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Original/*Obesidad*

Vitamin D levels and bone turnover markers are not related to non-alcoholic fatty liver disease in severely obese patients

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Abstract

Background: **Morbidly obese patients usually present vitamin D deficiency or secondary hyperparathyroidism. Low vitamin D levels have been recently related to non-alcoholic fatty liver disease (NAFLD). The aim of this study was to analyse the relationship between vitamin D, bone turnover markers and non-alcoholic fatty liver disease and metabolic syndrome in severely obese patients.**

Methods: **One hundred and ten patients who underwent bariatric surgery were included. Liver biopsy was taken during surgery. Two univariate analyses were carried out in order to i) analyse the relationship between liver histology and vitamin D-bone turnover markers (intact parathyroid hormone (PTH), osteocalcin and Carboxy-terminal collagen crosslinks) and ii) establish the association between metabolic syndrome components-insulin resistance (HOMA) and vitamin D-bone turnover markers.**

Results: **70% of the patients had lower levels of vitamin D or secondary hyperparathyroidism. None of the components of liver histology were associated with levels of vitamin D or with bone turnover parameters. Patients with metabolic syndrome showed lower levels of PTH and osteocalcin (72,42 (29,47) vs 61.25(19.59) p-Value: 0.022; 19.79 (10.43) vs 16.87(10.25) p-Value: 0,028, respectively). HOMA was not related to Vitamin D or bone turnover markers.**

Conclusion: **Low levels of vitamin D or hyperparathyroidism are common in severely obese patients. Vitamin D and bone metabolism markers were associated neither to NAFLD nor with metabolic syndrome in our series of obese morbid patients.**

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Key words: *Vitamin D Deficiency. Morbid obesity. Non alcoholic fatty liver Disease*.

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LOS NIVELES DE VITAMINA D Y MARCADORES DE RECAMBIO OSEO NO SE ENCUENTRAN RELACIONADOS CON EL HÍGADO GRASO NO ALCÓHOLICO EN OBESOS MÓRBIDOS

Resumen

Antecedentes: **los pacientes con obesidad mórbida presentan frecuentemente déficit de vitamina D o hiperparatiroidismo secundario. Presentar niveles bajos de vitamina D se ha asociado recientemente con el hígado graso no alcohólico (EHNA). El objetivo de este estudio fue analizar la relación de la vitamina D y los marcadores de recambio óseo con el hígado graso no alcohólico y el síndrome metabólico, en pacientes con obesidad mórbida.**

Métodos: **Ciento diez pacientes sometidos a cirugía bariátrica fueron incluidos, obteniéndose una biopsia hepática durante la cirugía. Dos análisis univariados se llevaron a cabo con el fin de: i) analizar la relación de la histología hepática con la vitamina D y marcadores de recambio óseo (hormona paratiroidea intacta (PTH), osteocalcina y enlaces cruzados de colágeno carboxi-terminal) y ii) establecer la asociación de los componentes del síndrome metabólico y resistencia a la insulina (HOMA) con los marcadores de recambio óseo y vitamina D.**

Resultados: **El 70% de los pacientes presentaron niveles bajos de vitamina D o hiperparatiroidismo secundario. Ninguno de los componentes de la histología hepática resultó asociciado con los niveles de vitamina D o con los parámetros de recambio óseo. Los pacientes con síndrome metabólico mostraron un nivel menor de PTH (72,42 (29,47) vs 61,25 (19,59) Valor p: 0.022) y de osteocalcina 19,79 (10,43) vs 16,87 (10,25) p-valor: 0.028). El HOMA no resultó relacionado con la vitamina D o con los marcadores de recambio óseo.**

Conclusión: **Niveles bajos de vitamina D e hiperparatiroidismo secundario son hallazgos frecuentes en pacientes con obesidad mórbida en nuestro medio. Los marcadores de la vitamina D y recambio óseo no resultaron asociados con el hígado graso no alcohólico, ni con el síndrome metabólico en nuestra serie de pacientes obesos mórbidos.**

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Palabras clave: *Deficit de vitamina D. Obesidad mórbida. Hígado graso no alcohólico*.

Abbreviations

ALT: Alanine transaminase.
AST: Aspartate aminotransferase.
BMI: Body mass index.
DM: Diabetes Mellitus.
CRP: C reactive protein.
CTX: Carboxy-terminal collagen crosslinks.
GGT: Gamma-Glutaryl transferase.
HBP: High blood pressure.
HOMA: Homeostasis model assessment.
IR: Insulin resistance.
MS: Metabolic syndrome.
NAFLD: Non-alcoholic fatty liver disease.
NASH: Non-alcoholic steatohepatitis.
PTH: Intact parathyroid hormone.
Vit D 25: 25-hydroxyvitamin D.
WC: Waist circumference.

Introduction

Vitamin D is essential to the regulation of the osteometabolic system, but it has other extra skeleton effects. Several evidences have shown that vitamin D deficient individuals are more likely to develop glucose intolerance, metabolic syndrome and type 2 diabetes mellitus[1], and also Vitamin D levels have been related to cardiovascular disease[2,3].

The prevalence of hypovitaminosis D in adult population is about 30-60%[4,5] and in severely obese patients decreased levels of Vitamin D have been reported in 80% of the cases[6]. The relationship between obesity and vitamin D remains unclear. It may have a multifactorial etiology: suboptimal synthesis (inadequate sun exposure or less exercise due to reduced mobility), increased storage of vitamin D in adipose tissue, and impaired production of 25-hydroxyvitamin in the liver because of hepatic steatosis[7,8]. A recent study suggests that vitamin D deficiency is associated with increased risk of developing obesity[5].

The prevalence of non-alcoholic fatty liver disease (NAFLD) in patients undergoing bariatric surgery exceeds 90%[9,10] and the range of prevalence of non-alcoholic steatohepatitis (NASH) in obese population was 10-56 %[11]. NAFLD has been associated to insulin resistance, metabolic syndrome and diabetes, but the reason to progress from simple steatosis to NASH or cirrhosis remains unclear. A multi-hit hypothesis has been proposed, where multiple factors (nutritional, genetic factors mainly) might contribute to liver inflammation development[12].

Decreased levels of Vitamin D have been related to NAFLD[13], so vitamin D deficiency is thought to play a role in the development of NALFD and in the progression of liver inflammation[14]. Less information about the role of intact parathyroid hormone (PTH) levels and bone turnover markers in NALFD has been reported.

The relationship between vitamin D, bone turnover markers and NAFLD has not been evaluated in morbidly obese patients, so the aim of this study was to analyse the relationship between vitamin D and bone turn over markers, non-alcoholic liver disease and metabolic syndrome in severely obese patients candidates to undergo bariatric surgery.

Material and methods

Patients

Patients who underwent bariatric surgery at third level hospital between June 2008 and March 2013 were prospectively included. The patients were included if they fulfilled SEEDO (Spanish Society for Obesity study) criteria for bariatric surgery[15]: body mass index (BMI) above 40 kg/m^2 or above 35 kg/m^2 with obesity associated comorbidities. Scopinaro´s laparoscopic biliopancreatic diversion was performed and a liver biopsy was obtained during surgery.

The exclusion criteria were: patients with primary hyperparathyroidism or renal disease, alcohol intake higher than 20g/day and other causes of liver disease (Hepatitis C, hepatitis B, autoimmune liver disease, hemochromatosis and treatment with steatosis inducing drugs). The study was approved by the Clinical research ethics committee in our hospital and written informed consent was obtained from all patients.

Clinical and laboratory

Clinical and anthropometric data were collected 2-4 weeks before surgery. Body mass index (BMI) was calculated using the following equation: Weight (Kg)/height (meters)^2. Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest.

The diagnosis of type 2 diabetes was based on the criteria of the American Diabetes Association, using a value of fasting blood glucose ≥ 126mg/dL at least twice[16]. The diagnosis of high blood pressure (HBP) was based on the following criteria: systolic blood pressure ≥135mmHg, and diastolic ≥85mmHg measured, at least, three times. Metabolic syndrome (MS) was diagnosed according ATPIII criteria[17].

A 8 hour-overnight fasting blood sample was obtained as a part of preoperative routine study to measure serum levels of: 25-hydroxyvitamin D (vit D 25) (ng/mL), intact parathyroid hormone (PTH) (pg/mL), osteocalcin, Carboxy-terminal collagen crosslinks (CTX) (ng/mL), alanine transaminase (ALT) (UI/L), aspartate aminotransferase (AST) (UI/L), Gamma-Glutaryl transferase (GGT) (UI/L), total cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL), glucose (mg/dL) and insulin (µU/mL). VitD 25 was measured by electro immunochemoluminescence, CTX

by enzyme-linked immunosorbent assay, Osteocalcin by radioimmuno assay and PTH by immunochemoluminescence. Insulin resistance (IR) was determined by homeostasis model assessment (HOMA) using the following equation: fasting insulin (μU/ml) x fasting glucose (mmol/L)/22.5).

Secondary hyperparathyroidism and vitamin D deficiency were considered whenever PTH levels were above 72 pg/ml, and VitD 25 ≤ 20 ng/ml, according to Endocrine Society recommendation[18].

Histology

A liver biopsy of at least 15 mm was obtained during surgery to ensure that it was feasible[19], and analysed by an expert pathologist. The Kleiner classification was used to establish NAS score (from 0 to 8), including steatosis (0 to 3), lobular inflammation (0 to 3) and hepatocellular ballooning (0 to 2). Fibrosis stage was classified from 0-4[20]. NASH was considered if NAS score was ≥5, not NASH if NAS score 0-2, and indeterminate if 3-4.

Statistics

Continuous variables were expressed as mean (standard deviation), and categorical variables as frequency (percentage). A p-Value < 0.05 was considered as significant. Statistical analysis was performed using SPSS 15.0 for Windows.

The normality of the distribution was determined by the Kolmogorov-Smirnov test. Before statistical analysis, skewed variables (vitamin D, HOMA, PTH, osteocalcin, CTX) were logarithmically transformed to improve normality. In text and tables these variables are represented in their natural units.

Two univariate analyses were carried out to valuate the relationships between i) liver histology and vitamin D-bone turnover markers and ii) between metabolic syndrome components and vitamin D-bone turnover markers. For a quantitative predictor and more than two levels of exposure variables, analysis of variance (ANOVA) was applied. Student t-test was used to compare two means or U the Mann- Whitney if they did not follow a normal distribution. Chi-squared or Fisher exact test were used for qualitative predictor. Pearson correlation coefficient was utilised to study the relationship between two continuous variables.

Results

From the 136 patients analysed, 26 were excluded because vitamin D, PTH levels or bone turnover markers data were not available. Patient's characteristics and pathological features are shown in table I and II, respectively. 48.2% (53/110) had HBP, 28.1% (24/110) DM and 48.2% met criteria for metabolic syndrome. 33.6% (37/110) had hyperparathyroidism, and 60.9%(67/110) vitamin D deficiency. 30% (33/110) of the patients had neither hyperparathyroidism nor vitamin D deficiency.

Table III shows the relationship between liver histology, vitamin D levels and bone metabolism. None of the analysed parameters were related to liver histology. In table IV, the analysis between vitamin D, bone metabolism, metabolic syndrome and other features are represented. Patients with MS had lower levels of PTH (61.25 vs 72.42, p-Value 0.022) and lower levels of osteocalcin (16.87 vs 19.79, p-Value 0.028).

Attending to NAS score, the distribution of patients with vitamin D deficiency was: NAS score 0-2: 40/63(63.5%); NAS score 2-4: 14/29(48.3%); NAS score≥ 5: 12/18(66.7%), p-Value: 0.314; and the distribution of patients with hyperparathyroidism was: NAS score 0-2: 22/63(34.5%); NAS score 2-4: 9/29(31%); NAS score ≥ 5: 6/18(33.3%), p-Value:0.935.

Table I
Characteristics of the patients included

N	110
Age (years)	44.18 (10.16)
Sex (female)	80 (72.7%)
IMC (kg/m^2)	46.86 (6.1)
Waist (cm)	13.,39 (12.78)
Glucemia (mg/dL)	109.33 (30.6)
Insulin (μU/mL)	23.17 (12.03)
HOMA	6.4 (4.1)
Cholesterol (mg/dL)	191 (32.82)
HDL (mg/dL)	49.97 (12.47)
Triglycerides (mg/dL)	139.05 (64.85)
ALT (UI/L)	22.91 (9.13)
AST (UI/L)	32.65 (20.15)
GGT (UI/L)	33.37 (2.14)
Total proteins (g/dL)	7.16 (0.42)
Albumina (g/dL)	4.38 (0.26)
Vit D 25 (ng/mL)	23.23 (16.61)
PTH (pg/mL)	68.85 (39.3)
Osteocalcin (ng/mL)	18.39 (10.4)
CTX (ng/mL)	0.26 (0.13)

Continuous variables are expressed as mean(SD) and categorical variables are expressed as frequency (percentage). BMI: body mass index. DM: Diabetes mellitus. HBP: high blood pressure. HOMA: homeostasis model assessment. VAI: visceral adipose index. HDL: high-density lipoprotein, ALT: alanine transaminase, AST: aspartate aminotransferase, GGT: gamma-glutaryl transferase, CRP: C-reactive protein.

Table II
Liver histological features of patients included

Steatosis	Grade 0	18 (16.4%)
	Grade 1	51 (46.6%)
	Grade 2	26 (23.6%)
	Grade 3	15 (13.6%)
Inflammation	Grade 0	38 (34.5%)
	Grade 1	61 (55.5%)
	Grade 2	8 (7.3%)
	Grade 3	3 (2.7%)
Ballooning	Grade 0	72 (65.5%)
	Grade 1	29 (26.4%)
	Grade 2	9 (8.2%)
NASH (NAS scale)	Grade 0-2	63 (57.3%)
	Grade 2-4	29 (26.4%)
	Grade ≥5	18 (16.4%)
Fibrosis	Grade 0	95 (83.6%)
	Grade 1	11 (10%)
	Grade 2	1 (0.9%)
	Grade 3	3 (2.7%)
Normal liver biopsy	15 (16.4%)	

Patients with vitamin D deficiency or with hyperparathyroidism did not show statistically significant higher HOMA levels (Vitamin D deficiency: 6.79(4.52) vs Non-Vitamin D deficiency: 5.81(3.36), p-Value: 0.328; hyperparathyroidism: 5.71(3.82) vs Non-Hyperparathyroidism: 6.75 (6.75), p-Value: 0.209). Hypovitaminosis D was present in 58.5%(31/53) of patients with MS and in 61.4%(35/57) of the patients without MS (p-Value 0.76); hyperparathyroidism was observed in the 24.5%(13/53) of patients with MS and in the 41.2%(24/57) of the patients without MS (p-Value 0.051).

Discusion

This cross-sectional study presents a novel approach to establish the relationship between vitamin D, bone turnover markers, metabolic syndrome and liver histology in obese patients candidates to bariatric surgery.

Nonetheless, this study has several limitations. Firstly, as this is not a controlled study and a cross-sectional design is used, results should be inter-

Table III
Liver histology, vitamin D levels, and bone turnover markers

		PTH	*Osteocalcin*	*CTX*	*VIT D 25*
Esteatosis grade	0	70.22(31.11)	20.91(15.59)	0.28(013)	25.16(19.23)
	1	69.39(31.15)	18.86(11)	0.24(0.12)	24.06(16.88)
	2	62.54(31.62)	18.08(5.28)	0.28(0.14)	23.93(17.67)
	3	63(21.4)	14.26(6.21)	0.14(0.12)	16.88(8.6)
	p-Value	0.768*	0.104*	0.588*	0.593*
Inflammation grade	0	71.16(30.4)	18.59(11.45)	0.25(0.13)	23.48(17.3)
	1	67.38(31)	19.15(10.32)	0.27(0.13)	22.71(16.16)
	2	51.38(16.82)	11.68(3.15)	0.18(6.53)	22.3(16.58)
	3	49.67(10)	18.24(6.54)	0.14(0.11)	33.03(23.7)
	p-Value	0.4*	0.131*	0.289*	0.745*
Balloning grade	0	66.94(31.22)	19.58(12.12)	0.27(0.13)	23.49(16.47)
	1	67.97(30.82)	16.67(5.61)	0.24(0.13)	25.5(18.6)
	2	64.78(14.67)	14.39(4.35)	0.13(0.1)	13.85(4.98)
	p-Value	0.93*	0.334*	0.493*	0.222*
NAS score	0-2	68.76(32.27)	19.73(12.64)	0.25(0.12)	22.65(15.98)
	3-4	67.45(29.36)	17.75(6.1)	0.27(0.15)	26.37(19.93)
	≥5	60.33(21.57)	14.73(5.34)	0.24(0.12)	20.2(12.51)
	p-Value	0,755*	0.182*	0.672*	0.797*
Fibrosis	No	67.68(28,68)	18.74(10.94)	0.27(0.13)	23.92(16.97)
	Yes	62.93(37,9)	16.11(5.72)	0.2(0.08)	18.83(13.87)
	p-Value	0.338+	0.448+	0.109+	0.228+
Bx Normal	No	65.93(29.6)	18.04(8.96)	0.25(0.13)	22.67(15.98)
	Yes	74.07(32.03)	20.58(17.24)	0.27(0.15)	26.79(20.48)
	p-Value	0.324+	0.595+	0.693+	0.507+

Continuous variables are expressed as mean(SD) and categorical variables are expressed as frequency (percentage). *p Value: analasys of variance; +p-Value: U the Mann- Whitney test. PTH: intact parathyroid hormone (ng/mL), Osteocalcine (ng/mL), CTX: Carboxy-terminal collagen crosslinks (ng/mL), Vit D 25: 25-hydroxyvitamin D (pg/mL). Bx Normal: Normal liver biopsy.

Table III
Metabolic syndrome, bone metabolism and vitamin D levels

		PTH	*Osteocalcin*	*CTX*	*VIT D 25*
Sex	Male	64.1 (37.15)	20.18 (12.13)	0.28 (0.11)	18.79 (11.28)
	Female	68.14 (26.9)	17.72 (9.68)	0.25 (0.13)	24.9 (18)
	p-Value	0.203+	0.151+	0.27+	0.104+
DM	No	69.8(29.82)	18.57 (9.37)	0.27 (0.12)	23.75 (16.58)
	Yes	57.13(28.72)	17.73 13.7)	0.22 (0.14)	21.36 (16.96)
	p-Value	0.066+	0.539+	0.09+	0.383+
HBP	No	71.88 (28.94)	19.9(13.06)	0.27 (0.14)	22.04 (15.96)
	Yes	61,83 (30.35)	16.76 (6.15)	0.24 (0.11)	22.5 (17.36)
	p-Value	0.019+	0.094+	0.44+	0.271+
MS	No	72.42 (29.47)	19.79 (10.43)	0.27 (0.13)	23.04 (15.25)
	Yes	61.25 (19.59)	16.87 (10.25)	0.24 (0.13)	23.43 (18.12)
	p-Value	0.022+	0.028+	0.219+	0.575+
Age		-0.131	-0.191	-0.218	0.079
	p-Value	0.173*	0.046*	0.013*	0.281*
HOMA		-0.05	-0.051	-0.108	-0.048
	p-Value	0.603*	0.594*	0.262*	0.617*
Waist		0.045	0.108	-0.077	-0.104
	p-Value	0.639*	0.26	0.421*	0.278*
BMI		0.134	0.092	-0.086	0.063
	p-Value	0.464*	0.338*	0.371*	0.512*
Triglycerides		0.052	0.012	0.013	-0.038
	p-Value	0.591*	0.904*	0.896*	0.693*
HDL		0.125	0.058	-0.041	0.054
	p-Value	0.195*	0.544*	0.672*	0.578*

*p Value and Pearson´s Correlation Coefficient; +p- Value: T-Student, cuantitative variables are expressed as mean (SD). PTH: intact parathyroid hormone (ng/mL), Osteocalcine (ng/mL), CTX: Carboxy-terminal collagen crosslinks (ng/mL), Vit D 25: 25-hydroxyvitamin D (pg/mL), DM: Diabetes Mellitus, HBP: high blood pressure, MS: Metabolic syndrome, HOMA: homeostasis model assessment, BMI: body mass index and HDL: high-density lipoprotein.

preted with caution. A control group of non severely obese patients with and without NAFLD would be needed to establish the role of morbid obesity in the co-relation between NAFLD and vitamin D levels. Moreover, the study did not take into account the season in which the levels of vitamin D were measured or the treatment with supplements of calcium and vitamin D.

In our study, higher levels of vitamin D were found among patients with normal biopsy, without NASH or without fibrosis, although the differences are not statistically significant. The low prevalence of NASH and high prevalence of low vitamin D levels in our environment could explain the difficulty to achieve statistical significance.

The relationship between NAFLD and vitamin D remains unclear. A recent meta-analysis observed that NAFLD patients were 1.26 times more likely to be vitamin D deficient (95% CI: 1.17-1.35)[14], but some studies did not find association between NAFLD and Vitamin D levels[21,22]. Only in four of the seventeen studies included in the meta-analysis, NAFLD was diagnosed by liver biopsy.

The pathogenesis of the association between NAFLD and vitamin D is not well explained. Evidences from animal studies support the hypothesis of an immunomodulatory role of vitamin D in NAFLD. Vitamin D deficient diet could produce expression of increased hepatic mRNA levels of IL-4, IL-6 or TNFα[23]. Moreover, phototherapy in animal models elevated vitamin D levels and could improve insulin resistance and reduce liver inflammation[24]. A recent controlled clinical trial patients with NAFLD showed no differences in liver function test, HOMA and grade of steatosis in ultrasound after four months of oral supplementation with vitamin D[25].

Osteocalcin has been related to insulin resistance and liver fibrosis[26]. Patients with chronic liver disea-

se show low levels of osteocalcin, but relationship among these parameters has not been clarified yet[27]. Lee et al[28] demonstrated that osteocalcin participates in adiposity and glucose homeostasis, suggesting skeleton influences on energy metabolism, so osteocalcin could be a surrogate marker of insulin resistance[26]. The relationship between osteocalcin and metabolic syndrome observed in our cohort, does not support this fact.

The association of vitamin D and PTH with metabolic syndrome also remains unclear. Some evidences support the association of PTH levels with metabolic syndrome based on the relationship between PTH and insulin resistance[29,30], but other studies have shown that PTH were not related to metabolic syndrome after adjust of vitamin D levels[31,32]. The co-relation between vitamin D deficiency and MS is supported by data from several observational studies, but the pathogenesis is not well defined and vitamin D supplementation in MS patients does not improve metabolic parameters[33]. In our cohort, PTH levels were inversely correlated to the presence of metabolic syndrome and vitamin D was not associated with metabolic syndrome.

Moreiro et al[34] found that 58% of the patients with normal presurgery PTH levels, after two years of Scopinaro´s Biliopancreatic diversion, developed secondary hyperparathyroidism in spite of calcium and vitamin D supplementation. Álvarez et *al*[35] observed hypovitaminosis D after an average of 26 months of a Sleeve gastrectomy in 43% of patients and 8% hyperparathyroidism. In our series 34% of the patients showed hyperparathyroidism, and 61% vitamin D deficiency. Checking vitamin D and PTH levels in severely obese patients presurgery is mandatory due to the high prevalence of vitamin D deficiency or secondary hyperparathyroidism observed.

Conclusion

In our cohort of severely obese patients, vitamin D and bone metabolism markers were associated neither to NAFLD nor with metabolic syndrome. About 70% of our obese patients showed low levels of vitamin D or hyperparathyroidism, so this should be tested pre-surgery and treated. Further research is needed to establish the relationship between NAFLD and vitamin D levels in severely obese patients.

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