ORIGINAL ARTICLE

Association between bone remodeling and inflammatory markers in obstructive sleep apnea in relation to disease severity

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KEY WORDS

bone mineral density, bone turnover, chitinase-3-like protein 1, obstructive sleep apnea

ABSTRACT

INTRODUCTION There is growing evidence that obstructive sleep apnea (OSA) influences both bone metabolism and structure. Chitinase-3-like protein 1 (YKL-40) is a novel inflammatory and remodeling marker, the levels of which were shown to increase in OSA. YKL-40 can probably alter the bone turnover.

OBJECTIVES The aim of the study was to assess a possible interplay between YKL-40 and bone turnover markers in patients with different stages of OSA, and to evaluate the relation between bone mass, severity of OSA, and YKL-40 levels.

PATIENTS AND METHODS The study involved 72 male patients with OSA. They were divided into 3 groups according to disease severity, using the apnea–hypopnea index (AHI): group 1 (n = 18; 5≤ AHI <15), group 2 (n = 25; 15≤ AHI <30), and group 3 (n = 29; AHI ≥30). All patients underwent polysomnography and densitometry. Fasting blood samples were collected for YKL-40, C-terminal telopeptide of type I collagen (CTX), procollagen type 1 N-terminal propeptide (P1NP), and other markers.

RESULTS P1NP differed between groups 1 and 2, as well as groups 1 and 3 ($P = 0.02$). Group 2 had higher CTX levels than group 1 (borderline significance, $P = 0.05$). A simple linear regression analysis showed that serum YKL-40 levels were associated with the levels of CTX ($P < 0.0001$, $\beta = 0.9871$) and P1NP ($P < 0.0001$, $\beta = 0.9780$).

CONCLUSIONS Our study might suggest that YKL-40 is associated with bone turnover in OSA. We may assume that this marker influences both bone formation and destruction; thus, OSA could be characterized by preserved bone mineral density.

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INTRODUCTION Obstructive sleep apnea (OSA) is characterized by recurrent episodes of arousals and oxyhemoglobin desaturation due to complete or partial obstruction of the airways during sleep.[1,2] It occurs in 4% to 20% of men and in 2% to 10% of women, and its incidence is rising because of aging and increased rates of obesity.[3,4] There is a growing evidence that OSA influences bone metabolism and structure. One of the postulated mechanisms by which OSA might affect the skeleton is inflammation. Pathological intermittent hypoxia characteristic for OSA leads to repetitive ischemic injury, which generates an acidic environment within the bones and triggers an inflammatory reaction, eventually resulting in susceptibility to fractures.[5]

Chitinase-3-like protein 1 (YKL-40) is a novel inflammatory and remodeling marker, which has been recently investigated in patients with OSA. YKL-40 levels were found to be higher in this population than in healthy controls. Wang et al[6] suggested that YKL-40 could be a predictor of

the occurrence and progression of OSA. Serum YKL-40 levels are also elevated in disorders associated with chronic inflammation and tissue remodeling. This encompasses diseases characterized by bone destruction. Mylin et al[7] demonstrated a connection between increased serum YKL-40 levels and bone turnover markers, with the dominance of bone resorption (the ratio of C-terminal telopeptide of type I collagen [CTX] to amino-terminal propeptide of type I collagen [P1NP]). Moreover, previous studies explored the role of YKL-40 in osteoclast function.[7] CTX is a peptide released as a product of bone degradation. Tomiyama et al[5] showed that CTX levels are increased in the urine of patients with OSA, but they decrease after continuous positive airway pressure therapy.

In the light of previous research, we aimed to assess a possible interplay between YKL-40 and bone turnover markers in patients with different stages of OSA. To our knowledge, there have been no previous studies investigating a potential association between YKL-40 and bone metabolism in such population. We also evaluated a relation between YKL-40, bone mass, and OSA severity. Considering the rising prevalence of OSA and osteoporosis, as well as the phenomenon of population aging in industrialized countries, it is important to investigate the possible links between those entities.[5]

PATIENTS AND METHODS

Study design and enrollment of patients We enrolled consecutive male patients aged older than 50 years who were admitted to outpatient sleep clinics between January 2016 and September 2016 due to symptoms suggesting OSA, and who obtained 10 points or more in the Epworth Sleepiness Scale and were thus referred for polysomnography. The total study sample comprised 72 patients with newly diagnosed OSA, who were divided into 3 groups according to disease severity assessed using the apnea–hypopnea index (AHI): group 1 (n = 18; 5≤ AHI <15), group 2 (n = 25; 15≤ AHI <30), and group 3 (n = 29; AHI ≥30). We also analyzed concomitant disorders with a special emphasis on cardiovascular disease (CVD).

The exclusion criteria were as follows: previous treatment for osteoporosis, active neoplastic process, central sleep apnea–hypopnea syndrome, thyroid dysfunction, and impaired liver or renal function (creatinine level >1.2 mg/dl).

Statistical analysis Statistical analysis was performed with the MedCalc Statistical Software version 16.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016). The D'Agostino–Pearson test was used to assess normality. Variables with a normal distribution were compared between the groups with the 1-way analysis of variance. The Krusal–Wallis test was performed when data did not fulfil the normality criteria. Data that did not follow a normal distribution were compared with the Mann–Whitney test. The χ^2 test was used to compare discrete variables. Relationships between data were analyzed with simple regression. Before inclusion to this statistical analysis, nonnormally distributed parameters were logarithmically transformed. A stepwise multiple regression analysis was used to confirm the association of YKL-40 concentrations and other analyzed markers of bone turnover after adjustment for body mass index (BMI). A *P* value of less than 0.05 was considered significant.

Laboratory analysis Fasting blood samples were collected for the measurement of YKL-40, CTX, P1NP, high-sensitivity C-reactive protein (hs-CRP), aspartate transaminase (AST), alanine aminotransferase (ALT), creatinine, and glycated hemoglobin A_{1c} (HbA_{1c}) levels. Serum YKL-40, CTX, and P1NP levels were assessed with an enzyme-linked immunosorbent assay kit (Sunred Biological Technology, Shanghai, China). The levels of hs-CRP (reference range <5 mg/dl [SI units, nmol/l]), AST (10–37 U/l [µmol/l]), ALT (10–41 U/l [µmol/l]), and creatinine (0.7–1.20 mg/dl [µmol/l]) were measured with an electrochemiluminescence method using Cobas 6000 (Roche Diagnostics, Mannheim, Germany). HbA_{1c} levels (<6.5% [proportion of total hemoglobin]) were assessed with an immunoturbidimetric assay using Architect i1000 (Abbot Diagnostics, Warsaw, Poland). Informed consent was obtained from all participants. The study was approved by a local bioethics committee and performed in accordance with the latest version of the Declaration of Helsinki.

Polysomnography Complete overnight polysomnography was performed by an experienced sleep technician at the Sleep Laboratory in the Department of Pulmonology, Allergology, and Respiratory Oncology at the Poznan University of Medical Sciences (Poznań, Poland) from 10 PM to 6 AM, using EMBLA S4000 – Remlogic, Somnologica Studio 5.0; Natus 2009 (Embla, Broomfield, Colorado, United States). An electroencephalogram, electromyogram, electrooculogram, electrocardiogram, hemoglobin oxygen saturation (finger pulsoximetry), the airflow through the nose and mouth (thermistor, nasal cannula), abdominal and thoracic movements, snoring sounds, and positions during sleep were observed and recorded. Apnea was defined as more than 90% and hypopnea as at least 30% reduction of airflow for more than 10 seconds and associated with a decrease of more than 4% in oxygen saturation. The AHI was defined as an average number of apneas and hypopneas per hour of sleep.[8]

Bone mineral density Bone mineral density (BMD) at the lumbar spine (L1–L4) was obtained with dual energy X-ray absorptiometry (Lunar Prodigy Primo, Warsaw, Poland). The results were presented as grams per square centimeter and T-score.

TABLE 1 Biochemical and clinical characteristics of the study groups divided according to the severity of obstructive sleep apnea

Parameter	Group 1 5≤ AHI <15 (n = 18)	Group 2 15≤ AHI <30 (n = 25)	Group 3 AHI ≥30 (n =29)	*P* value
Age, y	62 (57–69)	61 (56.5–65.2)	62 (56–67.2)	0.89
BMI, kg/m^2	31.3 (27.4–35.6)	29.7 (28.7–31.3)	33.2 (29.9–36.7)	0.02
HbA_{1c}, %	5.4 (5.3–6.5)	5.6 (5.5–6.0)	5.9 (5.6–6.1)	0.40
ALT, U/l	28 (23–42)	28 (23.2–34.5)	28 (20.7–44.5)	0.82
AST, U/l	25 (20–34)	22 (19–25)	22 (19–27.2)	0.37
Creatinine, mg/dl	0.9 (0.9–1.0)	0.9 (0.8–1.0)	1.0 (0.9–1.0)	0.28
Hs-CRP, mg/dl	1.5 (0.9–3.0)	2.3 (0.5–2.8)	2.2 (1.2–3.3)	0.20
AHI	8.5 (6–13.3)	21.9 (18.4–24.6)	50.3 (39.2–62.3)	<0.00
YKL-40, ng/ml	49.9 (42.1–54.4)	51.5 (46.2–151.8)	51 (46.2–89.1)	0.42
CTX, ng/ml	199 (182.0–216.0)	249.9 (205.825–729.1)	222.2 (181.4–420.3)	0.05
P1NP, ng/ml	72.2 (66.30–79.1)	81.6 (74.6–237.9)	79.2 (69.7–147.4)	0.02
L1–L4 T-score	−0.1 (−1.5 to 1.4)	−0.3 (−1.8 to 1.4)	−0.2 (−1.5 to 1.8)	0.79
L1–L4 BMD, g/cm^2	1.3 (1.1–1.4)	1.2 (1.1–1.4)	1.3 (1.1–1.5)	0.47

Data are presented as median (interquartile range).

Conversion factors to SI units are as follows: for ALT, 16.67; AST, 16.67; creatinine, 88.4; hs-CRP, 95.24; HbA_{1c}, 0.01.

Abbreviations: AHI, apnea–hypopnea index; ALT, alanine aminotransferase; AST, aspartate transaminase; BMD, bone mineral density; BMI, bone mass index; hs-CRP, high-sensitivity C-reactive protein; CTX, C-terminal telopeptide of type I collagen; HbA_{1c}, glycated hemoglobin A_{1c}; L1–L4, lumbar spine disks 1–4; OSA, obstructive sleep apnea; P1NP, procollagen type 1 N-terminal propeptide; YKL-40, chitinase-3-like protein 1

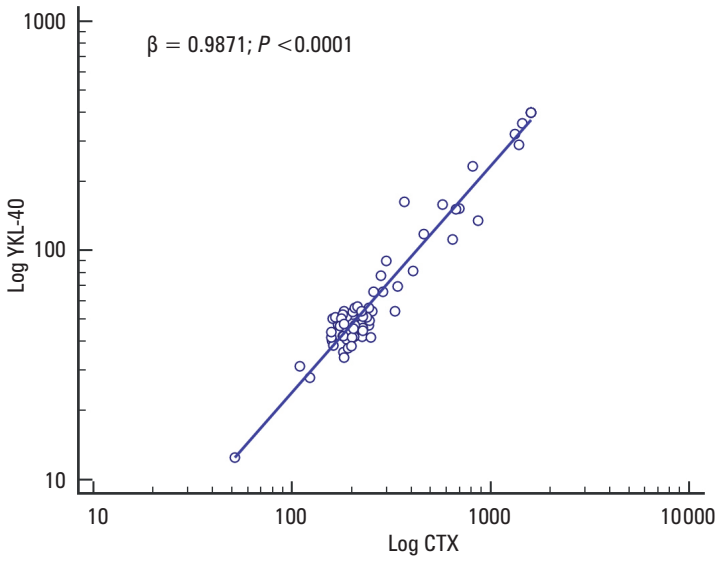

FIGURE 1 Association between chitinase-3-like protein 1 (YKL-40) and C-terminal telopeptide of type I collagen (CTX). Data were log-transformed to achieve a normal distribution.

RESULTS Biochemical and clinical characteristics of the groups are presented in TABLE 1. The groups did not differ in age or the levels of HbA_{1c}, ALT, AST, creatinine, hs-CRP, YKL-40, or L1–L4 BMD. Group 2 had a higher CTX level (P = 0.05) than group 1. P1NP levels differed between groups 1 and 2, and groups 1 and 3 (P = 0.02). BMI was higher in group 3 than in group 2 (P = 0.02) (TABLE 1).

The linear regression analysis showed that serum YKL-40 levels were significantly associated with CTX levels (P <0.0001, β = 0.99) (FIGURE 1) and P1NP levels (P <0.0001, β = 0.98) (FIGURE 2). These associations were observed also after adjustment for BMI. There were no associations between YKL-40 levels and AHI, hs-CRP, L1–L4 BMD, HbA_{1c}, T-score, age, or BMI (TABLE 2). CVD was present in 58 patients (group 1, 25% of the patients; group 2, 34.7%; group 3, 40.3%). Coronary heart disease was reported in 16 patients; hypertension, in 51; heart failure, in 4; stroke, in 5; arrhythmia in 6; and peripheral vascular disease, in 2. There was a trend for a higher incidence of CVD in patients with a more advanced stage of OSA (P = 0.05). However, YKL-40 levels did not differ between the groups with and without CVD (P = 0.27).

DISCUSSION YKL-40 is a glycoprotein expressed by a wide variety of cells, depending on their cellular activity. This encompasses osteoblasts and osteoclasts in tissues with intensive bone turnover. In a previous study, in a normal bone marrow, YKL-40 was detected by immunohistochemistry in both osteoblasts and osteoclasts.[9,10] YKL-40 is secreted by, for example, neoplastic cells, neutrophils, macrophages, chondrocytes, and vascular muscles.[11] The biomarker was first discovered by Mansell et al[12] as a protein secreted by human osteosarcoma-derived osteoblasts (cell line MG63). In that study, YKL-40 expression was stimulated by active vitamin D. Thus, the association between vital skeletal health promoter and YKL-40 synthesis by osteoblasts has been proved.[12] While normal fetal and adult osteoblast cultures did not secrete YKL-40, the expression of YKL-40 mRNA was seen in osteoblasts present within osteophytes in patients with osteoarthritis.[13] The YKL-40 expression increases with the stage of osteoclastogenesis in monocytes, stimulated to gain osteoclast differentiation. These observations imply that the expression of YKL-40 may be related to the stage of cell maturation.[14] It might also be associated with a reaction of these cells to an altered tissue environment.[15] Since OSA is known to change conditions in the bones, we hypothesized that there might be an association between YKL-40 levels and bone turnover markers.

Apart from triggering an inflammatory reaction, OSA influences the skeleton through several mechanisms. It alters sleep patterns, thus possibly leading to desynchrony in the clock gene expression in bones. It also stimulates

TABLE 2 Associations between serum chitinase-3-like protein 1 levels and selected parameters in the study group

Parameter	YKL-40	
	β	*P* value
Age	0.02	0.80
BMI	−0.13	0.82
AHI	0.11	0.35
Hs-CRP	−0.14	0.13
HbA_{1c}	−1.44	0.09
L1–L4 BMD	0.06	0.88
L1–L4 T-score	0.00	1.00
CTX	0.99	<0.0001
P1NP	0.98	<0.0001

Abbreviations: see **TABLE 1**

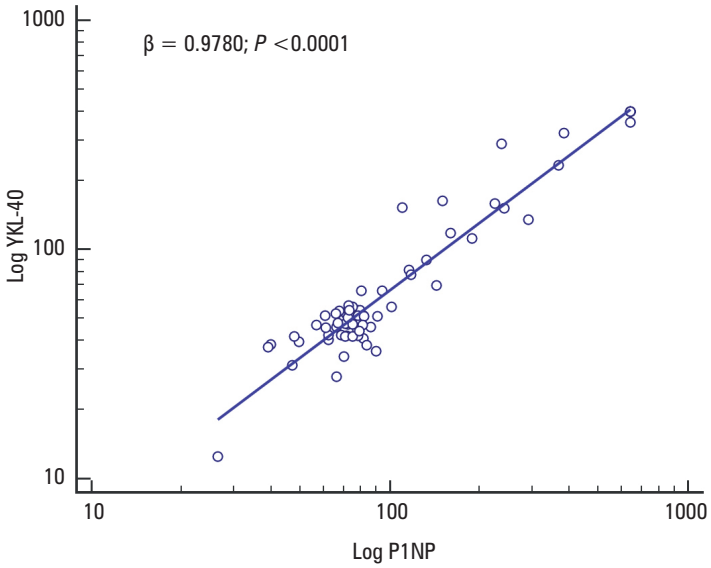

FIGURE 2 Associations between chitinase-3-like protein 1 (YKL-40) and procollagen type 1 N-terminal propeptide (P1NP). Data were log-transformed to achieve a normal distribution.

the sympathetic system by sleep fragmentation and by an increase in leptin concentrations. Excessive activation of sympathetic system hampers bone formation. Sleep disturbances alter the secretion cycle of melatonin, thus inhibiting its beneficial effects on the bones. Moreover, OSA reduces vitamin D production. This steroid not only plays a vital role in bone formation, but is also believed to have anticancer and anti-inflammatory properties.[16]

YKL-40 has been implicated in inflammation, endothelial dysfunction, and tissue remodeling. It was also shown to be associated with the presence and severity of OSA. Duru et al[17] underlined the usability of serum YKL-40 as an inflammatory biomarker in these patients. YKL-40 may be also a predictor of endothelial dysfunction and atherosclerosis in patients with OSA.[18,19] The median serum concentration of YKL-40 measured in 245 healthy adults (both men and women; mean age, 49 years) was 43 ng/ml.[7] However, since the reference range for YKL-40 levels has not been established yet, it is not possible to assess whether a slight elevation of YKL-40 levels observed in our study is clinically significant.

From among the bone turnover markers, we chose to assess CTX (resorption) and P1NP (formation), as they are regarded as the most useful.[20,21] However, the biological and laboratory variability in the values of bone turnover markers has limited their widespread clinical implementation.[22] The reference ranges for P1NP and CTX levels should be age- and sex-related. Several studies provided reference values for CTX and P1NP, but they were derived from serum automated methods.[23-26]

To date, there have been few studies investigating bone turnover in OSA and the results have been conflicting. Tomiyama et al[27] first provided the evidence for a link between OSA and abnormal bone metabolism. They described severity-dependent elevations in the serum and urinary levels of bone resorption markers (such as CTX) and their reversal following continuous positive airway pressure therapy in patients with OSA. Terzi et al[28] found that serum CTX levels, but not osteocalcin, were significantly higher in patients with OSA than in controls. However, Chen et al[29] did not find differences between patients with and without OSA in terms of bone turnover markers. We found that both CTX and P1NP levels increased with an increase in the AHI. It might be possible that not only the rates of bone destruction but also of bone formation rise with the AHI. OSA might contribute to an increased rate of bone metabolism, not just bone resorption. The lack of differences in BMD between the 3 groups may further support this finding.

Recent studies have examined the relationship between OSA and BMD in humans, with conflicting results. In a retrospective longitudinal cohort study, Chen et al[30] reported that patients with OSA are 2.7-fold more prone to develop osteoporosis than controls. A meta-analysis by Upala et al[31] showed that among the included cohort studies, the pooled odds ratio (OR) of osteoporosis was 1.92 (confidence interval [CI], 1.24–2.97) in patients with OSA in comparison with controls. However, a cross-sectional meta-analysis proved that the control group was more prone to develop osteoporosis (OR, 0.60; 95% CI, 0.42–0.87).[31] Mariani et al[32] did not find any correlation between the BMD of the lumbar spine, femoral neck, or total hip and the stage of OSA. In addition, Sforza et al[33] revealed higher BMD values in patients with OSA than in the control group. The strength of their study was a large sample size (n = 832). In animal models resembling OSA, intermittent hypoxia was not associated with differences in trabecular BMD in the femur in normoxic and hypoxic mice. The authors even suggested that chronic intermittent hypoxia might protect bone density. The limitations of the study include a short follow-up, while in

humans, OSA usually remains undiagnosed for many years.[34] We found differences in CTX and P1NP levels between the subgroups with mild--moderate and mild-severe OSA, but not when comparing patients with moderate and severe OSA. It might be attributed to the difference in BMI in those groups, as obesity may be associated with suppression of bone turnover.[35] It is also possible that the speed of bone turnover slows down in severe OSA. To sum up, studies investigating bone turnover markers and BMD have provided conflicting results. Our study supports the hypothesis that OSA might be associated with preserved BMD and an increase in CTX and P1NP levels. This indicates that both bone destruction and formation might be affected by OSA.

Interestingly, we found that YKL-40 levels are correlated with the serum concentrations of both P1NP and CTX. These correlations remain significant after correction for confounding factors such as BMI. The association of YKL-40 with bone turnover markers might just be an epiphenomenon. However, recent studies have suggested a possible interaction. First, YKL-40 possibly influences osteoclast function via matrix metalloproteinases.[14] Second, bovine YKL-40 was shown to attach specifically to type I collagen. It regulated the rate of fibril formation in vitro. Bovine YKL-40 has isoforms that exert different effects on the fibril structure of type I collagen. One has a suppressive effect on fibrillogenesis, while the other stimulates the fibril formation. This might support our finding that YKL-40 is associated both with CTX and P1NP levels.[36] However, it is unknown whether similar isoforms occur in humans and influence bone metabolism in the same way. Moreover, Di Rossa et al[14] reported that in human cultures of mature osteoclast silencing, YKL-40 using siRNA resulted in a reduced bone resorption in vitro. It further underlines the possibility that YKL-40 induces both bone destruction and formation.

There is a growing body of evidence that OSA is involved in the pathophysiology of CVD.[37,38] A meta-analysis and metaregression of 18 studies performer by Wang et al[39] indicated that particularly moderate-severe OSA appeared to reduce endothelial function, increase arterial stiffness, and cause chronic inflammation, leading to the development of CVD. YKL-40, similarly to other markers such as sclerostin and osteoprotegerin (OPG), may influence bone metabolism and may also affect the cardiovascular system.[40] We assessed the incidence of CVD in the studied group and found a trend for a higher incidence of CVD in patients with a more advanced stage of OSA (P = 0.05). YKL-40 levels did not differ between the groups with and without CVD (P = 0.27).

In future studies, it might be worthwhile to assess OPG and sclerostin in relation to bone turnover markers in a similar population. OPG is thought to be a mediator in the crosstalk between the vessels and bones. It is suggested that circulating OPG levels could be used as an independent biomarker of CVD.[41,42] Sclerostin is secreted mainly by osteocytes. In women with OSA, serum sclerostin levels correlated with the AHI and were higher in patients with CVD.[43]

In conclusion, to our knowledge, this is the first study to show a positive correlation between YKL-40 levels and bone turnover markers in patients with OSA. Our results also support a hypothesis that OSA may affect both bone destruction and formation. The possible association between YKL-40, bone metabolism, and severity of OSA requires further research.

Limitations The main limitations of this study are the small sample size and the lack of a healthy control group. However, this was a preliminary research aiming at the initial assessment of possible interactions and at designing future prospective studies based on the results. The strength of this study is the fact that the diagnosis of OSA was confirmed by polysomnography rather than being established on the basis of questionnaires assessing history and symptoms. What is more, to our knowledge, this is the first study to evaluate any interaction between BMD, bone turnover markers, and YKL-40 levels in patients with OSA. Our study might thus suggest that YKL-40 is associated with bone turnover in OSA. We may assume that OSA influences both bone formation and destruction markers; therefore, it could be characterized by preserved BMD.

CONTRIBUTION STATEMENT BB, EC-C, and BK-K conceived the concept of the study. MR, HBG, and ZK contributed to the design of the research. BB, EC-C, MK, HW, BK-K, AZ-K, and AH were involved in data collection. NS-G, EW, and BB analyzed and interpreted the data. BB, EC-C, BK-K, MK, HW, NS-G, and EW drafted the paper. AZ-K, AH, ZK, MR, and HB-G revised the manuscript critically for important intellectual content. All authors edited and approved the final version of the manuscript.

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