

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 IgE 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 immunoreaction 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), 100 µg/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 µg/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% CO₂. 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.

Table 2
Correlation between serum zinc levels and parameters among atopic asthmatics.

Variable	r	p
Age (y)	0.214	0.255
FEV1 (%)	0.950	< 0.001
Total IgE (IU/ml)	-0.898	< 0.001

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

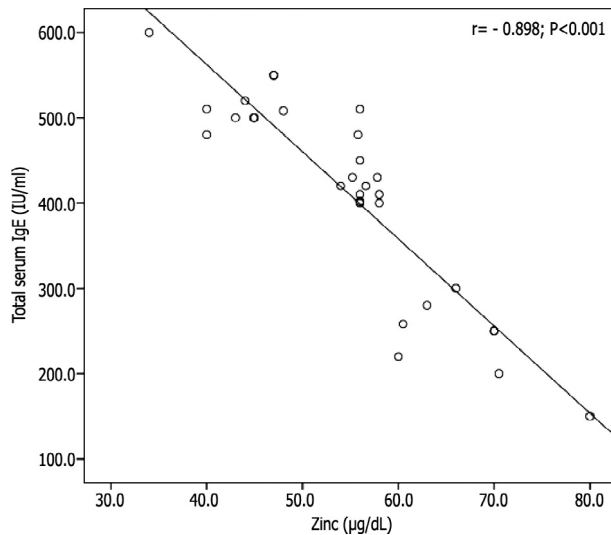


Fig. 1. Correlation between serum Zn and total IgE levels among atopic asthmatics.

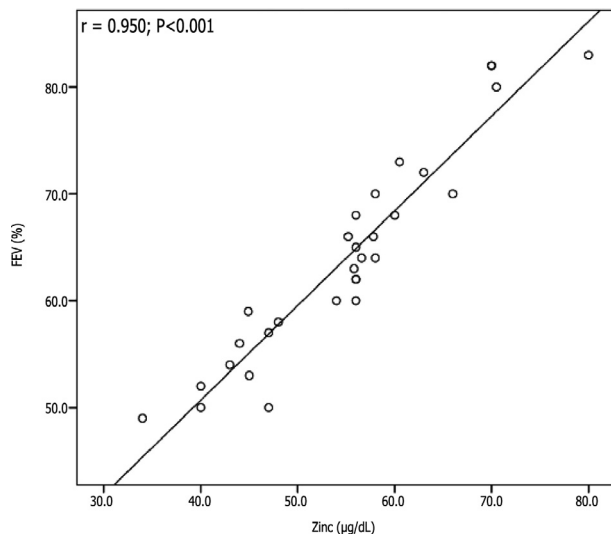


Fig. 2. Correlation between serum Zn levels and FEV1 values among atopic asthmatics.

correlate significantly with age (Table 2). On the other hand, a significant negative correlation was observed between serum zinc and total serum IgE levels among atopic asthmatics (Fig. 1). Additionally, a significant positive correlation was found between serum zinc and FEV1 values among atopic asthmatics (Fig. 2). On the other hand, no correlation was observed between serum zinc levels and total IgE or FEV1, among non-atopic asthmatics and healthy controls.

3.3. Cytokine production by PBMCs' culture

Among atopic asthmatics, both PHA-stimulated and unstimulated PBMCs produced significantly more IL10 compared to non-atopic asthmatics and healthy controls (Table 3).

Regarding IFN- γ , the spontaneous production of IFN- γ by unstimulated PBMCs was low in atopic, non-atopic asthmatics and controls without any significant difference between the three groups. Conversely, PHA-stimulated PBMCs produced significantly less IFN- γ in atopic asthmatics compared to non atopic asthmatics and healthy controls (Table 3).

Among atopic asthmatics, zinc significantly stimulated IFN- γ and slightly but not significantly decreased IL-10 production from PBMCs (Fig. 3). On the other hand, zinc triggered a slight alteration in cytokine levels in culture supernatant, among non-atopic asthmatics and healthy controls.

3.4. Effect of zinc status on cytokine production

Among atopic asthmatics, a significant positive correlation was found between serum zinc levels and IFN- γ ($r = 0.915$, $p < 0.001$). Moreover a significant negative correlation was observed between serum zinc levels and IL-10 ($r = -0.803$, $p < 0.001$). In case of incubation with zinc, we observed a significant correlation between serum zinc levels and IFN- γ ($r = 0.822$, $p < 0.001$) as well as IL-10 ($r = -0.425$, $p = 0.019$). On the other hand, no correlation was demonstrated between serum zinc levels and IFN- γ as well as IL-10, in non-atopic asthmatics and controls, neither in the presence nor absence of zinc in culture.

4. Discussion

To the best of our knowledge, no previous studies were conducted on the association between low zinc levels and atopy. In the present study, we found that serum zinc levels were significantly lower in atopic asthmatics as compared to non-atopic and controls. However, serum zinc levels did not differ between non-atopic asthmatics or controls. Our results were in partial agreement with Khanbabaee et al [21] who assessed the serum zinc level in 100 pediatric asthmatic patients in comparison with a control group. Although several studies demonstrated a lower concentration of zinc in the serum of asthmatic patients when compared to controls [22,23], some studies could not confirm this finding [24,25].

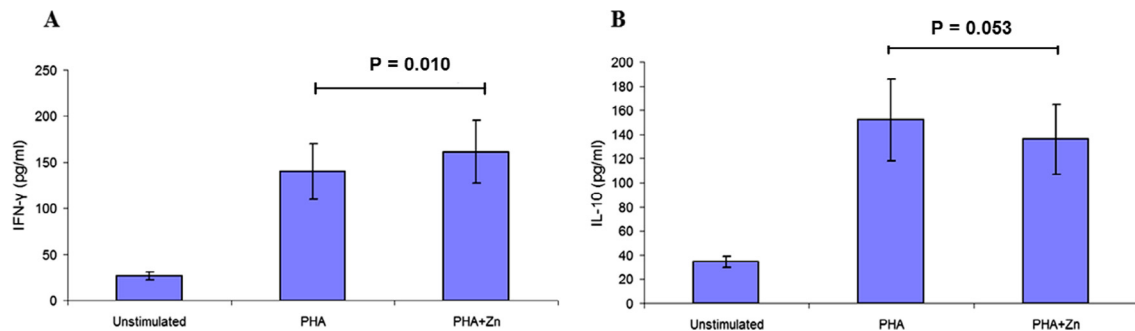
In the current study, the association between hypozincemia and atopy was confirmed by the presence of strong negative correlation between serum zinc and total IgE levels, among atopic asthmatics only. Additionally, this association was strengthened by the presence of a strong positive correlation between serum zinc and FEV1 values among atopic asthmatics only. This finding was in line with Khanbabaee et al. [21] who found that 90% of patients with severe persistent asthma had hypozincemia. These results suggest that zinc deficiency might play a role in the pathogenesis and contribution to the development of atopy.

As a result of zinc deficiency, nuclear factor-kB (NF-kB) activation, phosphorylation of I κ B, and binding of NF-kB to DNA are decreased and this results in decreased Th1 cytokine production [26]. Zn depletion leads to decreased IFN- γ release resulting in a shift from Th1 to Th2 cytokines [27]. This is strengthened by another study that demonstrated a reduction of childhood asthma by the intake of antioxidant vitamins, zinc and selenium during pregnancy and childhood [28].

Among atopic asthmatics, both PHA-stimulated and unstimulated PBMCs produced significantly more IL10 as compared to non-atopic asthmatics and healthy controls. Whereas, only PHA-stimulated PBMCs produced significantly less IFN- γ as compared to non-atopic asthmatics and healthy controls. Our findings were in accordance with several studies [29,30] that addressed cytokine difference between atopic and non-atopic asthmatics. The raised IL-10 production in atopic asthma

Table 3Comparison between the 3 study groups as regards IFN- γ and IL-10 levels.

Treatment	Mean cytokine production $\pm$ SD (pg/ml)							
	IFN- γ				IL-10			
	Atopic asthma, n = 30	Non-atopic asthma, n = 30	Control, n = 30	p	Atopic asthma, n = 30	Non-atopic asthma, n = 30	Control, n = 30	p
No stimulation	26.50 $\pm$ 4.38	28.33 $\pm$ 7.80	26.50 $\pm$ 6.84	> 0.05 [*]	34.33 $\pm$ 4.49	16.00 $\pm$ 6.35	15.66 $\pm$ 6.12	< 0.001 ^b
PHA	140.20 $\pm$ 30.31	254.67 $\pm$ 30.60	300.67 $\pm$ 58.89	< 0.001 ^a	152.00 $\pm$ 33.67	125.00 $\pm$ 31.38	127.00 $\pm$ 32.92	0.003 ^b
PHA + Zn	161.33 $\pm$ 34.21	269.67 $\pm$ 37.18	328.33 $\pm$ 57.66	< 0.001 ^a	136.00 $\pm$ 28.99	111.33 $\pm$ 26.62	113.33 $\pm$ 26.95	0.001 ^b

^a Post Hoc test: Control vs Atopic (S), Control vs non-atopic (S) and Atopic vs non-atopic (S).^b Post Hoc test: Control vs Atopic (S), Control vs non-atopic (NS) and Atopic vs non-atopic (S).^{*} Non-significant.**Fig. 3.** Addition of zinc to PBMCs culture results in significant increase in IFN- γ production (A) a slight but not significant decrease in IL-10 production (B), among atopic asthmatics.

confirms that the Th2 profile is believed to be essential in regulating airway inflammation in asthma [29]. On the other hand, the reduced IFN- γ secretion is completely in accord with previous data demonstrating that a deficient production of IFN- γ is crucial in moving the cytokine network toward the Th2 profile in atopy [30,31].

In the presence of Zn in culture, a significant stimulation of IFN- γ occurred and slightly but not significantly decrease of IL-10 production from PBMCs, in atopic asthmatics. On the other hand, zinc triggered a slight alteration in cytokine levels in culture supernatant, among non-atopic asthmatics and healthy controls. This confirms our observation that hypozincemia attribute to the development of asthma, especially in atopic patients.

Our results were in agreement with Metz et al. [32] who found that zinc triggered IFN- γ release and decreased interleukin-10 release. The increase in IFN- γ production after zinc supplementation is mediated by ZIP8 upregulation on T cells upon in vitro activation [33], however Metz et al. [32] attributed the enhanced IFN- γ release to the upregulation of natural killer cells after in vitro zinc supplementation.

Rosenkranz et al. [16] demonstrated that Zinc supplementation increases regulatory T cells in activated PBMCs from atopic subjects. This might explain the non-significant decrease in IL-10 demonstrated in our study, as activated T reg cells produce IL-10 [34].

Among atopic asthmatics, we examined the correlation between serum zinc levels and the concentration of cytokines produced in culture supernatant without zinc. A significant positive correlation was found between serum zinc levels and IFN- γ , moreover a significant negative correlation was observed between serum zinc levels and IL-10. In case of incubation with zinc, we observed a significant correlation between serum zinc levels and IFN- γ as well as IL-10. On the other hand, no correlation was demonstrated between serum zinc levels and IFN- γ as well as IL-10, in non-atopic asthmatics and controls, neither in the presence nor absence of zinc in culture. These findings reinforce the role of zinc supplementation in adjusting the Th1/Th2 imbalance among atopic asthmatics.

One of our study limitations was that we did not measure the serum

levels of IL-10 and IFN- γ in atopic asthma compared with non-atopic and control individuals.

In conclusion, we demonstrate a low serum zinc levels in atopic asthmatic patients, which was significantly linked to total serum IgE as a marker of atopy. Also, we observed an association of the hypozincemia observed among atopic asthmatics with alterations in the Th1 cytokine profile (IFN- γ), and the Th2 cytokine profile (IL-10). In conclusion, hypozincemia might play a role in the pathogenesis of atopic asthma. Additionally, zinc supplements might be useful in the treatment and prevention of this disease.

Ethical approval

Written informed consent was obtained from each of the participants after approving the study protocol by the Ethics Committee of Faculty of Medicine, Ain Shams University.

Conflict of interests

There is no conflict of interests. All authors do not have a direct financial relation with the commercial identities mentioned in the paper.

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