major systemic diseases, patients on oral steroid therapy within 6 weeks before enrollment and patients on antihistamine therapy within 7 days before enrollment, patients on oral contraceptives or antibiotics and iron or zinc supplements.

After 8 h fasting, two venous blood samples (5 mL each) were obtained by venipuncture from each participant. The first sample was collected into a gel vacutainer tube (Becton Dickinson, Oxford, UK). Blood was allowed to clot and serum was separated by centrifugation (3500 rpm, 15 min, 25 °C) and then stored in aliquots at −20 °C until used for measurement of zinc and total IgE levels. The second sample was collected on K3EDTA vacutainer tube (Becton Dickinson) and blood was used for separation of PBMCs to be used in culture.

2.2. Measurement of serum zinc and total IgE level

Total IgE was determined using an enzyme-linked immunosorbent assay (ELISA) kit (RIDASCREEN; R-Biopharm, Darmstadt, Germany) (Catalog number: A0141), while determination of zinc level was done by zinc colorimetric method (Quimica Clinica Aplicada S.A).

2.3. Assessment of pulmonary functions

This was performed at the Pulmonary Functions Laboratory at Ain Shams University Hospital, using the Flowmate V Plus spirometer (Spirometrics, Gray, ME). Spirometry was carried out according to the standards of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) [20].

2.4. Isolation of PBMCs and cell culture

The whole blood was diluted 1:1 with phosphate buffered saline (PBS) (Lonza, Walkersville, USA). PBMCs were isolated from buffy coats by Ficoll-Hypaque (Lonza) density-gradient centrifugation. The separated cells were washed twice with RPMI 1640 medium (Lonza). Then, cell pellets were suspended in 1 mL PBS buffer then counted and tested for viability. Viability exceeded 90% in all cases. PBMCs were suspended in complete medium which consisted of RPMI 1640 supplemented with 2 mM L-glutamine, 15% fetal calf serum (Biowest, Nuaillé, France), 100μ/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin –B (Biowest). PBMCs were then cultured at a density of 5×10^5/well in 200 μL of culture media in 96 wells U-bottom culture plates (Costar Corp., Cambridge, MA, USA) and stimulated with 10 ug/mL of Phytohemagglutinin (PHA) (Sigma Chemical Co., Poole, UK) in the absence or presence of 60 μM zinc in the form of zinc sulphate (Elnasr, Pharmaceutical Chemical Industries, Egypt) Cells were then incubated for 48 h in a humidified atmosphere at 37 °C and 5% CO_2. Then, the plate was centrifuged at 2000 rpm for 5 min and the supernatant fluid was collected and stored at −80 °C until used for measurement of IFN-γ and IL-10.

2.5. Measurement of IFN-γ and IL-10 in cell culture supernatants.

Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Novex, Life Technologies, Carlsbad, California, USA) were used for assessment of IFN-γ (Catalog number: KHC4021C) and IL-10 (Catalog number: KHC4021C).

2.6. Statistical analysis

Analysis of data was performed using the SPSS program, version 20. Data were expressed as mean ± standard deviation (SD) for quantitative parametric measures and both number and percentage for categorized data. For parametric data, Student *t*-test was used to compare the means of two groups, whereas the one-way analysis of variance (ANOVA) test was used to compare the means of three groups, followed by Tukey's test as a post-hoc test to identify significant differences between means. Chi-square test was used to compare categorical data. The strength of the relationship between serum zinc and the other studied variables was assessed using Pearson's method. All *p*-values are two-sided. A p value of < 0.05 was considered significant.

3. Results

3.1. Baseline characteristics of asthmatic patients

This study included 30 atopic asthmatic patients, 30 non-atopic asthmatics and 30 apparently healthy subjects as a control group. Demographic, clinical and biochemical parameters of enrolled subjects are summarized in Table 1. Age and gender were comparable between all three groups. Additionally, no significant difference was demonstrated between the 3 study groups as regards FEV1 values. Atopic asthmatics had significantly higher total IgE levels than non-atopic asthmatics and healthy controls (Table 1).

3.2. Levels of serum zinc in atopic asthmatics

Serum zinc levels were significantly lower in atopic asthmatics compared to non-atopic asthmatics and controls. However, serum zinc levels did not differ significantly between non-atopic asthmatics or controls (Table 1). Serum zinc levels among atopic asthmatics did not

Table 1
Demographic, clinical and biochemical data of the study population.

Variable	Atopic asthma, n = 30	Non-atopic asthma, n = 30	Control, n = 30	p
Age (y)[c]	45.23 ± 6.58	44.9 ± 6.54	44.9 ± 6.58	0.975
Sex, n (%)[d]				
Female	15(50%)	17(56.7%)	13(43.3%)	0.587
Male	15(50%)	13(43.3%)	17(56.7%)	
FEV1 (%)[c]	63.93 ± 9.68	63.87 ± 9.7	–	0.979
Total IgE (IU/ml)[c]	409.60 ± 118.19	60.03 ± 21.39	60.60 ± 25.78	< 0.001[a]
Zinc (μg/dL)[c]	54.94 ± 10.38	95.77 ± 6.81	96.20 ± 7.37	< 0.001[b]

FEV1, forced expiratory volume in 1st second; IgE, immunoglobulin E.

[a] Total IgE: Atopic asthma versus non-atopic asthma, $p < 0.001$; Atopic asthma versus controls, $p < 0.001$; Non-atopic asthma versus controls, $p = 0.926$.

[b] Zinc: Atopic asthma versus non-atopic asthma, $p < 0.001$; Atopic asthma versus controls, $p < 0.001$; Non-atopic asthma versus controls, $p = 0.815$.

[c] Data were presented as mean ± SD and compared together using ANOVA test.

[d] Data were presented as number (percentage) and compared together using Chi-square test.