AIR POLLUTION AND GENETIC INFLUENCES ON BONE MINERAL DENSITY AND OSTEOPOROSIS

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Abstract: Osteoporosis is a systemic skeletal disease characterized by reduced bone mineral density, disrupted bone microarchitecture and alterations in the amount and variety of proteins in bones. Bone turnover is a very complex process, depending on genetic and non genetic factors, such as diet, lifestyle or air pollution. The aim of the study was to explore genetic and environmental risk factors that contribute to osteoporosis by quantifying several factors related to bone mineral density. We assessed family history, vitamin D status, bone mineral density in subjects seeking advice on osteoporosis. Air pollution data were also obtained. Average concentrations of NO_2 and particulate matter PM_{10} were calculated. Due to their synergistic effect on the organism the maximum permissible concentration calculated for all air pollutants was exceeded. In our study total body bone mineral density was inversely associated with indicators of air pollution. The prevalence of vitamin D depletion was 64.76%. Genetic contribution to the etiology of osteoporosis was revealed by the positive family history for 36% affected subjects. Air pollution and vitamin D deficiency have a negative impact on bone mineral homeostasis.

Keywords: osteoporosis, air pollution, vitamin D deficiency

INTRODUCTION

Osteoporosis is a complex systemic skeletal disease that leads to an increased risk of fracture, usually of the vertebra, hip, wrist and humerus. The disorder is characterized by reduced bone mineral density (BMD), disrupted bone microarchitecture and alterations in the amount and variety of proteins in bone.

During the last years a better understanding of the cellular mechanisms operating in bone remodeling, both for healthy and affected bones, was gained [26]. Bone turnover is a very complex process, depending on different genes and signaling pathways that coordinate osteogenesis, but also on other environmental factors. It is estimated that more than 60% of bone mass variance is determined by genetic factors [23]. Environmental factors account for the non genetic influences and among these nutritional intakes, lifestyle or pollution play important roles. Thus, it is very important to know and recognize the risk factors of osteoporosis.

Clean air is a mixture of gases with constant rates in the lower layers of the atmosphere, which is an essential prerequisite in terms of maintenance and development of life on Earth.

Air pollution has different sources such as motor vehicle emissions, industrial combustion and heating, representing an important problem especially in urban areas. Exposure to air pollution is found to be associated with many adverse health outcomes, such as allergy, chronic obstructive pulmonary disease and increased mortality due to cardiovascular or respiratory diseases [12]. Despite higher fracture rates and more cases with osteoporosis in urban areas compared to rural populations, the relationship between air pollution and bone mineral density is not clearly known [7, 11, 15].

Heavy metals, such as cadmium and lead, have been proposed as risk factors for osteoporosis due to their ability to be deposited in the skeleton. Tobacco smoke was established as a risk factor for osteoporosis and osteoporotic fractures [17]. Inflammatory processes, which may be affected by air pollution, could also harm the skeleton [18]. These effects could give rise to the hypothesis that long-term exposure to air pollution is associated with risk of reduced bone mineral density.

Unlike stratospheric ozone that protects life on earth, tropospheric ozone is highly toxic and is the main pollutant of the atmosphere of industrialized countries and cities. Its precursors are emanated from industrial activities and traffic. Generated by lightning, photochemical reactions or with free radicals, it has a density of 1.66 times greater than air and is maintained close to the ground. It decomposes easily, generating free radicals with oxidizing power. The main primary pollutants leading to ozone formation are nitrogen oxides, volatile organic compounds and methane. The amount of tropospheric ozone varies in different areas and in time, its assessment is very difficult, monitoring of the precursor being necessary.

Atmospheric pollution, by blocking some of the ultraviolet rays can promote the development of vitamin D deficiency. Vitamin D regulates calcium homeostasis and bone mineralization and vitamin D action is mediated through a nuclear vitamin D receptor. The gene coding for this receptor was also widely studied as an osteoporosis candidate gene.

The National Osteoporosis Foundation recommends that adults under 50 get 400-800 International Units (IU) of vitamin D daily, and adults that are 50 and over get 800-1,000 IU of vitamin D daily. Some individuals may need more. There are two types of vitamin D supplements. These are vitamin D_3 and vitamin D_2. Previous studies suggested that vitamin D_3 was a better choice than vitamin D_2, but more recent studies revealed that vitamin D_3 and vitamin D_2 are equally good for bone health.

Sources of vitamin D are the **s**unlight, food and supplements and medications. Sunlight is an important source, as the skin synthesizes vitamin D from the ultra-violet light in sunlight. The body is able to store the vitamin and use it even later. The amount of vitamin D depends on time of day, season, latitude,

skin pigmentation and other factors. To protect bone health, optimal levels for vitamin D are 30-40 ng/ml or 75 nmol/L or higher. It is estimated that normally, a 15-minute exposure to sunlight three times weekly would provide adequate vitamin D. Dietary sources of vitamin D are reported to provide about 20 percent of the body's needs. A comparison between blood levels of vitamin D showed that average values in test samples taken between 1988-1994 were 30 ng/ml, whereas blood levels of vitamin D in the more recent samples averaged 24 ng/ml. Among newer blood samples, fewer people had levels of vitamin D above 30 ng/ml [13]. Different studies revealed a widespread prevalence of vitamin D deficiency or insufficiency, which has a deleterious effect on bone mineral homeostasis and peak bone mass achieved [6, 9, 10].

Family and twin studies have established a genetic contribution to the etiology of osteoporosis. The number of candidate genes has increased steadily in recent years genes but allelic variants conferring osteoporotic risk are largely undefined. As osteoporosis is a complex disease, allelic variation in many candidate genes including those that encode growth factors, cytokines, calciotropic hormones and bone matrix proteins are likely to also play a role. Most family and association studies to date have focused on the genetic contributions to bone density, a major determinant of bone strength and fracture risk [16].

The aim of this study was to investigate the associations between some genetic and non genetic osteoporosis risk factors and bone mineral density and osteoporosis in subjects with osteopenia and osteoporosis.

MATERIAL AND METHODS

We explored genetic and environmental risk factors that contributed to osteoporosis in Romanian subjects by quantifying the factors related to bone mineral density. Between January 2009 - December 2009 we evaluated 105 subjects treated in the Medical Rehabilitation Clinical Hospital Baile-Felix, who met the inclusion criteria, women that were before and after menopause and men diagnosed with the disorder, having one or more risks to develop this illness such as positive familial history: mother with osteoporosis, smoking, vitamin D deficiency due to air pollution, residency in Oradea (Romania).

Exclusion criteria were represented by subjects with obvious causes for vitamin D deficiency, such as previous history of gastrectomy, intestinal resection, liver disease, transplant bone disease or taking medications known to cause vitamin D depletion. Cases with biochemical evidence of primary hyperparathyroidism or renal impairment defined as a serum creatinine greater than 1.5 mg/dl were also excluded.

Bone mineral density measurement was done by DEXA method using LUNAR DPX-L of either the lumbar spine and/or the femoral neck (T Score), as well as X-ray examination of the thoracolumbar spine to identify subclinical vertebral fractures. According to WHO definition, subjects were subdivided by BMD values into two groups: with and without vertebral osteoporosis.

Laboratory methods evaluated serum calcium, creatinine, PTH and 25-hydroxyvitamin D in the hospital laboratory by standard methods. A cut-off level of 25 nmol/l for 25-hydroxyvitamin D was established to define vitamin D deficiency.

Outdoor air pollution exposure was estimated at the residential address of participants. Data regarding concentration of the following pollutants: particulate matter $PM_{10,}$ and nitrogen dioxide were received from Environmental Protection Agency Bihor. Average concentrations of NO_2 and particulate matter PM_{10} during the 5 year period 2005–2009 and monthly during 2009 were calculated.

Statistical methods: Descriptive statistics are presented as means ± SD or as percentages. Analysis was performed using SPS version 9.0 and Pearson correlation.

RESULTS

The studied subjects, who met the inclusion criteria, had mean age 62.2 years, ranging between 44 and 70 years (Table 1).

Table 1. General characteristics of the lot.

Characteristics	Total lot (*n*=105)
	Mean ± SD*
Age (yrs)	62.2±3.98
Range	44–70
Weight (kg)	68.9±12.4
Height (cm)	160.6±4.1
BMI (kg/m)	25.23±4.39
Month since menopause	50.042±33.43
Type of menopause (% surgical)	18.2
Menopause at 45 (% subjects)	31

The mean value for the risk factors was 3.27±1.24, with a mean of 25.23±4.39 kg/m^2 for the body mass index, mean T score for the hip -1.99±0.9 and mean T score for lumbar vertebrae - 3.06 ±1.1 (Table 2).

77.1 % of all subjects fulfilled inclusion criteria for the diagnosis of osteoporosis, the others were regarded as with osteopenia.

Table 2. BMD parameters in the subjects with osteoporosis.

BMD parameters	Total lot
	Mean ± SD* (n=160)
BMD, spine L1–L4, g/cm^2	0.867 ±0.95
BMD, spine L2–L4, g/cm^2	0.908± 0.105
BMD, hip total, g/cm^2	0.708±0.141
BMD, femoral neck, g/cm^2	0.762± 0.085

Note: BMD - bone mineral density

Vitamin D deficiency was found in 68 subjects, who had mean values for 25-hydroxyvitamin D of 24.6±10.0 ng/ml. For the entire study cohort, the mean serum PTH values were 47.7±32 pg/ml, calcium 9.2±0.3 mg/dl and creatinine 0.80±0.1 mg/dl.

Distribution of the risk factors for osteoporosis is presented in Tables 3 and 4.

Table 3. Risk factors for osteoporosis for the entire lot.

TOTAL -105 OP -81 Diagnosis (T Score)	Mother with O.P. N=38/105 36%	Vitamin D deficiency N=68/105 64%	Tobacco consumption N=20/105 19.04%
Number of OP/total lot	21.9% (23/105)	41.9% (44/105)	13.3% (14/105)
Normal T >-2.5	15/38 29.5%	24/68 35.2%	6/20 30%
OP T below -2.5	23/38 60.5%	44/68 64.7%	14/20 70%
Osteopenia	39.1%	61.36%	64.2%
Vertebral osteoporosis	30.4%	15.9%	14.2%
General osteoporosis	30.4%	22.7%	21.4%

Note: OP - osteoporosis

Table 4. Clinical and lifestyle risk factors at baseline in subjects with osteoporosis (n-81) (% of subjects).

Risk factors	Total population nr of OP *n* =81
History of fracture in mother	30.4%
History of osteoporotic hip fracture in mother	5.1%
Vitamin D deficit	64.7%
Current smoker	19.04%
T-score (lumbar spine)	–3.06 ±1.1
T-score (femoral neck)	–1.9±0.9

Note: OP - osteoporosis

The genetic contribution to the etiology of osteoporosis is revealed by the positive family history for many affected subjects. A parental history of fracture was associated with a modest but significantly increased risk of any fracture, osteoporotic fracture and hip fracture both in men and women. The risk ratio was higher at younger ages but not statistically significant ($p>0.05$). Thus, we noticed that a parental history of fracture, especially a family history of hip fracture confers an increased risk of fracture that is independent of bone mineral density.

The admitted concentration for ozone is 100 mg/m³, but especially in summer months, this amount is exceeded. NO, NO_2, CO, CH_4, O_2 and atomic oxygen, produced mainly by photolysis of NO_2 have a contribution to ozone formation. Although air contains more than 78% nitrogen, it is considered that unpolluted air has a concentration of 0.01-0.05 mg/m^3 NO and 0.1-0.5µg/m^3 NO_2.

Maximum permissible concentration (MPC) per day for NO_2 is 100 µg/mc and per year is 40 µg/mc. Maximum permissible concentration per day for PM_{10} is 50 µg/mc and per year was 40 µg/mc until 2009 and now is 20 µg/mc.

Monthly evolutions of mean concentration of NO_2 and PM_{10} in 2009 are presented in Figures 1 and 2.

Evolutions of mean annual concentrations of NO_2 and PM_{10} between 2005-2009 are presented in Figures 3 and 4.

These pollutants have a synergistic effect on the organism, in combination their effect is greater than the effect of the individual substances. Maximum permissible concentration of substances with synergis-

Monthly Evolution of the NO_2 Mean Concentration in 2009

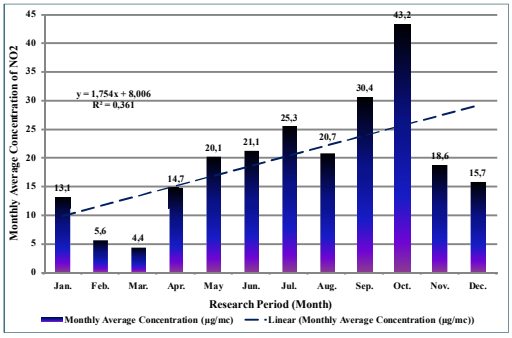

Figure 1. Monthly evolutions of mean concentrations of NO_2 in 2009.

Monthly Evolution of Mean Concentration of PM_{10} in 2009

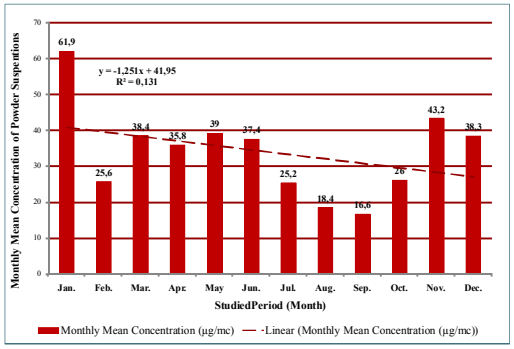

Figure 2. Monthly evolutions of mean concentrations of PM_{10} in 2009.

Annual Evolution of the NO_2 Mean Concentration between 2005-2009

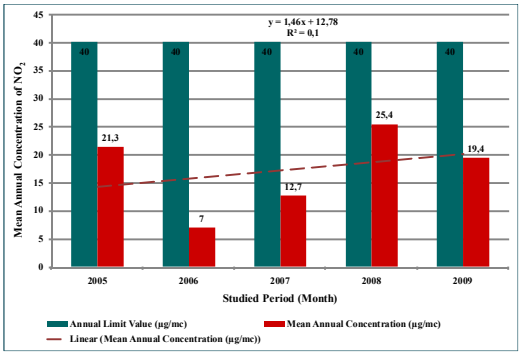

Figure 3. Annual evolutions of mean concentrations of NO_2 between 2005-2009.

Annual Evolution of Mean Concentration of PM_{10} between 2005-2009

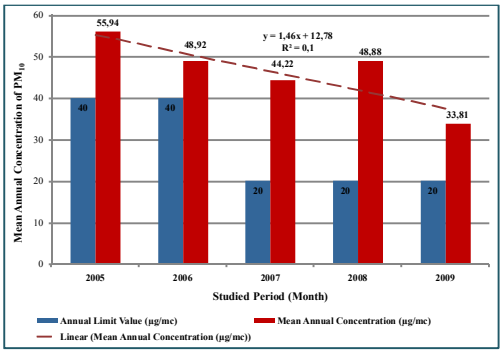

Figure 4. Annual evolutions of mean concentrations of PM_{10} between 2005-2009.

Evolution of ∑ Cx/CMAx

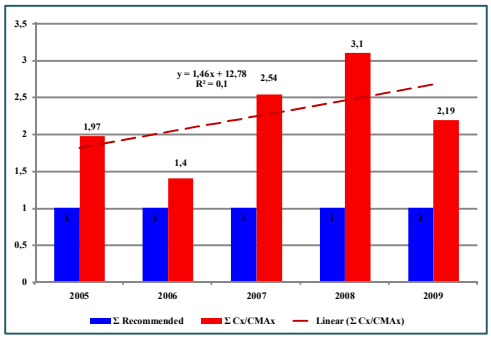

Figure 5. Evolution of the sum of the pollutants between 2005-2009.

tic action, simultaneously present in the air, is calculated as: C1/MPC1 + C2 / MPC2 + ... + Cx / C max ≤ 1, where C1, C2 .... Cx represent the concentration of pollutants 1, 2 .... x in the air. Even if each of the pollutants had values that did not exceed maximum permissible concentration, analyzing their sum and the synergistic effect, a value of 3.10 was obtained for 2008 and 2.19 for 2009 (Fig. 5).

In our study total body bone mineral density was inversely associated with indicators of air pollution (Table 5).

Table 5. Association between total body and hip bone mineral density and indicators of air pollution [a].

Measurement site		Correlation pearson	
		Correlation	(95% CI)
Total body BM (n=68)	PM_{10}	9.48683E-08	(−46, −1)*
	NO_2	5.47723E-08	(−12, 2)
Hip BMD (n=68)	PM_{10}	4.47214E-08	(−50, 15)
	NO_2	3.16228E-08	(−14, 6)

Note: - linear regression coefficients, change in bone mineral density (mg/cm^2) / units increase in air pollutants; *p<0.05, **p<0.01; [a)] NO_2, nitrogen dioxide and PM_{10}, particulate matter less than 10 µm in aerodynamic diameter, respectively. Annual mean during the period 2005–2009; CI - confidence interval

There was a strong correlation, statistically significant association between total body BMD, hip BMD and NO_2 and PM_{10}.

DISCUSSIONS

Osteoporosis is a complex multifactorial disease with genetic and non genetic factors involved in its etiology. The decrease of bone mass and the alteration of bone architecture, characteristics of osteoporosis, lead to increased bone fragility and a higher risk of fracture. The microarchitectural properties and overall size and geometry of bone influence skeletal strength.

Although a genetic influence on osteoporotic risk is well established, the number of genes involved, their chromosomal location, the magnitude of their effects, and the way they may interact with each other and with other risk factors are not well defined [27]. It is known that gender plays an important role in the development of osteoporosis, but genetic studies have almost exclusively focused on women, and have not tested whether gender modifies the association between genetic variation and osteoporotic risk. Therefore, future genetic studies will need to recruit men in the large samples used for studies.

Among the non genetic risk factors, vitamin D deficiency plays an important role in the etiology of osteoporosis and osteopenia [4].

Several reports have highlighted the high and rising prevalence of vitamin D depletion in different populations, in general [13], and in subjects with osteoporosis, in particular [5, 19]. Preliminary studies reported that a significant proportion of subjects seeking advice for osteoporosis are vitamin D depleted, while others have reported on the relationship between vitamin D nutrition, as assessed by serum 25-hydroxyvitamin D, and PTH, resorption markers, bone mineral density, and bone metabolism [25]. Various groups and experts have emphasized the importance of adequate vitamin D nutrition for optimal skeletal health and before initiating long-term therapy for osteoporosis [20].

Adequate sunlight is an essential part of the chemical process that helps organisms produce vitamin D, but many factors affect the amount of ultraviolet ray exposure received from the sun and can reduce

supplies of vitamin D. Severe vitamin D deficiency in children can cause rickets, which leads to permanent deformities of the bones. In adults, severe vitamin D deficiency results in softening of the bones (osteomalacia) and muscle weakness. A milder deficiency of the vitamin can lead to impaired balance, possibly increasing the risk of falling down [22, 24].

Pollution, which is settling over every major city in the world, is causing concern because it reduces the amount of vitamin D in many people. Vitamin D or calciferol is a fat-soluble vitamin that exists in several forms with a different biological activity. Pollution can affect the production of vitamin D in the body. In a cross-sectional study of postmenopausal women living in Belgium, urban residents were exposed to ozone levels three times higher than were rural residents. Compared with rural residents, urban residents had a higher prevalence of serum 25-hydroxyvitamin D levels > 75 nmol/L (84% vs. 38%), even though the urban residents had a significantly higher mean sun exposure index (113 vs. 87; $p < 0.001$). After adjusting for sun exposure index, the mean serum 25-hydroxyvitamin D level was more than twice as high in rural residents as in urban residents. This study suggests that ozone or some other component of polluted urban air filters ultraviolet rays and thereby decreases the synthesis of vitamin D in the skin. Previous studies have also found that residents of areas with a high degree of air pollution have lower vitamin D status, when compared with residents of less polluted areas [21].

Many studies have documented a rising prevalence of vitamin D depletion in different populations (Table 6) [8, 12, 14]. In some, the estimates may have been influenced by the inclusion of chronically ill subjects or others with fractures and established osteoporosis in whom calcium or vitamin D supplementation was advised. Thus, the wide variation in the reported prevalence of vitamin D depletion among different studies can be explained by the heterogeneity of the studied individuals, geographical variation, the cut-off levels used to define vitamin D depletion and differences in serum 25-hydroxyvitamin D assays.

Table 6. Reported prevalences of vitamin D depletion in different studies.

Author	Study population	Sample size (*n*)	Age (years ± SD)	Cut-off value (25-OHD)	Time of year	Prevalence (%)
Lips	Nursing home residents	109	Not Reported	<10 ng/ml	Beginning of summer	35%
Lips	PMOP	7564	66±7	<10 ng/ml	Winter, fall, summer	4%
Bettica	PM	570	59.2±7	<12 ng/ml	All seasons	28%
Isaia	Elderly women	700	67	<12 ng/ml	Winter	72%
Chapuy	General adult urban	1569	60±6	<12 ng/ml	Winter	14%
LeBoff	PM with hip fractures	30	Not Reported	<12 ng/ml	All seasons	50%
Villareal	PMOP	539	64±2	<15 ng/ml	All seasons	9%
Thomas	Medical inpatients	290	62±19	<15 ng/ml	End of winter and end of summer	57%
Haden	Osteoporotic women	237	56±13	<15 ng/ml	All seasons	16%
Holick	PMOP	1536	71±9	<30 ng/ml	Winter	52%
Guardia	Current study	2924	68±10	<15 ng/ml	All seasons	15%
Guardia	Current study	2924	68±10	<30 ng/ml	All seasons	72%

Note: PMOP: post-menopausal osteoporotic women; PM: postmenopausal women.

In another study, blood levels of vitamin D were measured in two areas of India, one with high levels of air pollution and the other with significantly less pollution and the average vitamin D concentration was 54% lower in the former than in the latter. Forty-six percent of the children in the heavily polluted area had subnormal blood levels of vitamin D and in 12% of the children the deficiency was severe enough to cause rickets. In contrast, none of the 31 children living in the less polluted area had vitamin D deficiency [1]. More recently, studies carried across different countries in South and South East Asia showed, with few exceptions, widespread prevalence of hypovitaminosis D, in both sexes and all age groups of the population [2, 3].

Air pollution probably also plays a role in large cities. Some studies revealed that hypovitaminosis D is occurs more often in urban subjects [28]. Limited outdoor activity may represent another compound of this problem in the urban population.

Vitamin D deficiency has a deleterious effect on bone mineral homeostasis and peak bone mass achieved, and may subsequently reflect as low bone mineral density [8]. Vitamin D depletion is high among ambulatory subjects seeking advice on osteoporosis. These observations suggest that air pollution may promote the development of osteoporosis.

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