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Differential inflammatory environment in patients with osteoporosis and type 2 diabetes mellitus

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Summary

Objetive: Type 2 diabetes mellitus (DM2) and osteoporosis are diseases associated with a pro-inflammatory environment, the prevention of which through new therapeutic strategies could prevent their development. However, there are few studies that evaluate the inflammatory profile of osteoporosis in patients with DM2.

This study focuses on evaluating the inflammatory immune response through serum concentrations of nine cytokines, two of them anti-inflammatory (IL-10, IL-5) and six pro-inflammatory (IL-2, IL-6, IL-12 (p70), IL-17A, TNFα and IFNγ) in 163 individuals with DM2 and 47 controls. A subpopulation, made up of 43 DM2 patients without osteoporosis, and 33 with osteoporosis, was analyzed in greater depth at the level of bone parameters. Furthermore, we have assessed the calciotropic hormones, bone remodeling markers, bone mineral density and vertebral fractures in the population, and we have analyzed the relationship of the cytokines tested with DM2, osteoporosis and prevalent vertebral fractures.

Patients with DM2 had significantly higher serum concentrations of IL-10 compared to the control group (0.5±1 vs. 0.14±0.3 pg/ml; p=0.016) and the levels of IL12 p70 were shown lower in patients with DM2 compared to controls (2.9±1.6 vs. 3.9±3.1 pg/ml; p=0.027).

In the group of patients with DM2 and osteoporosis, the levels of the cytokine IL-6 were elevated compared to the group with DM2 without osteoporosis (10.9±14.6 vs. 4.5±7.0; p=0.017). An association of IL-5 was also observed, with its lowest levels in the DM2 group with osteoporosis (1.7±0.2 vs. 3.8±0.6; p=0.032). Furthermore, IL-5 showed a direct correlation with the levels of the bone formation biomarker alkaline bone phosphatase (r=0.277, p=0.004) in the subpopulation of patients with DM2. The rest of cytokines did not show significant differences.

In conclusion, our findings indicate that in our study population, patients with DM2 compared to healthy subjects present an inflammatory profile opposite to what is expected in a hyperglycemic situation, probably as a compensatory response to the inflammation caused. The cytokine profile is modified in the subpopulation of diabetic patients, depending on the presence of osteoporosis. In this case, the inflammatory profile in the presence of osteoporosis is consistent with the expected response.

Key words: type 2 diabetes mellitus, osteoporosis, inflammation, cytokines.

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Introduction

Diabetes mellitus (DM2) and osteoporosis are increasing prevalence diseases due to the aging of the population, and gender, genetic and environmental factors, such as an unbalanced diet, obesity and a sedentary life. DM2 increased alarmingly in 2014, affecting more than 420 million people worldwide[1]. Patients with DM2 present a higher risk of falls and increased prevalence and incidence of fragility fractures have been observed in these patients[2-5], causing significant mortality, morbidity and increased healthcare costs.

DM2 affects bone homeostasis[6,7], and is associated with a higher risk of fractures[8], despite the fact that patients exhibit higher bone mineral density (BMD)[4,9-12]. Furthermore, reduced circulating levels of bone turnover markers have been observed in DM2[13], which should influence the high fracture risk in patients with DM2.

On the other hand, inflammation is gaining prominence in the development of the disease and its complications. Multiple studies show an increase in inflammatory cytokines in DM2, which confer a chronic state of low-grade inflammation.

In DM2, it is common for patients to have an inadequate lifestyle, with excessive caloric intake and lack of physical exercise, which promotes central adiposity and obesity, so that there is a greater infiltration of macrophages in the adipose tissue, potentially altering the secretion of cytokines[14]. The release of these inflammation-mediating proteins is thus the result of the activation of immune cells accumulated in metabolic tissues and that by altering the secretion of cytokines, promote systemic insulin resistance (IR) and damage to β cells. producing insulin. Thus, an inflammatory environment is associated with altered levels of circulating cytokines, which could alter insulin sensitivity, leading to a greater risk of suffering from DM2[15]. On the other hand, patients with DM2 have accelerated aging, a process that leads to an increased risk of developing bone fragility prematurely, especially in patients with poorly controlled blood glucose[16]. Inflammatory cytokines also increase their production during aging, being crucial for skeletal homeostasis. Inflammatory cytokines have been observed to alter RANKL: OPG ratios and may result in increased osteoclastogenesis[17]. Thus, the immune system is strongly linked to maintaining healthy bones.

In order to prevent the progression of osteoporosis and related fractures in patients with DM2, bone health should be evaluated and interventions for the prevention of fractures should be implemented in this population, and if DM2 and osteoporosis are established, pharmacological interventions should be found and effective lifestyles. In this sense, the most innovative treatments for DM2 include blocking the pathological overproduction of pro-inflammatory cytokines by antagonists of the receptor of the cytokine of interest, or by neutralizing antibodies to it. Currently, vaccine treatments are being developed, consisting of repeated injection of the cytokine to produce an overexpression of neutralizing antibodies against the injected cytokine. Specifically, drugs that block the effect of the cytokine IL-1β have emerged as first-line therapy. Monoclonal antibodies directed against IL-1 β[18,19] and vaccines[20] are being tested, which turn out to be beneficial in terms of glycemic and inflammatory parameters in patients with DM2.

Due to the increasing prevalence of DM2 and its comorbidities, such as osteoporosis, there is a growing demand for personalized therapies, the efficiency of which is periodically monitored by evaluating biomarkers of disease progression.

This study aims to expand the knowledge of the mechanisms involved in bone homeostasis, by evaluating inflammatory cytokines associated with osteoporosis in patients with DM2. We have focused on 9 circulating cytokines, which could be involved in the systemic inflammation of osteoporosis in patients with DM2. In this way, we intend to contribute to the knowledge of the cytokines involved in the pathogenesis of both diseases, facilitating and simplifying the design of anti-inflammatory therapies to prevent the progression of osteoporosis in patients with DM2.

Population and methods

Design and study population

This cross-sectional study encompasses a total of 210 participants, which include 47 control individuals and 163 patients with DM2 diagnosed with diabetes, according to the criteria of the American Diabetes Association. Diabetic patients were on therapy for their disease, including metformin, sulfonylureas, insulin, or a combination of these. Patients treated with thiazolidinediones were excluded because they affected bone metabolism and cytokine release.

The specific study in presence vs. absence of osteoporosis in the DM2 population was performed on 43 patients without osteoporosis and 33 patients without osteoporosis. We use the World Health Organization Criteria for osteoporosis[21]. Due to the special characteristics of the pathophysiology of type 2 diabetes condition the appearance of fractures without densitometric alterations, patients with osteoporosis will also be those with prevalent vertebral fractures, even without meeting the criteria of BMD ≤−2.5 standard deviations (SD) of the T-score in the lumbar spine, total hip or femoral neck.

All participants were Caucasian, 35 to 65 years old.

Exclusion criteria for the population of patients with DM2 include a previous history of systemic inflammation due to other diseases or chronic diseases different from DM2, anti-inflammatory treatments or high alcohol consumption. None of the subjects were treated with medication known to modify bone mass.

The population was recruited at the San Cecilio University Hospital in Granada, Spain, and the samples were managed by the Biobank of the Andalusian Public Health System. The study was approved by the Andalusian Biomedical Research Ethics Committee.

Anthropometric, clinical and biochemical measurements

Anthropometric data were collected, including body mass index (BMI) (weight in kilograms divided by the square of height in meters).

For the measurement of various biochemical parameters in serum, venous blood samples were taken in the morning after an overnight fast. Sera were stored at -80°C until examination.

The biochemical parameters of fasting glucose, glycated hemoglobin (HbA1c), calcium, phosphorus, and creatinine were measured using standard automated laboratory techniques. Calciotropic hormones measured were iPTH (immunoassay; Roche Diagnostics SL) and 25-hydroxyvitamin D (RIA; DiaSorin). The biomarkers of bone turnover measured were osteocalcin (RIA, DiaSorin Stillwater, MN); Bone alkaline phosphatase - (immunoassay, Hybritech Europe), CTX (immunoassay,

Elecsys CrossLaps; Roche Diagnóstica) and tartrate-resistant acid phosphatase 5b -TRAP5b- (immunoassay, IDS Ltd.).

For the measurement of bone density and vertebral fractures, bone mineral density (BMD) was evaluated in the lumbar spine (LS) L2-L4, in the femoral neck (CF) and in the total hip (TH) by means of dual absorptiometry of X-ray of (DEXA) with a Hologic QDR 4500 densitometer (Waltham, MA; coefficient of variation 1%). We use the World Health Organization Criteria for osteoporosis[21]. The presence of prevalent vertebral bills was evaluated in conventional lateral view radiographs of the spine, at the thorax level and at the lumbar level (T4-L5). Traumatic vertebral fractures were excluded. Vertebral fractures were identified according to the method of Genant et al.[22] Only moderate and severe fractures were considered in our study.

Cytokine measurement

The concentration of nine cytokines (IL-10, IL-4, IL-5, IL-2, IL-6, IL-12 (p70), IL-17, TNFα and IFNγ) was measured by multiplex assays with luminex technology, using the Millipore Human Th17 Magnetic Bead Panel kit (Cat. # HTH17MAG-14K), according to manufacturer's instructions. The reading was carried out on the Bio-Plex® 200 system (Bio-Rad). Data are expressed in pg x mL^{-1}. The intra-assay coefficient of variation was less than 10% and the inter-assay coefficient of variation was less than 15% for all the analytes studied. The assayed kit incorporates internal cytokine controls designed for use in quality control during accuracy and precision monitoring of cytokine analyzes carried out.

Statistic analysis

Data were analyzed using SPSS-23 software (SPSS, Inc.). Continuous variables were expressed by means and standard deviation, and categorical variables by percentages. The normal distribution was evaluated using the Kolmogorov-Smirnov test. The variables with normal distribution were studied using the Student's t-test, and the variables that did not meet normality were analyzed using the Mann-Whitney U test. The x^2 tests were used to compare categorical variables. Values of $p<0.05$ were accepted as statistically significant values.

Results

Clinical characteristics of the population of patients with DM2 and controls

The baseline characteristics of the entire study population, both the group of patients with DM2 and controls, are described in table 1. Due to the inclusion criteria, individuals with DM2 have significantly higher levels of glucose and HbA1c than the control group ($p<0.001$). Calciotropic hormones iPTH, osteocalcin and biomarkers CTX and TRAP5b were higher in controls.

Cytokine profile in patients with DM2 and controls

As presented in table 1, the cytokines that show differences in the comparison of their serum concentrations correspond to IL-10 and IL-12 p70. Serum IL-10 concentrations are higher in the group of patients with DM2 compared to the control group (0.5±1 vs. 0.14±0.3 pg/ml; $p<0.05$).

In the case of IL-12 p70, lower serum values are shown in patients with DM2 compared to healthy controls (2.9±1.6 vs. 3.9±3.1 pg/ml; $p<0.05$) . On the other hand, the values of the cytokines IL-5, IL-6, IL17A, TNFα and IFNγ do not show differences between the study groups, although IL-5 and IFNγ approach significance in the comparison of groups. In addition, the levels of the cytokine IL-4, IL-2 and IL-17A were not detectable in most cases. Therefore the data have not been presented in this study.

In figure 1, the comparison of the serum levels in the DM2 groups and the cytokine controls is graphically shown, being visualized in A) IL-10 and in B) IL-12 (p70).

Clinical characteristics of the group of patients with DM2 and its relationship with bone metabolism

The characteristics of the patient population with type 2 diabetes mellitus, based on the presence or absence of osteoporosis, are presented in table 2.

Regarding the calcium hormones 25 (OH) vitamin D and parathyroid hormone, no differences were observed for the first one. However, parathyroid hormone levels are elevated in the DM2 group in the presence of osteoporosis compared to the DM2 group without osteoporosis (45.9±4.0 vs. 31.1±1.4; $p=0.01$).

The bone remodeling markers CTX and TRAP5b and bone alkaline phosphatase show no differences between groups.

Regarding the DEXA measurement parameters, it can be verified both in the T-scores and in BMD, that these values correspond to the selection criteria of this sample of patients with DM2, according to their bone status. The group with osteoporosis presented all the parameters of BMD and T-score with a lower value compared to the group without osteoporosis.

Cytokine profile in patients according to the presence of osteoporosis in the DM2 population

Among the cytokines studied, IL-6 is shown with a higher serum concentration in the DM2 group with osteoporosis, compared to the DM2 group without osteoporosis (10.9±14.6 vs. 4.5±7.0; $p=0.01$). On the contrary, the cytokine IL-5 presented lower values in the same group of diabetics with osteoporosis (1.7±0.2 vs. 3.8±0.6; $p=0.032$). The cytokines studied IL-10, IL-12 (p70), TNFα and IFNγ did not show differences in the comparison between both groups.

In figure 2C, the levels of IL-6 are graphically shown in both DM2 groups, with and without osteoporosis, and in figure 2D the levels of IL-5 in the same groups are shown.

On the other hand, we found a lack of association in the analysis between the presence of fractures and the cytokines studied in the group of osteoporotic diabetics.

Relationship between cytokines and markers of bone formation and resorption

A correlation study has been carried out between the cytokines tested and the biomarkers of formation (bone alkaline phosphatase and osteocalcin) and bone resorption (TRAP5b and CTX), in the total population, in the type 2 diabetic population and in the osteoporotic diabetic population with prevalent vertebral fractures. The results indicate a significant direct correlation in the case of alkaline phosphatase and interleukin 5, both for the total population ($r=0.162$, $p=0.049$), and for the type 2 diabetic population ($r=0.276$, $p=0.004$) . This last correlation is shown in figure 2. In the case of the population of osteoporotic diabetics with prevalent vertebral fractures, the correlation is lost.

Table 1. Anthropometric and biochemical parameters and cytokine concentrations in the study population of patients with type 2 diabetes mellitus (DM2) and the control group

	DM2 group (n=163)	Control group (n=47)	P value
Age (years)	63±9	54±8	≤0.001*
Male/female (n)	91/72	29/18	0.028*
BMI (kg/m^2)	31.6±5.8	31.4±7.7	0.056
Glucose (mg/dL)	159±59	90±11	≤0.001*
HbA1c (%)	8.2±1.9	4.9±0.4	≤0,001*
Creatinine (mg/dL)	1.8±8.0	0.8±0.2	0.106
Calcium (mg/dL)	10.8±9.9	9.3±0.4	≤0.001*
Phosphorus (mg/dL)	3.8±3.3	3.4±0.48	0.779
25 (OH) D (ng/mL)	18.2±9.9	21.3±10.8	0.069
iPTH (pg/mL)	46.3±43.9	51.7±18.7	0.003*
Osteocalcin (ng/mL)	1.4±1.2	4.3±4.9	0.002*
Bone alkaline phosphatase (µg/L)	15.9±9.8	13.7±7.3	0.06
CTX (ng/ml)	0.23±0.13	0.35±0.15	≤0.001*
TRAP5b (UI/L)	1.4±0.92	1,8±0,87	0.019*
Vertebral fracture (%)	27.7	0	≤0.001*
Osteoporosis (%)	43.42	0	≤0.001*
Cardiovascular disease (%)	49	0	≤0.001*
Cytokines:			
IL-5 (pg/mL)	3.2±4.2	4.1±4.2	0.07
IL-10 (pg/mL)	0.5±1	0.14±0.3	0.016*
IL-2 (pg/mL)	1.3±2.8	0.3±0.6	0.57
IL-6 (pg/mL)	6.7±11.1	9.8±18	0.66
IL-12 (p70) (pg/mL)	2.9±1.6	3.9±3.1	0.027*
IL-17A (pg/mL)	2.7±2.2	2.1±1.7	0.41
TNF-a (pg/mL)	1.8±4.5	1.0±1.9	0.65
IFN-g (pg/mL)	1.3±1.4	0.8±1.2	0.07

Data are shown as mean ± standard deviation, percentages or total number (n) *: p-value <0.05 between groups.
BMI: body mass index; HbA1c: hemoglobin A1c; 25 (OH) D: 25 hydroxy-vitamin D; IL: interleukin; TNFα: tumor necrosis factor alpha; IFNγ: interferon gamma.

Discussion

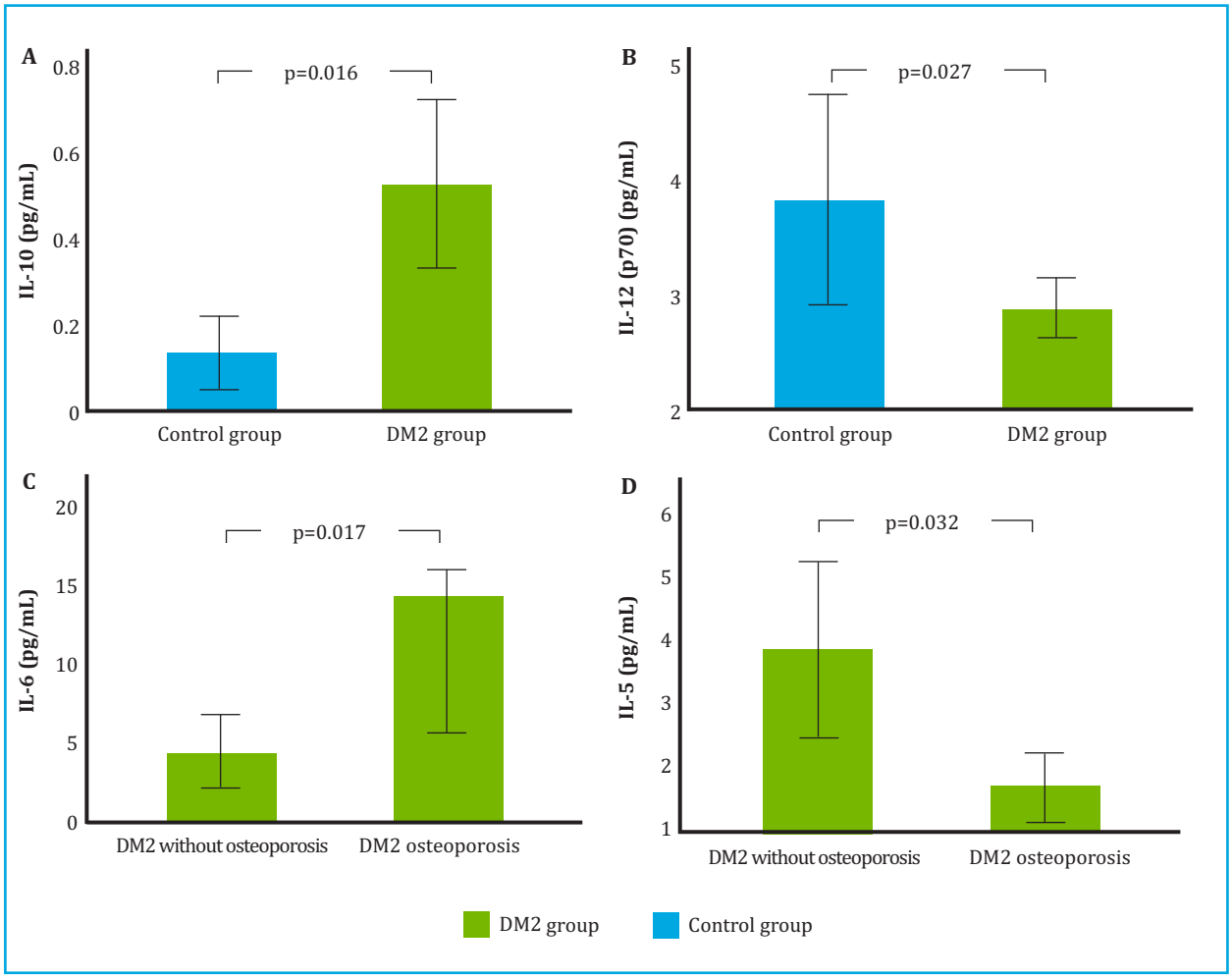
DM2 is an extremely complex and multifactorial chronic and systemic inflammatory disease. Clinical evidence shows that the risk of other complications such as osteoporosis is greatly increased in these patients. Insulin resistance can affect abnormal cytokine secretion and, in turn, produce alterations in bone metabolism, resulting in bone deterioration and osteoporosis[23]. However, the specific factors and molecular mechanisms that cause osteoporosis in patients with DM2 have not yet been elucidated.

In this study we have explored the relationship of the inflammatory environment with the presence of DM2 and osteoporosis. First, we have evaluated the association of the levels of various pro-inflammatory and anti-inflammatory serum cytokines (IL-2, IL-4, IL-17, IL-5, IL-6, IL-10, IL12 p70, TNFα and IFNɣ) in 210 individuals, of which 163 corresponded to patients with DM2 and 47 healthy individuals. Second, in the DM2 population, we have analyzed the association of these cytokines with osteoporosis, characterizing the population from the point of view of bone metabolism.

The results show higher serum IL-10 concentrations in DM2 compared to the control group, lower levels of IL-12 (p70) in patients with DM2, as well as higher circulating concentrations of IL-6 and lower IL-5 in the DM2 population with osteoporosis compared to DM2 patients without osteoporosis (see figure 1).

An outstanding finding of the present study involves increased levels of the anti-inflammatory cytokine IL-10, which has been shown to be elevated in patients with DM2 compared to the control group. Previously, it has been suggested that this anti-inflammatory cytokine is part of a complex interaction between pro-inflammatory

Figure 1. Association in the entire population, between control groups and patients with type 2 diabetes mellitus, with serum concentrations of: A) IL-10 and B) IL-12 (p70). C) association in the group of patients with type 2 diabetes mellitus, in relation to the presence and absence of osteoporosis, with the circulating concentration of IL-6 and D) of IL-5



and anti-inflammatory molecules, where the high levels of the latter would compensate and limit the damage caused by the inflammatory environment. This hypothesis was formulated in the context of both DM2, in a recent study with a low number of patients (n=25)[24], and in a study of osteoarthritis, where it is observed that inflammatory cytokines such as IL-6 and TNFα were expressed in parallel with the anti-inflammatory cytokine IL-10 as a compensatory mechanism for inflammation[25]. In fact, the physiological role of IL-10 is to limit the immune inflammatory response, inhibiting the activity of various cell types, especially the activation of macrophages and also preventing the production of other pro-inflammatory mediators such as IL-6 or TNFα[26]. On the other hand, it has been possible to verify that macrophages exposed to high levels of glucose show a resistance or low response to the effect of IL-10, preventing its anti-inflammatory action[27], so that the high levels of IL-10 could also be due to an attempt In addition, our findings are in line with those found by Wang et al.[28], who observed a progressive increase in IL-10 among patients without DM2, prediabetics and with type 2 diabetes. There are studies in contrast to our results, such as the one carried out with 15 patients with DM2 with respect to the same number of controls, in which a low expression of IL-10 is observed in DM2, and its levels were correlated with the levels of Glycosylated hemoglobin, for which it was proposed as a predictor of glycemia[29].

The pro-inflammatory cytokine IL-12 is a heterodimeric glycoprotein formed by the p40 and p35 subunits, its bioactive form being IL12 p70[30]. In our study we found a low serum concentration of the pro-inflammatory cytokine IL-12 p70 in patients with DM2 compared to controls. There are several studies with conflicting results. Thus, it has been shown that IL-12 increases in DM2 and is involved in the pathogenesis of atherosclerosis, macrovascular complications, diabetic retinopathy and endothelial dysfunction, especially in those patients with greater insulin resistance[31-33]. Several studies establish that the interruption in the expression of IL-12 triggers angiogenesis, protecting the endothelial tissues in type 2 diabetes. In addition, studies have been shown in murine models of DM2 in which IL-12[33] deficiency promotes overload. -expression of anti-inflammatory cytokines and reduces the expression of pro-inflammatory ones.

In line with our results, an increase in IL-10 and a decrease in IL-12 p70[34] have been observed in patients with DM2. In this study, it was suggested that interleukin IL-10 suppresses the activation of Th1 cells, which require IL-12 for their differentiation. In this way, the reduced level of IL-12 and the high concentration of IL-10 found in our study in patients with DM2, would contribute to

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Table 2. Anthropometric, physical and biochemical parameters of bone metabolism and serum cytokine concentrations in a subpopulation of the group of patients with type 2 diabetes mellitus (DM2), in relation to the presence and absence of osteoporosis

	Group DM2 and OP (n=33)	Group DM2 without OP (n=43)	P value
Age (years)	59.5±5.2	56.33±6.8	0.02*
Male/female (n)	19/14	22/21	0.37
BMI (kg/m^2)	33.1±6.5	29.8±4.4	0.02*
HbA1c (%)	7.6±1.8	8.1±1.8	0.4
Glucose (mg/dL)	163.3±65	180.5±58.5	0.08
Creatinine (mg/dL)	0.88±0.17	0.9±0.21	0.27
Calcium (mg/dL)	9.5±0.5	9.6±0.5	0.24
Phosphorus (mg/dL)	3.6±0.4	3.7±0.6	0.84
25 (OH) D (ng/mL)	19.2±11.8	16.5±10.6	0.38
Osteocalcin (ng/mL)	1.7±1.4	1.3±1.0	0.43
iPTH (pg/mL)	45.9±4.0	31.1±1.4	0.013*
Bone alkaline phosphatase (µg/L)	15.1±7.5	14.7±5.6	0.76
CTX (ng/ml)	0.24±0.1	0.18±0.09	0.14
TRAP5b (UI/L)	1.3±0.9	1.4±1.01	0.58
Fracture (%)	60.6	0	≤0.001*
DEXA parameters			
BMD CL (g/cm^2)	0.9±0.1	1.0±0.1	0.001*
BMD CF (g/cm^2)	0.7±0.1	0.8±0.1	0.005*
BMD CT (g/cm^2)	0.8±0.1	0.9±0.1	0.007
T-score CL	-2.0±1.4	-0.9±1.1	0.001*
T-score CF	-1.1±1.0	-0.27±0.7	0.001*
T-score CT	-1.1 ±1.0	-0.3±0.7	0.002*
Cytokines			
IL-5 (pg/mL)	1.7±0.2	3.8±0.6	0.032*
IL-10 (pg/mL)	0.7±1.2	0.4±0.7	0.97
IL-6 (pg/mL)	10.9±14.6	4.5±7.0	0.017*
IL-12 (p70) (pg/mL)	2.7±0.2	2.8±0.2	0.328
TNF-a (pg/mL)	1.0±2.1	1.2±1.7	0.41
IFN-g (pg/mL)	1.3±1.7	1.4±1.3	0.38

Data are shown as mean ± standard deviation, percentages, or total number (n). *: P value <0.05 between groups.
25 (OH) D: 25 hydroxy-vitamin D; iPTH: intact parathormone; CTX: carboxy-terminal telopeptide; TRAP5b: tartrate-resistant acid phosphatase 5b; FAO: bone alkaline phosphatase; BMD: bone mineral density; CL: lumbar spine; CF: Femoral neck; CT: total hip; IL: interleukin; TNFα: tumor necrosis factor alpha; IFNɣ: interferon gamma.

stopping the activation of the subpopulation of Th1 cells, the main producers of the pro-inflammatory cytokine IFNɣ, resulting in homeostasis of relevant tissues.

Thus, in our study we observed that the differences found in cytokine levels between patients with DM2 and controls seem to be the opposite of what would be expected in hyperglycemia, an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines. This could indicate a response to the increase in inflammation derived from hyperglycemia, rather than to the factors intrinsic to DM2.

The cytokine IL-6 has been described in multiple epidemiological studies as a powerful predictor of diabetes, suggesting that it interferes with the insulin signal and alters the function of beta cells[35,36]. We have investigated the potential of IL-6 as a factor involved in osteoporosis in patients with DM2, but did not find extensive references in the literature on this subject. The cytokine IL-6 performs two parallel functions that aggravate the osteoporotic condition: it stimulates the osteoclasts and inhibits the activity of the osteoblasts, resulting in a loss of bone density[37]. This effect of loss of bone density has been shown

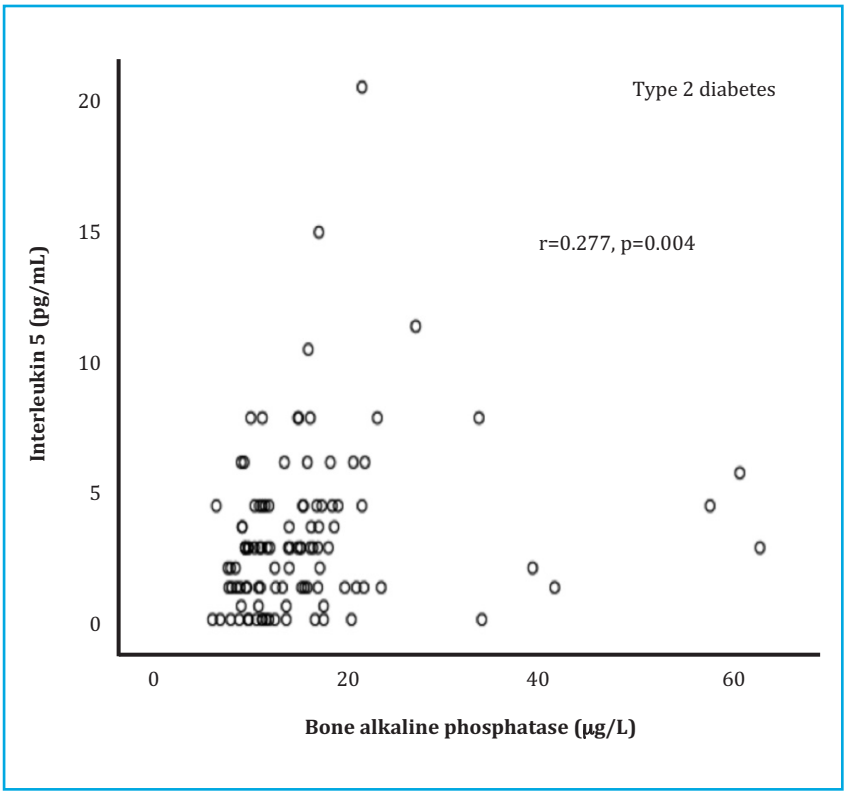
mainly in menopausal women[38]. Here it is shown that IL-6 is also increased in DM2 patients with osteoporosis. The anti-inflammatory interleukin IL-5 has been shown to lower levels in patients with DM2 and osteoporosis. Furthermore, we have observed a direct relationship of this IL-5 with the osteoblastic activity marker bone alkaline phosphatase.

These results should be expanded with larger studies that clarify the role of the association of inflammatory markers, DM2 and osteoporosis, as well as its possible extension to therapeutic intervention.

Conclusions

DM2 is a disease with a low degree of chronic inflammation, being extremely complex and multifactorial. In this study we have shown that patients with DM2 have altered levels of some pro-inflammatory and anti-inflammatory cytokines, which could be factors involved in the evolution of the disease. The inflammatory profile varies depending on the progression of the disease, the presence or absence of osteoporosis in patients with DM2. Taking into account the existence of differentiated profiles, it would be necessary to develop more precise options for the treatment of patients and include them in clinical practice guidelines.

Figure 2. Correlation graph showing the relationship between IL-5 and the bone formation biomarker alkaline bone phosphatase in the subpopulation of patients with type 2 diabetes mellitus



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Conflict of interests: The authors declare no conflict of interest.

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