ORIGINAL ARTICLES

Asthmatic Patients and the Risk of Osteoporosis

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ABSTRACT

Background: Corticosteroids are the most effective anti-inflammatory medications for the treatment of asthma. Systemic corticosteroid therapy is associated with significant adverse effects, including the promotion of osteoporosis. The effects of inhaled corticosteroid therapy on bone metabolism are not certain but are a concern with the increasing use of moderate and high doses for longer periods of time. Objectives: Study the effect of chronic inflammation and corticosteroid therapy on the bone mineral density in asthmatic patients. Methods: 111 subjects were divided into a control group and three asthmatic groups (1st one was on bronchodilator therapy only, the 2nd group was on inhaled steroids and the 3rd one was on inhaled and systemic steroids). Determination of serum level of receptor activator of the nuclear factor kB ligand (RANKL), osteoprotegerin (OPG) and assessment of bone mineral density by DEXA at proximal femur and lumbar spines were done. Results: There was statistically significant difference concerning the prevalence of osteoporosis of the proximal femur of the third group of asthmatic patients when compared to the other studied groups. There was statistically significant difference concerning the mean value of RANKL in the asthmatic patients groups when compared to the control group. Conclusion: This study indicated that the inflammatory load by asthma and the steroid therapy are risk factors for osteoporosis. So, asthmatic patients are at risk of osteoporosis. These patients should be investigated for the status of their bone mineral density. This study suggests the proximal femur to be the optimal site for assessment of osteoporosis in asthmatic patients.

Key words: Osteoporosis, asthma, corticosteroid, inhaled corticosteroid, bone density

Introduction

Corticosteroids (CS) are the most effective anti-inflammatory medications for the treatment of asthma. Systemic CS therapy is associated with significant adverse effects, including the promotion of osteoporosis (Bouvard et al., 2010). In contrast to systemic CS therapy, inhaled CSs provide effective therapy for asthma with few adverse effects (Expert Panel Report II, 1997).

The effects of inhaled CS therapy on bone metabolism are not certain but are a concern with the increasing use of moderate and high doses for longer periods of time. The relative risk of osteoporosis for different inhaled CS preparations and dosages has not been defined (Bouvard et al., 2010).

Patients on CS therapy are at increased risk of fractures. In asthma, the incidence of vertebral (Luengo et al., 1991) and rib (Hassel and Dacre, 2002) fractures was increased in patients on long-term CS compared with those who were not on CS.

Corticosteroids directly affect bone cells in a number of ways. This may be by stimulating osteoclastogenesis, decreasing osteoblast function and life span, increasing osteoblast apoptosis and impairing preosteoblast formation (Yao et al., 2008).

Corticosteroids act on osteoclastogenesis through osteoblastic signals on the receptor activator of the nuclear factor kB ligand (RANK-L) - osteoprotegerin (OPG) axis. Glucocorticoids enhance RANK-L, which binds and activates RANK on the surface of osteoclast precursors, and also inhibit OPG production, with a consequent induction of osteoclastogenesis and early increase in bone resorption in CS induced osteoporosis (Takuma et al., 2003), (Dore et al., 2010).

Understanding of bone remodeling process suggests that factors involved in inflammation influence bone physiology and remodeling, supporting the theory that inflammation significantly contributes in the pathogenesis of osteoporosis (Arron and Choi, 2000), (Lorenzo, 2000).

The purpose of bone density assessments is to identify individuals at risk of fractures and to monitor the treatment of osteoporosis. Various techniques are available to assess bone density. Dual-energy x-ray
absorptiometry (DEXA) and quantitated computed tomography (QCT) are most widely used. Presently, DEXA is preferred over QCT because it is less expensive, more accurate and requires less radiation exposure (Compston, 2010).

DEXA values are interpreted by comparing the number of standard deviations above or below the mean density to that of a young, adult, gender-matched population (T score). T score compares an individual with his or her ideal. Osteoporosis is defined as a T scores more than 2.5 SDs below the mean. Osteopenia is defined as a T score between –2.5 and –1.0. The relative risk for a fracture doubles for every decrease of 1 SD in bone mass (World Health Organization, 1994).

Aim of the work:

This work was carried out to study the effect of chronic inflammation and corticosteroids therapy on bone mineral density in asthmatic patients.

Subjects and Methods:

The present study was conducted at the Internal Medicine Department Medical Services Unit of the National Research Centre – Cairo - Egypt. The study was approved by ethical committee and all patients agreed informed consent for participation.

The study included 111 subjects; all of them were subjected to:
1. History taking and clinical examination.
2. Plain chest X-ray.
3. Laboratory Tests (S.creatinine, B.urea, Liver transaminases, Thyroid stimulating hormone, Tri & Tetraiodothyronin and Parathyroid Hormone).
4. Serum measurement of receptor activator of the nuclear factor kB ligand (RANKL) and human osteoprotegerin (OPG).
5. Diagnostic spirometric measurement of ventilatory functions pre & post bronchodilator using salbutamol (0.2 mg) by metered dose inhaler.
6. Measurements of bone mineral density by DEXA.

Among our subjects, 75 asthmatic patients were selected according to:
1. The presence of history of dyspnea, chest wheezes, cough, nocturnal attacks and/or reversible airway obstruction. Grading of bronchial asthma was done according to the criteria proposed by the WHO (World Health Organization, 1995).
2. Reversibility of airway obstruction in the pulmonary function tests (an increase in FEV1 more than 15% post bronchodilator) (Curtis et al., 1966).

The following subjects were excluded:
1. Smokers.
2. Patients with co-existent other medical diseases especially autoimmune and chronic inflammatory diseases (Walsh and Gravallese, 2004).
3. Patients with other risk factors for osteoporosis (Tobias, 1999):
   1. Age > 65 yr.
   2. Previous vertebral fracture or low trauma appendicular fracture
   3. Post-menopausal woman receiving hormonal replacing therapy.
   4. Premature menopause at <45 yr or male hypogonadism
   5. Low Body Mass Index (BMI): <20 kg/m²
4. Other causes of osteoporosis (e.g. alcohol excess, hyperparathyroidism, thyrotoxicosis).

The subjects were divided into 4 groups (one control and three asthmatic groups):
- Group I of asthmatic patients: It consisted of 21 patients with mild asthma receiving bronchodilators only (6mg per day of salbutamol or 24 mcg per day of formoterol and /or 200mg per day of aminophylline). (3 men and 18 women, age range: 44-65 years).
- Group II of asthmatic patients: It included 24 patients with moderate asthma receiving bronchodilators and inhaled glucocorticoids (600 to 800 mcg per day beclomethasone dipropionate or 200 to 800 mcg per day budesonide) (3 men and 21 women, age range: 40-64 years).
- Group III of asthmatic patients: It consisted of 30 patients with severe asthma maintained on bronchodilators, inhaled glucocorticoids (600 to 800 mcg per day beclomethasone dipropionate or 200 to 800 ...
mcg per day budesonide) and systemic glucocorticoids prednisolone (30-60 mg per day for 7 days tapered to 20 mg/day over 8 weeks and maintained on 10-15mg/day). (All were women, age range: 40-60 years).

Control group: It included 36 age, sex and culture matched healthy subjects.

Blood samples were collected using pyrogen free collecting tubes. Sera were separated by centrifugation at 1000xg for 30 minutes to remove particulates. Samples were aliquoted and stored frozen at -70ºC until analysis.

**Determination of serum level of RANKL and human osteoprotegerin (OPG):**

Total serum RANKL and human OPG were measured according to Chou et al., (2010). Total serum RANKL was measured by ELISA using Biovendeor Laboratory Medicine, Inc. CT Park Modrice, Evropska 873,664 42 Modrice, Czech Republic. The immunoassay was designed to measure the total (bound and free) amount of RANKL in serum. Human OPG was measured by ELISA using Biovendeor Laboratory Medicine, Inc. Palackecho tr. 56, 612 00 Brno, Czech Republic.

**Bone density measurement:**

For all patients and controls bone mineral density (BMD) g/cm² of the lumbar spine and the left proximal femur (if unaffected by disease, otherwise the right proximal femur) was measured by dual energy X-ray absorptiometry (DEXA) with the use of Norland, XR 46. The mean BMD value of the second, third and fourth lumbar vertebrae (lumbar spine BMD) and of the femoral neck of the proximal femur (femoral neck BMD) were used in the present analysis. T score > -1 was considered normal, T score between -1 and -2.5 was considered Osteopenia, and T score ≤ -2.5 was considered osteoporosis (Collier-J et al., 2002).

**Statistical Method:**

Data was presented by means ± SD and percentages. The compiled data were computerized and analyzed by EPI Info version 6.2 produced through the collaboration between CDC/WHO and by SPSS PC+, version 7.5. The following tests of significance were used: Analysis of variance (ANOVA) test between more than two means, t test between means was used to analyze mean difference, Z test between percentages to analyze percent difference. Chi –Square test ($\chi^2$) was used to study the pattern of distribution of different variables. A level of significance was with $p \leq 0.05$.

**Results:**

There were no statistically significant differences concerning the age, sex, number of post menopausal females, & duration of menopause & duration of bronchial asthma or duration of bronchial asthma therapy among the studied groups ($P>0.05$) (Table1).

**Table 1**: Statistical comparison among the studied groups as regard age and sex

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Asthma groups</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N 36</td>
<td>I 21</td>
<td>II 24</td>
<td>III 30</td>
</tr>
<tr>
<td>Age (mean±SD) (years)</td>
<td>55.3±8.2</td>
<td>54.7±7</td>
<td>52.3±8.6</td>
<td>50.4±7.3</td>
</tr>
<tr>
<td>Females (N (%))</td>
<td>24 (66.6)</td>
<td>18 (85.7)</td>
<td>21(87.5)</td>
<td>30(100)</td>
</tr>
<tr>
<td>Post Menopausal Females (N (%))</td>
<td>4(11.1)</td>
<td>3(14.2)</td>
<td>3(12.5)</td>
<td>5(16.6)</td>
</tr>
<tr>
<td>Duration of Menopause (mean±SD)(years)</td>
<td>8±3</td>
<td>8±2</td>
<td>7±3</td>
<td>6±4</td>
</tr>
<tr>
<td>Duration of Bronchial Asthma (mean±SD)(years)</td>
<td>-</td>
<td>25±6</td>
<td>24±6</td>
<td>22±5</td>
</tr>
<tr>
<td>Duration of Bronchial Asthma Therapy (mean±SD) (months)</td>
<td>-</td>
<td>5±1</td>
<td>5±2</td>
<td>5±2</td>
</tr>
</tbody>
</table>

N: Number NS: Non significant P is significant < 0.05
There were statistically significant differences ($P<0.05$) concerning the prevalence of osteoporosis of the proximal femur among the studied groups (Table 2). Meanwhile, there were no significant differences ($P>0.05$) concerning the prevalence of osteoporosis of the lumbar spines among the studied groups (Table 2).

### Table 2: Statistical comparison among the studied groups as regard Bone Mineral Density (BMD) in the proximal femur (PF) and the lumbar spines (LS).

<table>
<thead>
<tr>
<th></th>
<th>Normal BMD%</th>
<th>Osteopenia%</th>
<th>Osteoporosis%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF</td>
<td>LS</td>
<td>PF</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=36</td>
<td>25%</td>
<td>50%</td>
<td>66.7%</td>
</tr>
<tr>
<td>I</td>
<td>42.9%</td>
<td>28.6%</td>
<td>42.9%</td>
</tr>
<tr>
<td>II</td>
<td>25%</td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td>III</td>
<td>20%</td>
<td>30%</td>
<td>20%</td>
</tr>
<tr>
<td>P value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

P is significant $< 0.05$  S: Significant  NS: Non significant

There were statistically significant differences ($P<0.05$) concerning the prevalence of osteoporosis of the proximal femur of the third group of asthmatic patients when compared to the other studied groups ($P<0.05$) (Table 3).

### Table 3: Statistical comparison of the prevalence of osteoporosis in the proximal femur (PF) between the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &amp; Asthma II</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Control &amp; Asthma III</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Asthma II &amp; Asthma III</td>
<td>0.04</td>
<td>S</td>
</tr>
<tr>
<td>Osteoporosis in PF</td>
<td>0.03</td>
<td>S</td>
</tr>
</tbody>
</table>

P is significant $< 0.05$  NS: Non significant  S: Significant

There were statistically significant differences ($P<0.05$) concerning the mean values of RANKL among the groups classified according to their BMD (normal, osteopenia and osteoporosis) of proximal femur (PF) (Table 4 & 5), meanwhile there were no statistically significant differences ($P>0.05$) concerning the mean values of age and OPG among the same groups (Table 4).

There were no statistically significant differences concerning the mean values of age, OPG and RANKL among the groups classified according to their BMD (normal, osteopenia and osteoporosis) of lumbar spines (LS) (Table 4).

### Table 4: Statistical comparison of the mean values of age, osteoprotegerin (OPG) and RANKL among normal bone mineral density(BMD), osteopenia and osteoporosis of proximal femur (PF) and lumbar spines (LS) of all subjects.

<table>
<thead>
<tr>
<th>Variables ( Mean±SD)</th>
<th>Normal BMD</th>
<th>Osteopenia</th>
<th>Osteoporosis</th>
<th>P value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) PF</td>
<td>53.7±8.3</td>
<td>52.6±7.9</td>
<td>56.5±9</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>OPG (pmol/L) PF</td>
<td>5.2±6.3</td>
<td>5.2±6.3</td>
<td>5.2±5.3</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>RANKL (pg/ml) LS</td>
<td>2437.2±16439</td>
<td>2437.2±16439</td>
<td>2437.2±16439</td>
<td>0.02</td>
<td>S</td>
</tr>
</tbody>
</table>

P is significant $< 0.05$  NS: Non significant  S: Significant

### Table 5: Statistical comparison of the mean values of RANKL among normal bone mineral density (BMD), osteopenia and osteoporosis of proximal femur (PF) of all subjects.

<table>
<thead>
<tr>
<th>Site</th>
<th>Compared Groups (Mean±SD)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>3804.4±2945.9 &amp; 1891.7±16439</td>
<td>0.03</td>
<td>S</td>
</tr>
<tr>
<td>RANKL</td>
<td>0.04</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

S: Significant  P is significant $< 0.05$
There were statistically significant differences ($P<0.05$) concerning the mean values of RANKL among the studied groups, however there were no statistically significant differences ($P>0.05$) concerning the mean values of OPG among the studied groups (Table 6).

Table 6: Statistical comparison of the mean values of osteoprotegerin (OPG) and RANKL among the asthmatic groups and control

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Asthma groups</th>
<th>P value</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td>36</td>
<td>21</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>OPG (pmol/L)</td>
<td>6.8±5.7</td>
<td>3.7±1.1</td>
<td>4.2±2</td>
<td>4.8±3.1</td>
</tr>
<tr>
<td>RANKL (pg/ml)</td>
<td>336.3±123.1</td>
<td>4504.6±1641</td>
<td>3304.7±713</td>
<td>5143.7±2274</td>
</tr>
</tbody>
</table>

HS= Highly significant  NS: Non significant

There were statistically highly significant differences ($P<0.01$) concerning the mean value of RANKL among the asthmatic patients groups when compared to the control group, meanwhile there were no statistically significant differences ($P>0.05$) concerning the mean value of RANKL among the asthmatic patients groups (Table 7).

Table 7: Statistical comparison of the mean values of RANKL between the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &amp; Asthma I</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Control &amp; Asthma II</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>Control &amp; Asthma III</td>
<td>0.0001</td>
<td>HS</td>
</tr>
<tr>
<td>Asthma I &amp; Asthma II</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Asthma I &amp; Asthma III</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Asthma II &amp; Asthma III</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS= Highly significant  NS: Non significant

Discussion:

Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk (Consensus Development Conference on Osteoporosis, 1993).

The risk for an osteoporosis-related fracture is greatly increased by medications, diseases, or both that accelerate bone resorption or inhibit bone formation. The most common cause of secondary osteoporosis is CS therapy (Robert, 2011).

In this study there was no statistically significant difference concerning the duration of bronchial asthma therapy among the studied groups with average of 5 months duration. Grossman et al., (2010) stated that BMD rapidly decreased after 3 months of CS therapy and peaked at 6 months with slower deterioration over the following years.

In this study the prevalence of osteoporosis was increased significantly in the third asthmatic group of patients when compared to the other studied groups. The meta-analysis carried out by Van Staa et al., (2002) and Compston, (2010) confirmed that a dosage of 5 mg or more of prednisolone orally or its equivalent per day decreased bone mineral density and rapidly increased the risk of fracture over 3 to 6 months. This could explain our results of increased osteoporosis in the third asthmatic group who received oral prednisolone in a dose exceeded the above mentioned dose.

On comparing the second and the third groups of asthmatic patients in our study, there was significant increase in the prevalence of osteoporosis in the third group. This finding can be explained by the dose of inhaled CS in the second group, as Wong et al., (2000) presented an evidence for a dose-related decrease in bone-mineral density in patients with asthma who were taking inhaled corticosteroids. They studied the relation between cumulative inhaled corticosteroid dose and bone-mineral density at the lumbar spine and proximal femur in adults with asthma. They found out that a high dose of inhaled CS (> 2,000 μg/day) over years (median 6 years) was associated with a decrease in bone-mineral density, but lower doses of inhaled CS showed negative association with bone-mineral density. In our work, the patients of the second asthmatic group were receiving inhaled CS at nearly 800 μg per day which is far below the suggested dose by Wong and his team to affect bone-mineral density.

In the current study, there was no statistical significant difference regarding the prevalence of osteoporosis in the second asthmatic group when compared to the first asthmatic group (not receiving CS), this supported the evidences regarding the limited systemic effects of mild to moderate doses of inhaled CS. Van Staa et al., 2001 compared the risk of fracture in patients taking inhaled glucocorticoids with patients taking inhaled nonglucocorticoid bronchodilators and controls not using inhalers. They found no differences between the inhaled glucocorticoid and nonglucocorticoid bronchodilator groups in the risk of nonvertebral fracture. Users of inhaled glucocorticoids had a higher risk of fracture, particularly of the hip and spine, than did the controls,
but this might be related more to the severity of the underlying respiratory disease than to the inhaled glucocorticoids.

In this study there were statistically significant differences concerning the mean values of RANKL among the studied groups classified according to their BMD (normal, osteopenia and osteoporosis) of proximal femur (PF). Meanwhile, there were no statistically significant differences concerning the mean values of OPG among the same groups. Teitelbaum, (2007) stated in his research on osteoclasts that RANKL is a membrane-bound tumor necrosis factor (TNF) receptor expressed on osteoblast precursor cells that recognizes RANK on the osteoclast surface through a direct cell-cell interaction. This process is essential for osteoclast differentiation, activation and survival. RANKL is considered the key osteoclastogenic cytokine. Also Mundy, (2007) pointed out in his research regarding osteoporosis and inflammation that OPG ligand is a membrane-bound TNF-related factor that acts as an endogenous inhibitor for RANKL specifically binding it and blocking its interaction with RANK. Thus, osteoporosis detected in this study could be explained by the increased level of RANKL which was not associated with similar increase in the level of OPG.

In this study there were no statistically significant differences concerning the prevalence of osteoporosis of the lumbar spines (LS) among the studied groups. Assessment of BMD at LS appears to be less significance in evaluating osteoporosis induced by asthma or CS therapy. In support to our view, Leslie and his colleagues, 2007 studied the effectiveness of bone density measurement for predicting osteoporotic fractures in clinical practice and they recommended the femur (neck or total hip) to be the optimum site for predicting the risk of fractures. In addition, WHO had developed the Fracture Risk Assessment Tool (FRAX) calculator to evaluate fracture risk of patients. It was based on individual patient models that integrate the risks associated with clinical risk factors as well as BMD at the femoral neck (Kanis et al., 2010). Also, Kanis et al., (2005) in their study for assessment of fracture risk found out that the spine was the optimum site for monitoring response to treatment and recommended the hip measure alone for the fracture risk assessment.

In this study there was no statistically significant difference (P>0.05) concerning the mean values of age between normal BMD, osteopenia and osteoporosis of proximal femur (PF) and lumbar spines (LS) of all subjects. This finding is supported by Luengo and his research team (Luengo et al., 1991) who carried out a comparative study to evaluate vertebral fractures in steroid dependant asthma and involutional osteoporosis. They found out that the influences of sex and age on fracture risk and BMD were minor.

In this work there were statistically significant differences concerning the mean value of RANKL among the asthmatic patients groups when compared to the control group, meanwhile there were no statistically significant differences concerning the mean value of RANKL among the asthmatic patients groups. This could be explained by the inflammatory load by the primary disease which -in this study- was the bronchial asthma. This explanation was supported by Yun and Lee, (2004) who studied the link between inflammation and bone turnover and also, by Bultink et al., (2005) who studied the prevalence of and risk factors for low bone mineral density and vertebral fractures in patients with systemic lupus erythematosus, they discovered several clinical observations that revealed coincidence of systemic osteoporosis with period of systemic inflammation and reported an increase in the risk of developing osteoporosis in various inflammatory conditions. Also, Elmstahl et al., (2003) - during their research concerning the association between inhaled CS and bone density in postmenopausal women- found out that the inflammatory mediator nitric oxide (NO) was involved in the pathogenesis of osteoporosis. The activation of the inducible NO synthesis (iNOS) pathway by cytokines, such as IL-1 and TNF-, inhibited osteoblast function in vitro and stimulated osteoblast apoptosis.

In conclusion, this study indicated that the steroid therapy and the inflammatory load by asthma are risk factors for osteoporosis. So, asthmatic patients are at risk of osteoporosis. These patients should be investigated for the status of their bone mineral density. This study suggests the proximal femur to be the optimal site for assessment of bone mineral density in asthmatic patients.

Acknowledgment

We are grateful to Ghada Mahmoud El Qattan, National Research Centre for her help in preparing this article.

References


