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REGULAR ARTICLE

Evaluation of bone mineral density and 25-hydroxyvitamin D levels in subjects with silica exposure

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Abstract
Objective The purpose of the study was to evaluate the bone mineral density (BMD) and 25-hydroxyvitamin D (25(OH)D) levels in patients with silica exposure.
Materials and methods The study included 104 male subjects with silica exposure and 36 healthy subjects. Posterior–anterior radiographs were classified according to the International Labour Office (ILO) Classification. Category 0 patients were classified as Group I ($n = 54$), category I patients were classified as Group II ($n = 25$), Category II and III patients were classified as Group III ($n = 25$).
Results Femoral neck BMD values were significantly lower in Group III ($p = 0.007$). Lumbar vertebrae BMD values were significantly lower in all groups with silica exposure than in the control group ($p = 0.000$). The osteoporosis rate was significantly higher in Group III ($p = 0.000$). Subjects with silica exposure were determined to have diminished 25(OH)D levels ($p = 0.012$).
Conclusion The results of this study demonstrated that subjects with silica exposure have diminished BMD and 25(OH)D levels.

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Introduction

Silicosis is a type of pneumoconiosis seen after the inhalation of silicate dust in its SiO_2 form [1]. After inhalation, alveolar macrophages, phagocytate silica particles and an inflammatory response occurs by releasing tumor necrosis factors (TNF), interleukin-1 (IL), leukotriene B4, and other cytokines. If the exposure is continuous and chronic, this course eventually results in fibrosis and silicotic nodule formations which are composed of collagen bundles [2]. In addition, silicosis is one of the most common and oldest occupational lung diseases, particularly in developing countries, with high mortality and morbidity. Silicosis also affects life quality adversely [3–6]. It is frequently seen in cement and ceramic industries, quartz mining, and denim sandblasting [1, 3, 4].

Chronic bronchitis, pulmonary hypertension, airflow limitation, tuberculosis and other infections, pneumothorax, emphysema (compensatory to silicosis), increased immune reaction and autoimmunity, and renal diseases have been reported to be the complications of silicosis in previous data [2–5]. Moreover, osteoporosis has been reported in silicotic horses, rodent models, and in a young man with silicosis, although the exact relationship and underlying mechanism are not clear yet [7–9]. It has been shown that pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and soluble receptor activators for nuclear factor-K B ligand (RANKL) have been released due to the inflammatory cascades in patients with silicosis [10, 11]. RANKL is the main cytokine which induces osteoclastic activity and promotes osteoclast resorptive activity [12]. Soluble

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RANKL has been shown to decrease BMD via triggering osteoclast activity and apoptosis inhibition [13]. However, to the best of our knowledge, bone mineral density (BMD) and 25-hydroxyvitamin D (25(OH)D) levels in subjects with silica exposure has not been previously studied. Therefore, the purpose of this study was to investigate whether there is a relationship between BMD, 25(OH)D levels and silicosis.

Materials and methods

Study design and participants

This cross-sectional and controlled study was conducted on a total of 104 male subjects with a history of likely silica exposure (stone carvers or quartz miners) and 36 male healthy subjects. Subjects with complicated silicosis or concomitant disorder causing osteoporosis (endocrine disorders, corticosteroid use, diabetes mellitus, immobility, more than two cups of coffee and more than 3 drinks of alcohol consumption in a day, with a history of malabsorption, weight loss, immobility), and patients who had previously received osteoporosis or vitamin D supplements were excluded from the study. Approval for the study was granted by the local Ethics Committee. Informed consent for participation in the study was obtained from all participants.

Data collection

Demographic characteristics (age, body mass index, occupation, working duration, history of smoking, drug use, history of any other disease) and the clinical features of the subjects were recorded. Posterior–anterior chest radiographs (digital) of all subjects with silica exposure were evaluated according to the International Labour Office (ILO) Classification. Laboratory assessments were performed to exclude secondary reasons of osteoporosis.

International Labour Office Classification

Parenchymal abnormalities can be classified as small opacities (with a diameter <10 mm) and large opacities (with a diameter >10 mm). Opacities are also classified into 4 major categories (0, 1, 2, or 3) according to profusion. Profusion refers to the concentration of the small opacities in the affected zone of the lungs. Category 0 refers to an absence of opacity and 3 refers to the most profuse of the small opacities [14]. In this study, category 0 subjects were classified as Group I, category I subjects as Group II, and Category II–III subjects as Group III.

Laboratory assessments

Complete blood count, erythrocyte sedimentation rate, liver and renal function tests, calcium, phosphor, alkaline phosphatase, 25(OH)D, plasma cortisol level, gonadotropins, ferritin, vitamin B12, thyroid hormone levels (TSH, free T3 and T4), parathyroid hormone, prolactin, free testosterone, immunoglobulin E, beta-2 microglobulin, urine tests, total protein, and albumin levels were assessed to determine secondary causes of osteoporosis. Venous blood samples were obtained after a 12-h overnight fast. 25(OH)D levels were measured using the chemiluminescence microparticle immunoassay method (ARCHITECT®, Biokit S.A., Barcelona, Spain) with an imprecision of <10 % within laboratory coefficient of variation. Photometry technique was used to determine calcium and phosphor levels. Alkaline phosphatase was measured using the enzymatic assay. Parathyroid hormone levels were determined by chemiluminescence immunoassay method.

Bone mineral density evaluation

Bone mineral densitometry was measured by Dual-Energy X-ray Absorptiometry (DEXA) at both the femoral neck and lumbar spine. The results of the measurements were expressed as grams per square centimeter (g/cm^2). *T* score refers to the BMD when compared to a reference mean. A *T* score of 0 features a bone density equal to the mean peak bone mass (a healthy man) and a *T* score of -1 indicates 1 standard deviation below this. Cases were classified into three categories according to the *T* scores of the either the femoral neck or lumbar vertebrae as follows [15, 16].

1. Normal: *T* score greater than or equal to -1 SD.
2. Osteopenia (low bone mass): *T* score <-1 and >-2.5 SD.
3. Osteoporosis: *T* score ≤ -2.5 SD.

Statistical analysis

SPSS version 16 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Data were expressed as mean ± standard deviation or percentage. Normal distribution was analyzed with the Kolmogorov–Smirnov test. The Chi-square test or Fisher's exact test were used for categorical variables between the groups where appropriate. One-way ANOVA analysis was used to determine whether there were any significant differences between the means of the groups. Post hoc Tukey was used to determine which of these groups differed from the others. Correlations between clinical parameters and BMD were analyzed using Pearson's correlation coefficients.

Bonferroni correction was also applied to avoid type 1 error using $p < 0.0125$. Multivariate analysis with logistic regression was applied if age was a risk factor for osteoporosis.

Results

The study sample comprised 54 subjects in Group I, 25 subjects in Group II and 25 subjects in Group III. The demographic and clinical characteristics of the subjects are given in Table 1. The mean age of the subjects was significantly higher in Group III than in all the other groups ($p = 0.001$). The influence of the age difference on osteoporosis was not statistically significant ($p = 0.598$). The BMD values of the femoral neck were significantly lower in Group III than in the other groups ($p = 0.007$). The lumbar BMD values were significantly lower in the groups with silica exposure than in the control group ($p = 0.000$), although no significant difference was determined between Groups I, II and III ($p > 0.05$). Tenure was significantly lower in Group I ($p = 0.002$). There was a negative correlation between tenure and BMD values, but this difference was not statistically significant.

The Bone Mineral Density classifications of the subjects according to the *T* score of either the femoral neck or lumbar vertebrae are given in Table 2. The osteoporosis rate was significantly higher in Group III ($p = 0.00$).

The laboratory parameters of the groups are given in Table 3. The 25(OH)D and Ca levels were significantly lower in the silica-exposed groups than in the control group ($p = 0.001$ and $p = 0.012$, respectively).

Discussion

The objective of this study was to evaluate BMD and 25(OH)D levels in subjects with silica exposure, which to the best of our knowledge, has not been previously reported in literature. Three important findings were determined in this study. First, subjects with silica exposure had decreased BMD. Second, the osteoporosis rate was higher in subjects with silica exposure and third, the silica-exposed groups had diminished 25(OH)D levels.

Inhalation of silica particles triggers an inflammatory cascade by releasing soluble receptor activators for nuclear factor-K B ligand (RANKL) and pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) [10, 11]. Soluble

Table 1 Demographic and clinical characteristics, bone mineral density parameters of the patients (mean ± SD)

	Group I ($N = 54$)	Group II ($N = 25$)	Group III ($N = 25$)	Control ($N = 36$)	*p*
Age (years)	36.5 ± 5.9	39.0 ± 7.0	**44.0 ± 5.3**	36.6 ± 6.9	**0.001***
BMI (kg/m^2)	25.5 ± 2.7	25.0 ± 4.0	26.8 ± 7.5	26.8 ± 3.2	0.340
BMD femoral neck (g/cm^2)	0.98 ± 0.12	0.98 ± 0.16	**0.89 ± 0.11**	1.01 ± 0.10	**0.007***
BMD lumbar spine (g/cm^2)	**1.06 ± 0.17**	**1.04 ± 0.14**	**0.99 ± 0.14**	1.15 ± 0.12	**0.000***
Tenure in silica-exposed job (years)	**12.9 ± 7.5**	15.9 ± 5.6	17.8 ± 5.4	–	**0.002***
Smoking *n*, (%)	36 (66.7)	17 (68)	14 (56)	20 (56)	0.594

One-way ANOVA was used to determine whether there are any significant differences between the means of the groups. Post hoc Tukey was used for determining which of these groups differ from each other

The influence of the age difference on osteoporosis was not statistically significant ($p = 0.598$). Relative risk was 1.038 (%95 CI) (0.904–1.192) (*RR* = odds ratio, *CI* confidence interval)

Bold data/*p* values denote significance

BMI Body Mass Index, *BMD* bone mineral density

* $p < 0.05$

Table 2 Bone mineral density classification of the patients according to *T* score of the either femoral necks or lumbar vertebra (*n*, %)

	Group I ($N = 54$)	Group II ($N = 25$)	Group III ($N = 25$)	Control ($N = 36$)	*p* values
Normal	21 (38.9)	10 (40.0)	5 (20.0)	22 (61.1)	0.06
Osteopenia	32 (59.3)	12 (48.0)	14 (56.0)	14 (38.9)	0.07
Osteoporosis	1 (1.9)	3 (12.0)	**6 (24.0)***	0	**0.00***

Bold *p* value denotes significance

Chi-square/Fisher's exact test was used to compare osteoporosis rate between the groups

* $p < 0.0125$

Table 3 Laboratory results of the groups

	Group I	Group II	Group III	Control
Calcium	**9.2 ± 0.3***	**9.3 ± 0.3***	**9.1 ± 0.3***	9.6 ± 0.3
25(OH)D	**12.1 ± 5.7****	**10.8 ± 5.3****	**12.8 ± 6.7****	18.1 ± 5.6
Osteocalcin	20.5 ± 7.1	22.5 ± 7.6	19.5 ± 5.6	21.4 ± 6.5
Cortisol	13.7 ± 5.8	15.5 ± 6.4	15.1 ± 6.7	14.2 ± 6.1
PTH	35.5 ± 12.7	37.4 ± 11.6	35.3 ± 12.4	38.6 ± 18.0
ALP	187.6 ± 37.5	205.6 ± 86.8	171.8 ± 44.0	209.4 ± 62.3

PTH parathormone, *ALP* alkaline phosphatase, *25(OH)D* 25-hydroxyvitamin D

One-way ANOVA was used to determine whether there are any significant differences between the means of the groups. Post hoc Tukey was used for determining which of these groups differ from each other

* $p = 0.001$, ** $p = 0.012$

RANKL decreases BMD by triggering osteoclast activity and apoptosis inhibition [13]. On the other hand, in silicosis there is an increased immune response and risk of autoimmune diseases such as scleroderma, rheumatoid arthritis, and systemic lupus erythematosus. These rheumatic disorders and corticosteroid use for their treatment can cause osteoporosis [10, 11]. Immobility with decreased life quality in subjects with silicosis can also cause osteoporosis [4]. In the current study, uncomplicated silicotic subjects were included. They had no previous history of immobility, corticosteroid use or autoimmune disorders. If subjects with complicated silicosis had been included to show the association between the disease severity and BMD, silicosis would have been accompanied by the medical treatment, immobility and other risk factors for osteoporosis. Therefore, only subjects with uncomplicated silicosis were included.

According to the results of the current study, the BMD values of the lumbar vertebrae were significantly lower in all the groups with silica exposure than in the control group and those of femoral neck were significantly lower only in Group III. There was a significant difference in Group III in respect of age. A significant decrease in BMD in that age group is not expected [17]. Nevertheless, multivariate analysis with logistic regression was applied and the age did not significantly affect the BMD. Nevertheless, the coexistence of increased age and silica exposure might engender a predisposition to osteoporosis. In the current study, the 25(OH)D and calcium levels were found to be lower compared to healthy controls. Since the study subjects worked in closed areas, low 25(OH)D levels could be attributed to inadequate exposure to sunlight. Although low 25(OH)D levels in young adult men due to dietary habits are not expected, the lack of evaluation of socioeconomic status and dietary habits is a limitation of the current study. Overall, the results gave rise to the thought that the decrease in BMD and *T* score of the femoral neck or lumbar vertebrae was due to the silica exposure and was associated with the disease severity. Together with the inflammatory process of silicosis, diminished 25(OH)D levels might have also caused decreased BMD levels.

Group I had a significantly shorter tenure ($p = 0.002$) than the other groups which was in accordance with the ILO classifications. Group II and III had longer tenure than Group I but this was not significant.

There are some important limitations to this study. First, the sample size showed heterogeneity and the groups were not age matched. Second, the study was limited by the lack of better definitions of exposure assessment. Although the workers were from similar jobs, the exact socioeconomic status of the workers, which might have been associated with vitamin D levels and osteoporosis, was unknown. The absence of a bone resorption marker (such as serum CTX) to characterize bone metabolism is the third limitation.

Conclusion

The results of this study indicate that subjects with silica exposure have diminished BMD and 25(OH)D levels. Therefore, osteoporosis must be taken into account while planning medical treatment for silicosis and clinicians should perform BMD measurements in relevant subjects. Further studies considering bone metabolism and markers in patients with silicosis are awaited.

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Compliance with ethical standards

Conflict of interest None.

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