

Research Article

Inhaled Glucocorticoid Use and the Risk of Osteoporosis in Asthmatic Patients

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Abstract

Introduction: Many recent studies have focused on the possible role of corticosteroid inhalation in osteoporosis in asthmatic patients.

Aim: This study aimed to determine whether the indicators of bone formation or resorption are different between asthmatic patients and healthy subjects.

Materials and Methods: To achieve this outcome, 21 middle-aged patients with mild to moderate asthma treated with inhaled corticosteroid and the same number of healthy individuals matched for age (38 ± 6.5 years) were enrolled in this study by accessible sampling. All the subjects were non-trained and non-smokers. The serum levels of osteocalcin (OC), alkaline phosphatase (ALP), and cross-linked telopeptides of type I collagen (CTX) were measured to assess and compare bone formation and resorption between the two groups. An independent-sample *t* test was used to compare all variables between the patients with asthma and the healthy subjects.

Results: Significant differences in body weight and other anthropometrical markers were not observed between the two groups ($p > 0.05$). Serum osteocalcin level was borderline significantly lower in the asthmatic patients than in the healthy subjects ($p = 0.051$). The ALP level was significantly lower ($p = 0.021$) but the serum CTX levels were higher in the asthmatic patients than in the healthy subjects ($p = 0.014$).

Conclusion: On the basis of these findings, inhaled corticosteroid can affect bone turnover in asthmatic patients, although more research is needed to further explore any potential link between corticosteroids and osteoporosis.

Keywords: Asthma, Bone turnover, Osteocalcin, Corticosteroid

1. Introduction

Clinical studies have shown that patients with asthma and chronic obstructive pulmonary disease, especially those who undergo long-term corticosteroid therapy, are more exposed to an increased risk of osteoporosis [1]. The findings indicate that the risks of vertebral and non-vertebral fractures are respectively 6.2 and 4.1 times more in patients with asthma than in healthy people [2]. Meanwhile, patients with asthma

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who use oral or inhaled corticosteroids have the lowest bone density and doubled osteoporosis risk in comparison with patients without history of corticosteroid use [2].

The results of a previous study indicated that the treatment of asthma with inhaled corticosteroids influences the serum or plasma levels of the biochemical indexes of bone resorption and formation. In this regard, Jan et al. found that the use of corticosteroids in asthmatic patients decreases osteocalcin bone content [3]. Osteocalcin is the major bone matrix non-collagen protein [2] produced by osteoblasts [4]. Osteocalcin is the special marker of osteoblast function, whose major part takes place in the extracellular bone matrix after synthesis and its minor part has access to the blood stream [5]. Apart from osteocalcin, alkaline phosphatase has also been known to be a marker of bone formation; that is, its increase is representative of new bone tissue formation by osteoblasts [6]. On the other hand, the increases in the levels of biochemical markers of bone resorption, including the C-terminal telopeptide of type 1 collagen (CTX), hydroxyproline, and pyridinoline, and the decreases in the levels of the markers of bone formation, including osteocalcin and alkaline phosphatase, are all associated with bone loss and osteoporosis [5]. Almost 55% of the differences in bone density, strength, and osteoporosis severity in asthmatic patients are dependent on the amount of inhaled corticosteroid consumption [7]. In this regard, studies have shown that one-session corticosteroid injection in 36 asthmatic children leads to immediate suppression of osteoblast cell activities and decreased levels of osteocalcin and alkaline phosphatase [3]. These evidences support the consumption of inhaled corticosteroid as a predictive indicator of osteoporosis in asthmatic patients.

Despite the aforementioned evidences, some studies indicated that the consumption of corticosteroids does not have any effect on the markers of bone turnover and metabolisms. For instance, a study found no changes in the serum levels of osteocalcin, potassium, calcium, and magnesium after 9 months of corticosteroid consumption as compared with the baseline levels in patients with asthmatic bronchitis [8]. Harmanci et al. (2001) found that 6 months of corticosteroid therapy in asthmatic patients does not change the biochemical indexes of bone formation and resorption, including osteocalcin, bone and serum alkaline phosphatase, deoxypyridinoline, parathyroid hormone, calcium, and fasting glucose [9]. Aljubran (2014) found no changes in serum CTX level in spite of the 3-week corticosteroid consumption [10].

A review of the aforementioned findings revealed, to some extent, a contradiction in the response of markers of bone formation and destruction to inhaled corticosteroids in asthmatic patients. That is, some studies indicated that inhaled corticosteroid consumption in asthmatic patients causes osteoporosis by affecting the markers of bone metabolism [2, 3]. Whereas some other studies showed the lack of response of the markers of bone metabolism to inhaled corticosteroids [8, 9]. According to these contradictory evidences, the present study intended to compare the baseline levels of some markers of bone formation and destruction (osteocalcin, alkaline phosphatase, and CTX) between adult men with asthma treated with corticosteroids and healthy men.

2. Materials and Methods

2.1. Subjects and patients

In this comparative study, 42 non-active middle-aged men (38 ± 6.5 years old) who either had asthma ($n = 21$) or were healthy ($n = 21$) were enrolled by accessible sampling.

$$n = \frac{(\sigma_1^2 + \sigma_2^2) \left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right)^2}{\lambda^2}$$

($\alpha = 0.05$, $1 - \beta = 0.8$, $\Delta = \text{effect size} = 0.36$)

($\Delta = d/\delta$)

Asthma diagnosis and its severity were determined on the basis of forced expiratory volume in 1 second/forced vital capacity (FEV₁/FVC) and other respiratory volumes, and evaluation of clinical protests by a specialist. Thus, the mean of FVC, FEV₁, and FEV₁/FVC ratio were $71\% \pm 5.61\%$, $72\% \pm 6.45\%$, and $69.8\% \pm 2.86\%$, respectively. On the basis of this information, asthma severity was diagnosed as mild to moderate. Approval for this study was granted by the ethics committee of Islamic Azad University, Saveh branch, Iran. After explaining the nature of the study in detail, informed consent was obtained from all the participants.

2.2. Inclusion and exclusion criteria

Medical history taking was conducted to retrieve information about health status, current medications, and physical examination for all the patients. All the subjects in the two groups were inactive, non-smoker, and non-alcoholic. The inclusion criteria for the asthma group were determined as existing asthma for at least 3 years and use of inhaled corticosteroids for 1 year. However, the inhaled corticosteroid dose differed by 2–4 puffs among the patients. The participants were included if they had no regular physical activity in the previous 6 months. Subjects with a history or clinical evidence of impaired fasting glucose level or diabetes, a recent myocardial infarction, an active liver or kidney disease, and other chronic diseases were excluded.

2.3. Anthropometry

The anthropometrical markers were measured in each patient. Height was measured without shoes on standing position while the shoulders were tangent with the wall. Body weight was measured on the same day to the nearest 0.1 kg. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Abdominal circumference (WC) was measured at the superior border of the iliac crest and rounded off to the nearest 0.1 cm after normal expiration. Percentage body fat was measured using a body composition monitor (Omron, Finland).

TABLE 1: Mean and standard deviation of the anthropometric characteristics of study subjects.

Variables	Asthmatic patients	Healthy subjects
Age (years)	37.8 ± 5.61	38 ± 6.23
Height (cm)	174.6 ± 2.69	175 ± 3.26
Weight (kg)	93.4 ± 8.32	93.6 ± 9.48
AC (cm)	104 ± 9.65	103 ± 10.32
HC (cm)	105 ± 7.68	106 ± 7.83
BMI (kg/m ²)	30.02 ± 3.11	30.59 ± 2.44
Body fat (%)	29.14 ± 3.23	29.31 ± 2.32

AC, abdominal circumference; AH, hip circumference; BMI, body mass index

3. Statistics

3.1. Blood sampling and analyses

Blood samples were collected for measurement of the serum levels of osteocalcin, CTX, and ALP in the 2 groups. A venous blood sample was collected from each subject after a 10- to 12-h overnight fast between the hours of 8 a.m. and 9 a.m. The subjects were asked to avoid any physical activity for 48 hours before blood sampling. After sampling in EDTA or serum tubes, blood samples were immediately chilled on ice and centrifuged, and aliquots were stored at -80°C until biochemical analyses were performed. ALP level was measured using a photometric method (Pars Azmoon-Tehran, Iran) by AutoAnalyzer (RA-100, Canada). Serum samples were used to measure osteocalcin with the ELISA (enzyme-linked immunosorbent assay for quantitative detection of human osteocalcin-CTX, Biovendor, Austria). The inter- and intra-assay coefficients of variance and sensitivity of osteocalcin level were 1.3, 5.1%, and 0.5 ng/ml respectively. The inter- and intra-assay coefficients of variance and sensitivity of CTX were 3/0%, 10/9%, and 40 ng/ml, respectively.

3.2. Data analysis

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc, Chicago, IL, USA). Normal distribution of data was analyzed using the Kolmogorov-Smirnov normality test. The independent Student *t* test was used to compare the variables between the two groups. *P* values of <0.05 were considered statistically significant.

4. Results

The physical characteristics of the subjects are shown in Table 1. Data are expressed as mean \pm SD. No statistically significant differences were observed between the 2 groups with regard to the anthropometrical markers ($P > 0.05$).

Comparison of serum osteocalcin and CTX levels between middle-aged men with asthma and healthy subjects was the main aim of the present study. On the basis of

TABLE 2: Mean and standard deviation of bone formation and resorption in the study subjects.

Variables	Asthmatic patients	Healthy subjects
Osteocalcin (ng/ml)	27.8 ± 4.36	33.6 ± 4.25
ALP (U/l)	251 ± 56*	281 ± 51
CTX (ng/ml)	0.35 ± 0.11*	0.29 ± 0.09

ALP, alkaline phosphatase; CTX, cross-linked telopeptides of type I collagen
*Significant difference with the healthy subjects.

the independent sample *t* test, the asthmatic patients showed significantly lower ALP levels than the healthy subjects ($P = 0.021$, Figure 1).

In addition, serum CTX concentrations were significantly higher in asthmatic patients than healthy subjects ($P = 0.014$, Figure 2).

Despite the significant differences in ALP and CTX levels between the 2 groups, a borderline significantly lower serum osteocalcin level was found in the asthmatic patients than in the healthy individuals ($P = 0.051$, Figure 3).

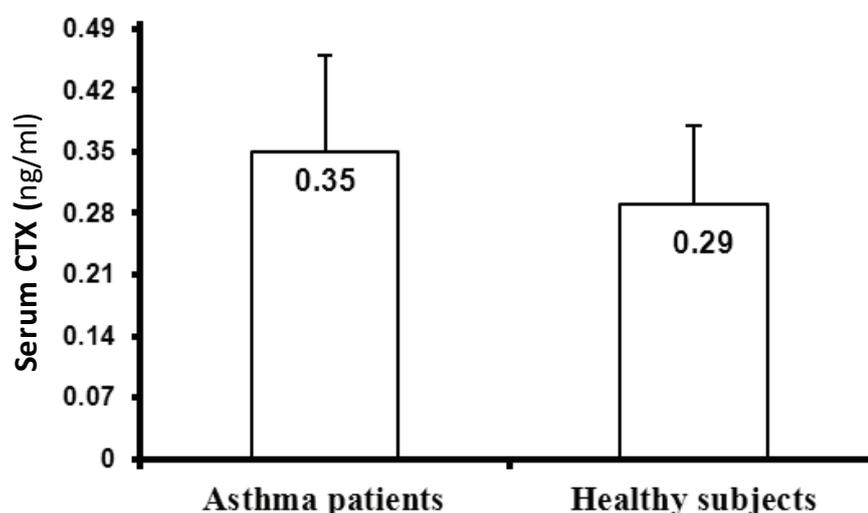


Figure 1: Significantly higher serum CTX levels in the asthmatic patients than in the healthy individuals ($P = 0.014$).

5. Discussion

On the basis of our data, although the findings of the present study indicated a borderline significantly lower osteocalcin level in the asthmatic patients than in the healthy subjects, the serum CTX level was far higher in the asthmatic patients. In other words, the results of the present study showed that the asthmatic patients had higher CTX levels than the healthy subjects. On the other hand, the asthmatic patients had lower alkaline phosphatase level, another marker of bone formation, than the healthy men. Although inhaled corticoids are introduced as the most effective and most accessible medicinal intervention to control asthma, their entry from the lungs to the blood

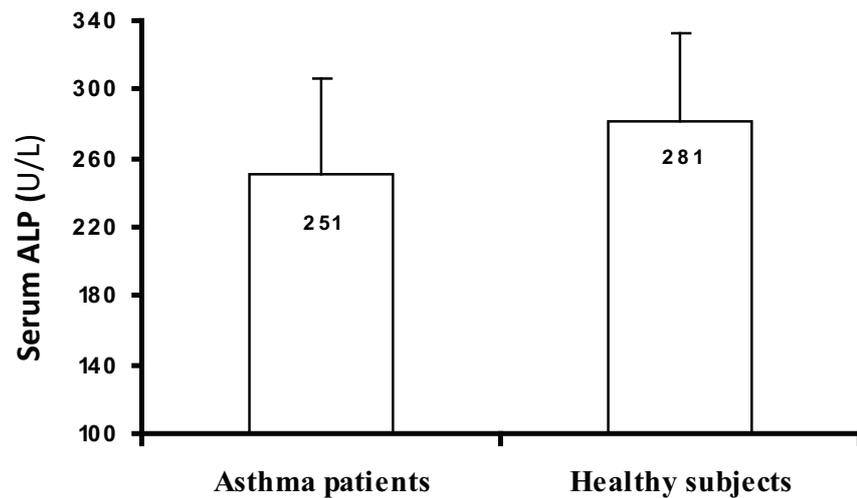


Figure 2: Significant lower of ALP levels in the asthmatic patients than in the healthy individuals ($P = 0.021$).

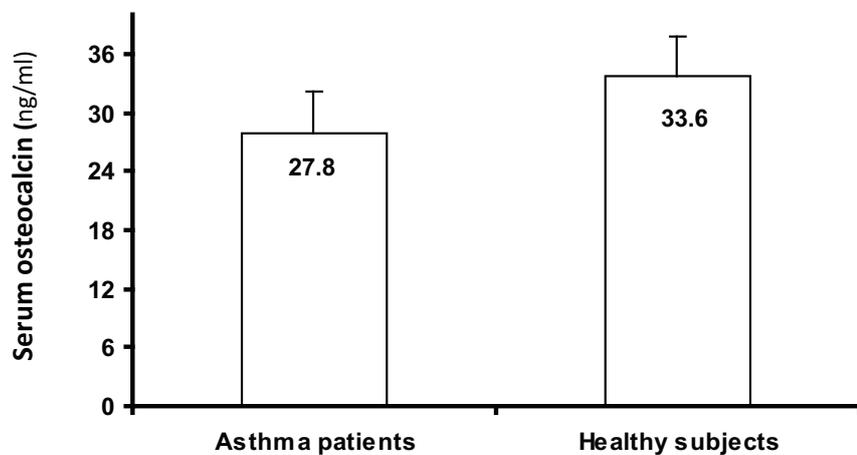


Figure 3: Borderline significantly lower serum osteocalcin levels in the asthmatic patients than in the healthy individuals ($P = 0.051$).

stream has some non-negligible side effects [11]. The inhibition of airway inflammation by corticosteroids decreases the hyperresponse of airways and controls asthma symptoms. Glucocorticoids penetrate to the cytoplasm through the cell membrane and are connected to its receptors in the cytoplasm [12]. Their entry into the respiratory tract can activate or deactivate (inhibit) some genes associated with asthma by their function [13].

Epithelial cells, the main location of the asthma symptoms, are the most important target locations of inhaled corticosteroids. Thus, inhaled corticosteroids inhibit the transcription of several inflammatory genes in airway epithelial cells and reduce airway wall inflammation [11]. They also increase the gene transcriptions of lipocalin 1, adrenergic receptors, leukocyte protease inhibitor, and anti-inflammatory cytokines, including interleukin-10, interleukin-12, and interleukin-1 receptor antagonists, and inhibit the MAP kinase-dependent pathways in the airways. Furthermore, they also decrease

the transcription of inflammatory cytokines (including interleukine-2 and interleukine-6, interleukine-11, interleukine-15, and TNF- α), chemokines (including interleukine-8 and E-otaxin), inflammatory peptides (including endothelin-1), and transcription of adhesion molecules, including ICAM-1 and VCAM-1 in the airways. At a cellular level, inhaled corticosteroids reduce inflammatory cells in the airways such as eosinophils, lymphocyte T-cells, mast cells, and dendritic cells [11]. The inhibition of mucosal inflammation by inhaled corticosteroids occur rapidly, which is associated with the significant decrease in eosinophils in a 6-hour period and decrease in hyper-response of the airways [14].

However, the use of high doses of corticosteroids has negative systematic effects, including bone diseases such as rickets, osteoporosis, and bone necrosis. Corticoids have destructive effects on the function and survival of osteoblasts and osteocytes, and on the maintenance and prolongation of osteoclasts, which are associated with metabolic bone diseases. Glucocorticoids reduce osteoblastogenesis, increase osteoblast cell death, and decrease their bone formation ability [15]. It has been recognized that the use of glucocorticoids, which changes the potential of bone marrow mesenchymal stem cells (aka adipogenic differentiation), is associated with the reduction of osteoblastogenesis [16]. Moreover, the increased production of reactive oxygen species caused by steroids leads to osteoblast cell death [17].

Studies on rat species showed that the activity of glucocorticoid receptors in osteoblasts reduces bone mass, thick bands of bone connective tissue (tendon), number of osteoblasts, and colony-forming units [18]. The inhibition of the activity of cytokine-derived osteoblasts is caused by steroids such as interleukin-11 and by damages to osteoblast differentiation. These factors reduce osteocalcin and alkaline phosphatase levels by long-term use of corticosteroids. In the present study, although the lower osteocalcin levels in the asthmatic patients were statistically insignificant in comparison with those in the healthy subjects, these were significant from a clinical perspective. On the one hand, the insignificance of this difference may be attributed to the small number of study samples or score distribution. These patients had lower alkaline phosphatase level, one of the markers of bone formation, than the healthy group. In bones, osteoblasts are a great source of alkaline phosphatase, and their levels in cells are indicative of the osteogenic ability of osteoblasts [19]. Moreover, the decreased levels of alkaline phosphatase along with other markers of bone formation are associated with bone destruction or decreased bone formation [20].

Osteocyte cell death caused by the use of corticosteroids was first proposed by Weinstein (1998) [21]. Approximately 15% to 20% of osteoblast cell deaths have been reported in rats treated with steroids. Increased osteocyte lacunar size and loss of minerals in osteocytes have also been observed in rats treated with steroids [22]. Xia et al. (2010) reported a 50% decrease in the number of osteocytes in rats treated with steroids [23]. Increased autophagy of osteocytes has also been introduced as one of the mechanisms caused by osteocyte cell death as the result of the use of glucocorticosteroids. Osteocalcin gene expression in osteoclasts and bone resorption cells have been found to be reduced by glucocorticosteroid administration [24]. However, the precursors of osteoclasts, macrophages, and monocytes derived from bones

can result in mature osteoclast formation by using glucocorticosteroids [24]. Proving the aforementioned evidences, the findings of the present study indicate that the asthmatic patients who used inhaled corticosteroids had higher CTX levels, a marker of bone destruction, than healthy men.

The responses of metabolic markers or determinants of bone to corticosteroids are, to some extent, different depending on the age and sex of the patients. In this regard, Monadi et al. (2015) intended to determine the effect of oral use of corticosteroids for 5–6 years on bone density in the lumbar spine and femoral neck of 44 asthmatic patients at different age ranges (with a mean age of 50 years). They found that the bone densities in both areas of the body were lower in the asthmatic patients than in the healthy group. Moreover, the decrease in bone density was higher in the younger asthmatic patients. On the basis of these evidences, the researchers concluded that the effect of corticoids on bone density in asthmatic patients is greatly dependent on the patients' age. That is, younger patients are more exposed to decreased bone density and osteoporosis in response to corticosteroid consumption than older patients [25].

On the basis of the data presented herein, we can conclude that inhaled corticosteroids can affect bone metabolism in asthmatic patients. Thus, osteoporosis or disturbance in bone formation and resorption mechanisms in these patients can be attributed to inhaled corticosteroids. Overall, inhaled glucocorticoids contribute to the development of osteoporosis in asthmatic patients. However, inhaled corticosteroids are advocated for the treatment of asthmatic patients. Therefore, new solutions that can attenuate the side effects of inhaled corticosteroids in asthmatic patients by compensatory mechanisms seem necessary.

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Authors' Contributions

All authors had equal roles in the design, work, statistical analysis, and manuscript writing.

Conflicts of Interest

The authors declare no conflict of interest.

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