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Original Article / Özgün Araştırma

Is anti-IgE therapy effective in preventing magnesium and selenium loss in bones of mice with chronic allergic asthma?

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Abstract

Objective: Chronic allergic asthma (CA) is a respiratory disease that affects millions of people worldwide. While there is evidence linking airway hyperresponsiveness and asthma to factors related to bone metabolism, the impact of asthma on bone health is not well understood. Therefore, to explore whether: (i) CA causes meaningful changes in bone magnesium (Mg) and selenium (Se) levels, and if any, (ii) anti-IgE (anti-immunoglobulin E) treatment has a protective effect against these changes.

Methods: In present study used tibia bones from a previous study on CA in mice. A murine model was used to generate CA. Thirty-two BALB/c male mice were randomly divided into four equal sized groups (eight mice/group): control group (intact), CA (treated with saline (0.9% NaCl), CA+L-AIgE (100 µg of anti-IgE), CA+H-AIgE (200 µg of anti-IgE). After immunization, saline was administered by inhalation three times a week. Anti-IgE applications were performed intraperitoneally for a total of 8 weeks in five sessions with 15-day intervals. Bone Mg and Se levels are determined by inductively coupled plasma mass spectrometry (ICP-MS), which is used to determine the elemental composition of various samples.

Results: Mg levels of CA and CA+L-AIgE groups were significantly decreased compared to the control ($P<0.01$ for both comparisons). The mean Mg level of the CA+H-AIgE group was close to the control, and the difference was not significant. In all study groups, Se levels were significantly reduced compared to the control ($P<0.05$ for the CA group, $P<0.01$ for CA+L-AIgE and CA+H-AIgE groups). No other significant difference was detected among the groups.

Conclusion: Our study provides solid evidence of an association between CA and lower levels of Mg and Se in bones. Current data also showed that anti-IgE therapy can partially and dose-dependently prevent Mg loss induced by CA. These results have significant implications for the treatment and management of bone problems associated with asthma, highlighting the potential for anti-IgE use as a viable treatment for preventing and treating bone mineral metabolism abnormalities in asthma patients.

Keywords: Asthma, Anti-IgE, ICP-MS, Mineral Loss, Magnesium, Selenium

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Anti-IgE tedavisi, kronik alerjik astımı olan farelerin kemiklerindeki magnezyum ve selenyum kaybını önlemede etkili midir?

Öz

Amaç: Kronik allerjik astım (CA), dünya genelinde milyonlarca insanı etkileyen bir solunum hastalığıdır. Solunum yolları hiperreaktivitesi ve astımın kemik metabolizmasıyla ilişkili faktörlerle bağlantısı olduğuna dair kanıtlar olsa da, astımın kemik sağlığı üzerindeki etkisi iyi anlaşılmamaktadır. Bu nedenle, bu çalışmada amaç, (i) CA'nın kemik magnezyum (Mg) ve selenyum (Se) düzeylerinde anlamlı değişikliklere neden olup olmadığını ve varsa, (ii) anti-IgE (anti-immunoglobulin E) tedavisinin bu değişikliklere karşı koruyucu etkisi olup olmadığını araştırmaktır.

Yöntemler: Bu çalışmada, daha önce farelerde CA üzerine yapılan bir çalışmadan tibia kemikleri kullanıldı. Farelerde CA modeli oluşturmak için BALB/c cinsiyeti erkek 32 fare dört eşit boyutta gruba (her biri sekiz fare) rastgele ayrıldı: kontrol grubu (sağlam), CA (salin (%0,9 NaCl) ile tedavi edilen), CA+L-AIgE (100 µg anti-IgE), CA+H-AIgE (200 µg anti-IgE). İmmunizasyondan sonra, salin üç kez haftada bir inhalasyon yoluyla uygulandı. Anti-IgE uygulamaları, beş seans halinde toplam 8 hafta boyunca 15 günlük aralıklarla intraperitoneal olarak yapıldı. Kemik Mg ve Se düzeyleri, çeşitli örneklerin elementel bileşimini belirlemek için kullanılan indüktif eşleştirilmiş plazma kütle spektrometresi (ICP-MS) ile belirlendi.

Bulgular: CA ve CA+L-AIgE gruplarının Mg düzeyleri kontrole göre anlamlı olarak azaldı (her iki karşılaştırma için $P<0.01$). CA+H-AIgE grubunun ortalama Mg düzeyi kontrole yakındı ve aradaki fark anlamlı değildi. Tüm çalışma gruplarında Se seviyeleri kontrole göre anlamlı olarak azaldı (CA grubu için $P<0.05$, CA+L-AIgE ve CA+H-AIgE grupları için $P<0.01$). Gruplar arasında başka anlamlı bir fark saptanmadı.

Sonuç: Çalışmamız, CA ile kemiklerde Mg ve Se seviyelerinin azalması arasında bir ilişki olduğuna dair sağlam kanıtlar sunmaktadır. Ayrıca, anti-IgE tedavisinin CA tarafından indüklenen Mg kaybını kısmen ve doza bağımlı olarak hafiflettiğini bulduk. Bu sonuçlar, astımla ilişkili kemik problemlerinin tedavisi ve yönetimi için önemli etkilere sahiptir ve anti-IgE kullanımının astım hastalarında kemik mineral metabolizması anormalliklerini önleme ve tedavi etmede geçerli bir tedavi olarak potansiyelini vurgulamaktadır.

Anahtar kelimeler: Astım, Anti-IgE, ICP-MS, Mineral Kaybı, Magnezyum, Selenyum.

INTRODUCTION

Asthma is a chronic condition that results in inflammation and constriction of the airways leading to airflow obstruction[1]. In the treatment of asthma, medications such as inhaled corticosteroids (ICS), long-acting beta-agonists, and oral medications (corticosteroid, antihistamine vs.) are commonly used[2]. However, for some individuals with moderate to severe asthma, traditional treatments may prove to be ineffective, prompting the exploration of alternative therapies such as anti-immunoglobulin E (anti-IgE) monoclonal antibody, an Food and Drug Administration (FDA) approved medication specifically for asthma[3]. It has also, approved by the European Medicines Agency (EMA) as an add-on therapy for patients aged 12 years and older with uncontrolled severe persistent allergic asthma[4]. While there is evidence linking chronic asthma to factors related to bone metabolism and homeostasis, the impact of asthma on bone health is not well understood[5-7]. Studies have indicated that chronic asthma may be a potential risk factor for bone health[7].

Given its interdependent relationship with calcium, magnesium (Mg) is considered one of the essential minerals for bone health[8]. On the other hand, selenium (Se), known for its antioxidant properties, has been identified as a crucial mineral that plays a key role in the pathogenesis of asthma and has proven to be necessary for bone health[9]. However, the relationship between chronic allergic asthma (CA) and bone diseases, specifically the role of Mg and Se in CA pathogenesis and bone metabolism, remains unclear. To address this gap in knowledge, this study aims to explore whether: (i) CA causes meaningful changes in bone Mg and Se levels, and if any, (ii) anti-IgE treatment has a protective effect against these changes using an experimental-chronic

inhalation exposure model of induced CA in mice. This study is the first to explore the impact of CA and anti-IgE treatments on Mg and Se levels in bone.

METHODS

Animal preparation, antigen sensitization and challenge protocol

Bones used in the present study were obtained from our previous study[10]. In the study, CA development in BALB/c mice was verified by histopathological evaluations of lung and morphometric analyzes of smooth muscle and bronchial wall thicknesses. From bronchoalveolar lavage fluid, IgE levels, airway cells, and principal cells that are identified in airway inflammation were determined for the verification[10]. The tibia bones were dissected and used for the current study following the completion of the experimental protocol. The treatment, antigen sensitization, and challenge protocols utilized in our prior study were briefly summarized.

In the present study, the murine model of asthma used to study CA was carried out in accordance with Temelkovski et al.[11] A total of 32 male BALB/c mice, weighing 22-24 g and aged between 8-10 weeks, were purchased and kept under standard housing conditions. After a 24-hour acclimation period with no observable clinical signs, the mice were randomly divided into four equal groups of eight mice each: the CA group, CA+L-AIgE group, CA+H-AIgE group, and the control group. The mice in the CA, CA+L-AIgE, and CA+H-AIgE groups were sensitized through intraperitoneal injection of 10 µg/mouse of OVA (C_45 H_74 N_10 O_13·×C_2 HF_3 O_2, CAS No:138831-86-4, Grade V, Sigma-Aldrich, MO, USA) in 200 µL saline (0.9% NaCl), 21 and 7 days before inhalation exposure. The mice in the control group were treated with saline by the same route and dosage. After immunization period, on the 21st day of the study, the mice in CA, CA+L-AIgE, and CA+H-AIgE were challenged with inhalation of nebulized OVA (2.5% OVA solution in aerosolized sterile saline) via a nebulizer system (InnoSpire Essence, Philips Respironics New Jersey Inc., NJ, USA) for 30 minutes of exposure time, three times a week, up to 8 weeks[10].

On the 21st day of the study, treatment protocol was begun. During nebulized OVA challenge, 100 µg and 200 µg of anti-IgE (Xolair®, Novartis Pharmaceuticals, Istanbul, TR) dissolved in 200 µL saline were given intraperitoneally to the mice in CA+L-AIgE and CA+H-AIgE, at 15 days intervals for a total of five sessions up to 8 weeks, respectively[10-13]. The mice in the control group were not received any medication.

48 hours following the last OVA exposure, the mice were sacrificed by thoracotomy under ketamine/xylazine (100/10 mg/kg; IP) anesthesia[10,13] and their right tibiae with surrounding muscle tissue were removed. Until the experiments were completed, the dissected bones were kept at -20°C in gauze pads saturated in ordinary saline.

Determination of bone Mg and Se levels by inductively-coupled plasma-mass-spectrometer (ICP-MS)

ICP-MS was conducted to determine the Mg and Se levels of bones. For this purpose, the muscle tissue cleaned bone samples were weighed 0.0001 g accuracy with a precision scale (Shimadzu-ATX224/Uni Block, Shimadzu Europa GmbH, Duisburg, GE) and placed directly into the microwave oven tube. 2 mL of nitric acid 65% (~14.34 M; Merck KGaA, Darmstadt, GE), 6 mL of hydrochloric acid 37% (~12.06 M; Catalogue No: 100317, Merck KGaA) and 1 mL of hydrogen peroxide 30% (~9.8 M; Catalogue No: 107209, Merck KGaA), respectively, were added to the microwave tube containing bone sample. After gas outlet completed, the tube was placed in the microwave oven. Oven heated at 160°C for 10

minutes, and then the tube was allowed to cool at the room temperature. Afterwards the sample was taken to falcon tube and was filled to 50 mL with deionized water. Appropriate dilutions were performed with deionized water to prepare the sample for the analysis. Analyze ready sample was analyzed using ICP-MS. The amount of element arising from the solvent was determined by using the blank. ICP-MS gave results in ppm or ppb and the amounts of Mg and Se were obtained by multiplying the dilution coefficients made while preparing the sample. Results of three times repeated measurements were averaged. The data was expressed as mg/g bone or µg/mg as applicable.

Properties of ICP-MS device: Brand/Model: Agilent-7500ce (Tokyo, Japan); Software: ChemStation (G1834B) (ensures that each sample is safely analyzed and the data obtained is interpreted correctly); System: Octopole reaction system; Purity of argon gas used: 99.998%; Calibration range: 0-to-90 ppb in 10 ppb steps (calibration was performed using multi-element calibration standards having internationally recognized standards); Elemental detection limits: <1 ppb.

Working conditions of the device during analyzes: RF Power: 1500 W; Sample Dept: 8.8 mm; Plasma Gas: 15 L/min; Carrier Gas: 0.9 L/min; MakeUP Gas: 0.14 L/min; Aux Gas: 1 L/min; Nebulizer Pump: 0.1 rps; S/C Temp: 2oC; Mod: No gas mod; Integration Time: 0.60 sec; Rep: 3; Interference Correction: ON.

Statistical analysis

The Shapiro-Wilk test was applied to the data to assess the distribution's normality. A one-way analysis of variance (ANOVA) was carried out to see if the data were normally distributed, and then the Tamhane T2 multiple comparison test was performed. The Kruskal-Wallis test was used to determine if the data were not normally distributed; it was then followed by multiple comparisons with Bonferroni correction. Differences were deemed statistically significant if their two-tailed p-value was less than 0.05. The data were shown as median [interquartile range] in the figures as opposed to mean + standard deviation (SD) in the table. Statistica 8.0 Program (StatSoft Inc., Tulsa, OK, USA) and SPSS for Windows (Release 20.0.0, Lead Technologies Inc., Chicago, IL, USA) were used to conduct the statistical analyzes, as applicable.

RESULTS

Mg levels of CA and CA+L-AIgE groups were significantly decreased compared to the control ($P<0.01$ for both comparisons; Table I and II, Fig. 1). The mean Mg level of the CA+H-AIgE group was close to the control, and the difference was not significant (Table I and II, Fig. 1). In all study groups, Se levels were significantly reduced compared to the control ($P<0.05$ for the CA group, $P<0.01$ for CA+L-AIgE and CA+H-AIgE groups; Table I and II, Fig. 2). No other significant difference was detected among the groups.

Table I: Mean ± SD values of bone Mg and Se levels of the groups.

	Control Group	Study Groups		
		CA	CA+L-AIgE	CA+H-AIgE
Magnesium (Mg; mg/g bone)	2.00 ± 0.05	1.79 ± 0.10	1.70 ± 0.12	1.88 ± 0.23
Selenium (Se; µg/g bone)	1.95 ± 1.07	0.69 ± 0.19	0.66 ± 0.16	0.69 ± 0.06

CA: Chronic Allergic Asthma; CA+L-AIgE: Chronic Allergic Asthma+100 µg of anti-IgE; CA+H-AIgE: Chronic Allergic Asthma+200 µg of anti-IgE.

Table II: Comparisons of bone Mg and Se levels among the groups.

Groups (I)	Multiple Comparisons for Mg Levels ANOVA test: F(3, 28) = 6.883, p = 0.001			Multiple Comparisons for Se Levels Kruskal-Wallis test: H(3, N=32) = 16.625, p = 0.0008			
	Group (J)	Mean Difference (I-J)	Sig.* (*P*-Value)	Group	Mean of Rank	z' Values	Sig.* (*P*-Value)
Control	CA	0.215*	< 0.01	CA	13.88	3.038*	< 0.05
	CA+L-AlgE	0.308*	< 0.01	CA+L-AlgE	11.63	3.518*	< 0.01
	CA+H-AlgE	0.124	0.689	CA+H-AlgE	12.38	3.358*	< 0.01
CA	Control	-0.215*	< 0.01	Control	28.13	3.038*	< 0.05
	CA+L-AlgE	0.093	0.541	CA+L-AlgE	11.63	0.479	1.000
	CA+H-AlgE	-0.092	0.908	CA+H-AlgE	12.38	0.320	1.000
CA+L-AlgE	Control	-0.308*	< 0.01	Control	28.13	3.518*	< 0.01
	CA	-0.093	0.541	CA	13.88	0.479	1.000
	CA+H-AlgE	-0.184	0.362	CA+H-AlgE	12.38	0.160	1.000
CA+H-AlgE	Control	-0.124	0.689	Control	28.13	3.358*	< 0.01
	CA	0.092	0.908	CA	13.88	0.320	1.000
	CA+L-AlgE	0.184	0.362	CA+L-AlgE	11.63	0.160	1.000

**Significant at the 0.05 level. CA: Chronic allergic asthma; CA+L-AlgE: CA+100 µg of anti-IgE mAb therapy; CA+H-AlgE: CA+200 µg of anti-IgE mAb therapy.*

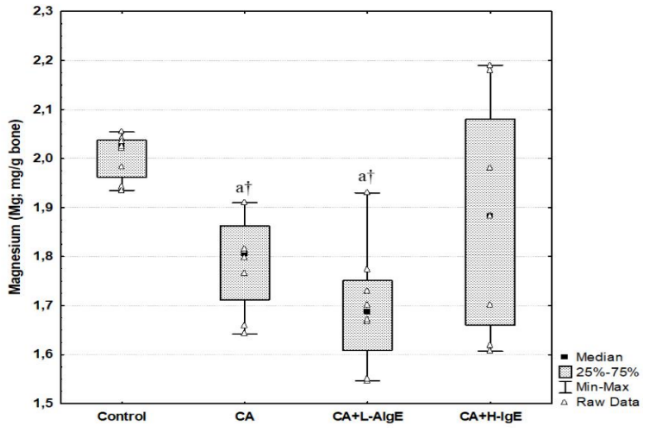

Fig. 1. Comparisons of bone Mg levels among the groups. Cases with statistically significant differences are shown in the figure.

[a]vs. Control †P<0.01

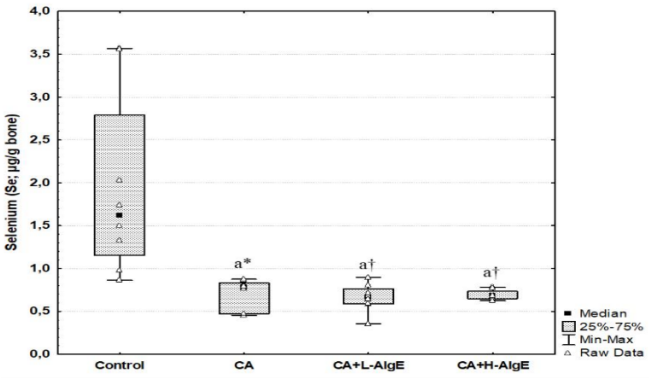

Fig. 2. Comparisons of bone Se levels among the groups. Cases with statistically significant differences are shown in the figure.

*[a]vs. Control *P<0.05 †P<0.01*

DISCUSSION

The study demonstrates that CA exposure leads to a reduction in bone Mg and Se content, indicating a detrimental impact on bone structure. Mg is a crucial mineral for bone development and mineralization, as it promotes osteoblast activity and the function of phosphatase enzymes involved in bone formation[8]. Mg serves as a cofactor for various biological processes, including glycolysis, lipid, protein, and nucleic acid synthesis, and functions as a signaling transducer due to its positively charged ion nature, while stabilizing cell membranes[14]. Mg also opposes calcium and can directly affect bone density by decreasing number of osteoblasts, increasing number of osteoclasts, and reducing bone stiffness[15]. Indirectly, it disrupts PTH and vitamin D metabolism, promotes inflammation and oxidative stress, and causes bone loss[8]. Thus, assessing bone Mg levels in both physiological and pathological states is crucial, as Mg can interfere with calcitropic hormones and is recognized as a natural calcium antagonist[16]. Mg deficiency is known to have a direct impact on bone density, as animal studies have shown that inadequate dietary Mg intake supports the development of osteoporosis[17,18]. Animals

deficient in Mg have fragile bones, microcracks in trabecular bone, and significantly impaired mechanical properties[7]. It is also important to note that low Mg intake delays cartilage and bone differentiation and matrix calcification[19]. Evaluating Mg levels in bone physiology and pathology situations is critical, given the vital role Mg plays in bone development and mineralization.

Se, like Mg, is an essential mineral that plays a vital role in maintaining bone health. Its role likely involves the functions of selenoproteins, which are antioxidant enzymes that regulate cellular redox balance, inflammation, and bone cell proliferation/differentiation[9,20]. At higher doses, Se can also play additional cellular roles, such as immune function, osteoclast inactivation, cell cycle arrest, and apoptosis, which help prevent bone resorption[9]. Observational studies also show a positive correlation between Se levels and bone health, with higher Se levels linked to increased bone mineral density and a reduced risk of osteoporotic fractures[9]. It is evident that both Mg and Se are critical minerals for maintaining healthy bones, and their deficiency resulting from asthma can have detrimental effects on bone structure and function. Therefore, our findings are of utmost importance as they shed light on the critical role that Mg and Se play in maintaining healthy bones, particularly in patients with asthma. By identifying the potential risks of deficiencies in these essential minerals, our research underscores the need for regular assessment of Mg and Se levels in individuals with asthma to prevent the development of bone-related complications.

Studies have revealed that chronic airway diseases can lead to the development of osteoporosis due to the systemic inflammatory response they induce[21]. The bone is a dynamic organ that undergoes cycles of resorption and deposition of matrix proteins and minerals[22]. Normally, these processes are balanced and do not result in a net loss or increase in bone mineral density. However, during an inflammatory attack, there is a shift towards bone resorption that leads to a net loss of bone tissue[22]. Inflammatory mediators play a crucial role in triggering bone resorption[23], and chronic low-grade inflammation can decrease bone quality[24]. The immune response produces many oxidative compounds to eliminate invading agents, and asthma is associated with strong oxidative stress due to increased oxidant forces and decreased antioxidant capacity[25]. Recently, a perspective article suggested that Mg and phosphate released by bone during resorption could have a nutritional function for activated immune cells[22]. Our findings align with the plausible proposition that Mg liberated during bone resorption.

Various long-term control therapies such as ICS, long-acting beta-agonists, oral medications (corticosteroid, antihistamine vs.), and inhaled bronchodilators are available for treating asthma[26]. However, these treatments may have serious side effects and may not be effective for some patients, prompting researchers to investigate alternative therapies[27]. One such alternative therapy is the use of anti-IgE monoclonal antibody, which has been shown to be efficacious and safe for moderate to severe asthma[28]. Anti-IgE therapy suppresses free IgE and inhibits inflammation associated with allergen-induced inflammation, including mast cell degranulation, histamine release, late-phase asthma responses, pulmonary eosinophil infiltration, and nitric oxide concentration in the respiratory tract[29]. In our previous study[7], we investigated the effects of anti-IgE therapy on asthma morbidity, bone mechanics, and mineral properties in a chronic inhalation exposure model of asthma in mice. Our results demonstrate the efficacy of anti-IgE therapy in promoting bone mineralization. However, selenium supplementation may be required in addition to anti-IgE therapy to prevent bone

loss and maintain optimal bone health in individuals with asthma.

CONCLUSION

Our study provides solid evidence of an association between CA and lower levels of Mg and Se in bones. Current data also showed that anti-IgE therapy can partially and dose-dependently prevent Mg loss induced by CA. These results have significant implications for the treatment and management of bone problems associated with asthma, highlighting the potential of anti-IgE treatment as a viable technique for preventing and treating bone strength and bone mineral metabolism abnormalities in asthma patients. However, further research is needed to validate our findings and gain a better understanding of the underlying processes that drive these observed alterations in bone physiology. Overall, present study represents an important step forward in our knowledge of the complex link between asthma and bone health.

Ethics Committee Approval: All experiments and protocols described in this study were executed under the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (CETS #123) and were confirmed by the local experimental ethics committee of Kahramanmaras Sutcu Imam University with the decision number 2013/01, dated 23.01.2013. All authors consent to participate in this research.

Conflict of Interest: The authors declared no conflicts of interest.

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