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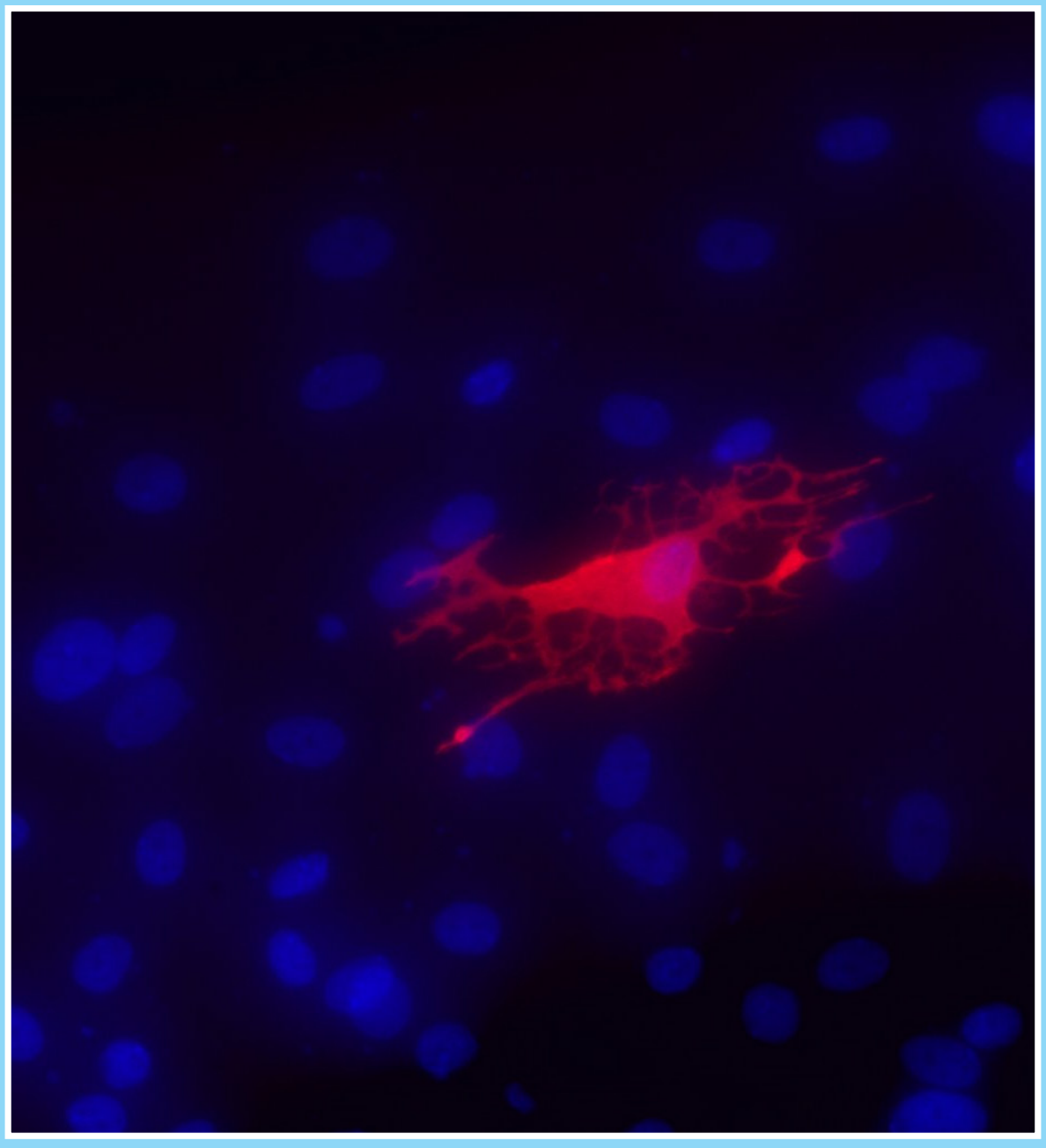

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Original

Prevalence of vitamin D deficiency in patients with risk factors: an observational, multicentre, cross-sectional study (PREVIFAC project)

Carmen Valdés Llorca

Centro de Salud Fuencarral. Madrid, Spain

Abstract

Introduction: vitamin D deficiency may be the cause or a predisposing factor in many diseases.

Objective: to determine the prevalence of vitamin D deficiency in patients with risk factors.

Materials and methods: a multicentre, cross-sectional observational study in which researchers from across the country selected the first three patients with risk factors for vitamin D deficiency on three consecutive days. During the consultation, the Vitamin D Test® (PRIMA Home Test) self-test was administered to classify vitamin D levels as "excessive", "sufficient", "insufficient" or "deficient".

Results: a total of 261 researchers selected 771 patients (76.3 % women) with a mean age of 62.0 ± 15.6 years and a body mass index (BMI) of 27.7 ± 5.3 kg/m^2. 70.8 % of patients had vitamin D levels classified as "insufficient" or "deficient". Significant risk factors in the bivariate analysis included: insufficient dietary intake, low exposure to sunlight, osteoporosis, obesity, chronic kidney disease and hyperparathyroidism. In the multivariate analysis, the independent risk factors were osteomalacia (odds ratio [OR] 4.12), low sun exposure (OR 2.49), osteoporosis (OR 1.97) and obesity (BMI > 30 kg/m^2) (OR 1.65).

Conclusions: a high prevalence of vitamin D deficiency (71 %) was detected using a screening test, and osteomalacia, low exposure to sunlight, osteoporosis and obesity were identified as factors associated with deficient or insufficient levels of vitamin D.

Keywords: Vitamin D deficiency. Risk factors. Obesity. Osteoporosis. Prevalence. Sun exposure.

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Ethical considerations: This study was conducted in full compliance with the Declaration of Helsinki. All participants provided informed consent before participating in the study.

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Artificial intelligence: The author declares that no artificial intelligence (AI) or AI-based tools were used in the preparation of this article.

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INTRODUCTION

Vitamin D is a fat-soluble vitamin essential for calcium homeostasis and bone mineralization; dietary intake and, especially, cutaneous synthesis through sunlight exposure are the main sources of cholecalciferol (vitamin D_3) (1-4). Measurement of the plasma concentration of 25-hydroxyvitamin D, abbreviated as 25(OH) D, is the standard method for determining vitamin D status, although some discrepancies remain regarding the minimum levels considered normal for the general population (> 20 ng/mL or 30-50 ng/mL) (5-7), as well as the criteria for hypovitaminosis D (20-30 ng/mL), insufficiency (10-20 ng/mL), and deficiency (< 10 ng/mL) (8).

The relevance of vitamin D deficiency as a causal factor or a factor associated with numerous chronic diseases has attracted increasing interest because of its rising prevalence in recent decades, affecting different age groups and substantially increasing the global burden of disease worldwide (9,10). Data from observational studies indicate that approximately 40 % of the European population has hypovitaminosis D and 13 % has severe vitamin D deficiency (11). In Spain, 4 out of 10 inhabitants younger than 65 years and 8 out of 10 people older than 65 years have vitamin D insufficiency or deficiency (12). In addition to its critical importance in calcium metabolism and musculoskeletal health, vitamin D is an essential nutrient involved in the regulation of cell growth, the cardiovascular system, and innate and acquired immunomodulation, and the nuclear vitamin D receptor has been identified in almost all cells in the body (13). Vitamin D deficiency stimulates parathyroid hormone (PTH) secretion, increases bone remodeling, reduces bone mineral density and bone quality, and thereby increases the risk of osteoporosis, bone fragility, and osteoporotic fractures. In a meta-analysis of 86 studies including more than 103 million individuals aged 15-105 years, the overall prevalence of osteoporosis was 18.3 %, increasing to 23.1 % among women compared with 11.7 % among men (14). The overall incidence of osteoporotic fractures ranges from 500 to 1000 per 100,000 person-years among adults older than 50 years, increases with age, and is higher among women (15). Therefore, osteoporosis associated with vitamin D deficiency represents a major health problem with important consequences for patients' quality of life and the economic burden on health care systems (16,17).

Risk factors for vitamin D deficiency are numerous and include low sun exposure, advanced age, dark skin, body mass index (BMI), reduced dietary intake or absorption of the vitamin (e.g., celiac disease), decreased endogenous synthesis due to liver cirrhosis, renal failure, or hypoparathyroidism, and increased hepatic catabolism due to the use of certain drugs that affect hepatic cytochrome P450 enzymes, thereby accelerating the degradation of vitamin D into inactive metabolites (18-21).

Although risk factors for vitamin D deficiency in general have been extensively studied, evidence on vitamin D deficiency in individuals with risk factors remains limited. Therefore, a real-world data study was conducted to determine the prevalence of vitamin D deficiency in Spain among patients with risk factors for, or factors associated with, hypovitaminosis D.

METHODS

DESIGN AND PARTICIPANTS

We designed an observational, cross-sectional, multicenter study covering the entire Spanish territory (PREVIFAC project, an acronym for prevalence of vitamin D deficiency in patients with risk factors) within the setting of medical consultations across different specialties, including Family and Community Medicine/Primary Care, Endocrinology and Nutrition, Internal/General Medicine, Rheumatology, and Geriatrics, among others. These specialties comprised health care professionals who routinely attended patients with risk factors for vitamin D deficiency. The primary endpoint of the study was to determine the prevalence of vitamin D deficiency among patients with risk factors. Secondary endpoint were to assess the sociodemographic and clinical characteristics of patients with vitamin D deficiency, identify the predominant risk factors for hypovitaminosis D, and analyze the relationship between risk factors and vitamin D deficiency.

The patient inclusion criteria were as follows: age > 18 years and the presence of at least 1 of the following risk factors for, or factors associated with, vitamin D deficiency: rickets, osteomalacia, osteoporosis, chronic kidney disease, intestinal malabsorption syndromes, celiac disease, obesity (BMI > 30 kg/m^2), low sun exposure (< 2 minutes/day without sun protection), advanced age (> 65 years), non-Caucasian ethnicity, use of drugs that could affect 25(OH)D levels (anticonvulsants, glucocorticoids, antifungals, cholestyramine, and antiretroviral agents used to treat HIV), hyperthyroidism, granulomatous diseases (tuberculosis, coccidioidomycosis, etc.), older adults with a history of falls or nontraumatic fractures (at least 2 falls or nontraumatic fractures in the previous year), pregnancy, and breastfeeding, as well as provision of signed informed consent. Exclusion criteria were age < 18 years, institutionalization, and refusal to provide signed informed consent.

The study protocol was approved by the Hospital Clínico San Carlos Drug Research Ethics Committee (Madrid, Spain) (code 22/698-E; approved on December 23, 2022). All patients provided written informed consent.

STUDY PROCEDURES

A total of 136 medical centers were selected to participate in the study, with at least 1 specialist practicing at each center. To ensure nationally representative participation, at least 1 center from each autonomous community in Spain was required to participate. Physician participation was anonymous and voluntary. After providing consent, each investigator recruited 3 patients who met the inclusion criteria and provided signed informed consent. To make patient selection as random as possible, investigators were instructed to include the first eligible patient attending the consultation on each of 3 consecutive days.

During the consultation, sociodemographic data (sex, age, marital status, living arrangements, educational level, and occupation), clinical and lifestyle variables (anthropometric data, smoking status, alcohol consumption, and caffeine consumption), and risk factors for, or factors associated with, vitamin D deficiency corresponding to the variables established in the inclusion criteria (rickets, osteomalacia, osteoporosis, etc.) were collected for each patient included in the study. In addition, vitamin D deficiency screening was performed using the Vitamin D Test® (PRIMA Home Test, PRIMA Lab, S.A., Balerna, Switzerland) (https://primalabsa.ch/en/home/check-up/vitamin-d-test/). This is a rapid and easy-to-perform self-test requiring a drop of blood obtained by finger prick. The test is an immunoassay based on the principle of competitive protein binding that classifies vitamin D levels as "excessive," "sufficient," "insufficient," or "deficient," with a measurement range of 10-100 ng/mL and an accuracy of 94.4 % (95 %CI, 87.6-97.6 %). Vitamin D values correspond to > 100 ng/mL for "excessive," 30-100 ng/mL for "sufficient," 10-30 ng/mL for "insufficient," and 0-10 ng/mL for "deficient," according to the information provided by the manufacturer.

The study was conducted over a 20-month period (May 2023-December 2024). The PREVIFAC project information leaflet contained the URL where the study materials were available and a personalized access password. In addition to patient information (Supplementary Table I - https://www.revistadeosteoporosisymetabolismomineral.com/files/535/ADMA2-00100-03.pdf), investigator data were also collected (age and sex, specialty, years of practice, and participation in training programs or research projects on vitamin D).

STATISTICAL ANALYSIS

To obtain an unbiased estimator of the distribution of Vitamin D Test® results in the population when the response variable was discrete, and assuming simple random sampling in the study area, a sample size of 822 participants was calculated, considering a 3 % margin of error, a 95 % confidence level, and 50 % heterogeneity. Categorical variables are expressed as frequencies and percentages, and continuous variables as means and SD, with 95 %CI. Fisher's exact test was used to compare qualitative variables, and Student's *t* test or one-way analysis of variance (ANOVA) was used for quantitative variables; alternatively, the nonparametric Mann-Whitney *U* or Kruskal-Wallis tests were used depending on the applicable conditions. In the bivariate analysis, the association between each risk factor and vitamin D deficiency was analyzed by combining the "insufficient" and "deficient" categories, and the results were expressed as odds ratios (ORs) with 95 %CI. Risk factors with a p value > 0.1 in the bivariate analysis were included in a logistic regression model to identify independent variables associated with vitamin D deficiency. A statistical significance level of .05 was accepted. All calculations were performed using SAS statistical software (Statistical Analysis System, SAS Institute, Cary, NC, United States), version 9.4.

RESULTS

INVESTIGATOR DATA

A total of 261 investigators from throughout Spain participated in this study, including 179 men and 82 women, with a mean age of 54.5 ± 10.9 years. The specialties with the highest participation were Family and Community Medicine, including Primary Care (43.3 %), General/Internal Medicine (26.8 %), Endocrinology and Nutrition (18.4 %), Rheumatology (6.1 %), Orthopedic Surgery and Traumatology (1.1 %), and Obstetrics and Gynecology (1.1 %). Regarding years of professional experience, 86.7 % had > 10 years of experience, 8 % had between 5 and 10 years, and 5 % had < 5 years. Furthermore, during the previous 12 months, 38.7 % of participants reported having participated in a vitamin D training program, but only 5.4 % had participated in a research project on vitamin D other than the present study.

PATIENT CHARACTERISTICS

A total of 771 patients (76.3 % women) were included in the study, with a mean age of 62.0 ± 15.6 years (95 %CI, 60.9-63.1) and a BMI of 27.7 ± 5.3 kg/m^2. The main sociodemographic, clinical, and lifestyle data are shown in table I. Most patients were married or living with a partner (62.0 %), lived with a partner or their own family (69.0 %), had completed university education (36.8 %), were employed (47.6 %) or retired (45.9 %), were nonsmokers (67.1 %), and did not consume alcohol daily (87.0 %). Overall, 12.2 % of participants were current smokers and 20.7 % were former

smokers. Current smokers had started smoking at a mean age of 20.2 ± 7.6 years, and former smokers had quit smoking a mean of 15.0 ± 19.3 years previously. Among the 13.0 % of patients who consumed alcoholic beverages daily, the mean number of standard drink units was 2.4 ± 1.6. Finally, most patients (63.8 %) consumed caffeine daily, with a mean consumption of 2.9 ± 1.7 cups of coffee and other caffeinated beverages.

Table I. Sociodemographic, clinical, and lifestyle characteristics of the 771 patients included in the study

Variables	Number (%)
Sex	
Male	183 (23.7)
Female	588 (76.3)
Age, years, mean ± SD (95 %CI)	62.0 ± 15.6 (60.9-63.1)
Marital status	
Married/living with a partner	478 (62.0)
Widowed	148 (19.2)
Single	91 (11.8)
Separated/divorced	54 (7.0)
Living arrangements	
With a partner or own family	532 (69.0)
Alone	175 (22.7)
With family of origin	64 (8.3)
Educational level	
Completed primary education, or equivalent	190 (24.6)
Secondary education, high school, or intermediate vocational training	129 (16.7)
Advanced vocational training/completed upper secondary education or pre-college education	130 (16.9)
Completed university education	284 (36.8)
No formal education	38 (4.9)
Employment status	
Work disability	9 (1.2)
Sick leave	13 (1.7)
Unemployed, without work disability	28 (3.6)
Employed/student/homemaker	367 (47.6)
Retired	354 (45.9)
Body mass index (BMI), kg/m^2, mean ± SD (95 %CI)	27.7 ± 5.3 (27.3-28.1)
Smoking status	
Current smoker	94 (12.2)
Age at smoking initiation, years, mean ± SD (95 %CI)	20.2 ± 7.6 (18.6-21.7)
Former smoker	160 (20.7)
Years since smoking cessation, mean ± SD (95 %CI)	15.0 ± 19.3 (13.4-16.6)
Duration of smoking, years, mean ± SD (95 %CI)	23.2 ± 11.7 (21.4-25.0)
Never smoker	517 (67.1)
Daily alcohol consumption	
No	671 (87.0)
Yes	100 (13.0)
Number of standard drink units consumed, mean ± SD (95 %CI)	2.4 ± 1.6 (2.1-2.7)
Daily caffeine consumption	
No	279 (36.2)
Yes	492 (63.8)
Cups of coffee, mean ± SD (95 %CI)	2.2 ± 1.1 (2.1-2.3)
Glasses of caffeinated beverages, mean ± SD (95 %CI)	0.7 ± 1.2 (0.6-0.8)
Total of both, mean ± SD (95 %CI)	2.9 ± 1.7 (2.7-3.0)

SD, standard deviation; 95 %CI, 95 % confidence interval; standard drink unit, equivalent to 10 g of alcohol. Data are expressed as frequencies and percentages unless otherwise indicated.

SCREENING FOR VITAMIN D DEFICIENCY AND RISK FACTORS

According to the Vitamin D Test® results, 70.8 % of patients had vitamin D levels corresponding to the "insufficient" or "deficient" categories, whereas only 28.5 % had "sufficient" levels (Fig. 1).

The distribution of risk factors for vitamin D deficiency in the study population is shown in table II. The most frequent factors were low sun exposure (66.5 %), advanced age (> 65 years) (50.8 %), use of sunscreen (sun protection factor > 8) (49.7 %), osteoporosis (42.0 %), obesity (BMI > 30 kg/m^2) (34.0 %), generally insufficient dietary intake (22.1 %), chronic renal failure (14.4 %), a history of falls or nontraumatic fractures in older adults (14.1 %), and glucocorticoid therapy (10.5 %). In the bivariate analysis, the risk factors showing a statistically significant association with vitamin D deficiency were hyperparathyroidism (OR, 3.13), low sun exposure (OR, 2.43), insufficient dietary intake (OR, 1.89), osteoporosis (OR, 1.81), chronic renal failure (OR, 1.78), and obesity (OR, 1.52) (Table III). In the multivariate analysis, independent risk factors for vitamin D deficiency were osteomalacia (OR, 4.12), low sun exposure (OR, 2.49), osteoporosis (OR, 1.97), and obesity (OR, 1.65) (Table IV).

DISCUSSION

The results of this study show that 71 % of the Spanish population sample with risk factors had vitamin D deficiency. Risk factors for, or factors associated with, vitamin D deficiency that reached statistical significance were low sun exposure, osteoporosis, osteomalacia, insufficient dietary vitamin D intake, obesity, chronic renal failure, and hyperparathyroidism. These findings are consistent with data from other studies assessing vitamin D deficiency in populations with specific risk factors.

Low sun exposure, defined as less than a few minutes per day without protection, was present as a risk factor in 66.5 % of patients and emerged as one of the predominant risk factors. Furthermore, the presence of

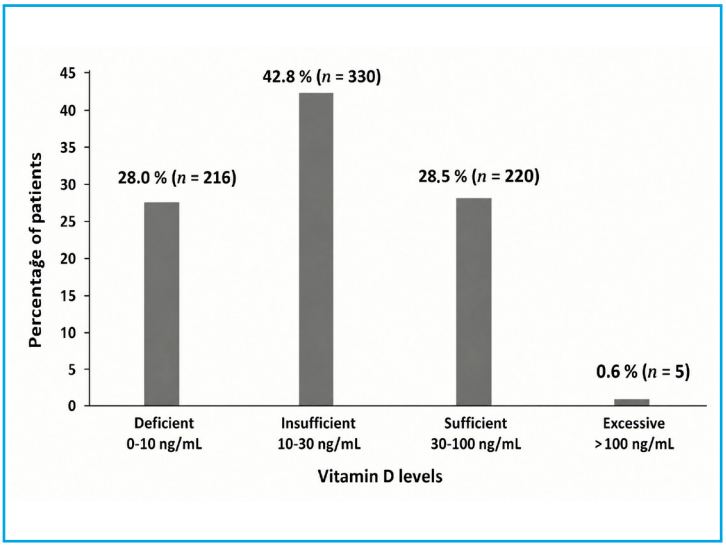

Figure 1. Distribution of patients according to Vitamin D Test® (PRIMA Home Test) results.

Table II. Distribution of risk factors for, or factors associated with, vitamin D deficiency in the study population

Risk factor	Number (%)
Low sun exposure (less than a few minutes per day without protection) (*n* = 770)	512 (66.5)
Advanced age (> 65 years) (*n* = 770)	391 (50.8)
Use of UV radiation-filtering creams (sun protection factor > 8) (*n* = 726)	361 (49.7)
Osteoporosis (*n* = 742)	312 (42.0)
Obesity (BMI > 30 kg/m^2) (*n* = 768)	261 (34.0)
Insufficient dietary intake (*n* = 728)	161 (22.1)

(Continues on next page)

Table II (cont). Distribution of risk factors for, or factors associated with, vitamin D deficiency in the study population

Risk factor	Number (%)
Chronic renal failure (*n* = 765)	110 (14.4)
Older adult with a history of falls or nontraumatic fractures (at least 2 falls or nontraumatic fractures in the previous year) (*n* = 767)	108 (14.1)
Glucocorticoid therapy (*n* = 764)	80 (10.5)
Intestinal diseases and GI surgery (*n* = 764)	72 (9.4)
Non-Caucasian population (*n* = 763)	63 (8.3)
Hyperthyroidism (*n* = 765)	50 (6.5)
Pregnancy and breastfeeding (*n* = 765)	43 (5.6)
Hyperparathyroidism (*n* = 760)	41 (5.4)
Chronic cholestasis and primary biliary cirrhosis (*n* = 763)	40 (5.2)
Osteomalacia (*n* = 757)	36 (4.8)
Nephrotic syndrome (*n* = 765)	23 (3.0)
Hypoparathyroidism (*n* = 759)	22 (2.9)
Anticonvulsant therapy (*n* = 762)	21 (2.8)
Severe chronic liver disease/liver cirrhosis (*n* = 766)	20 (2.6)
Primary biliary cirrhosis (*n* = 763)	18 (2.4)
Antifungal therapy (*n* = 761)	16 (2.1)
Cholestyramine therapy (*n* = 763)	15 (2.0)
Pancreatic insufficiency (e.g., cystic fibrosis) (*n* = 763)	14 (1.8)
Antiretroviral therapy for HIV (*n* = 764)	12 (1.6)
Pseudohyperparathyroidism (*n* = 760)	12 (1.6)
Rickets (*n* = 762)	12 (1.6)
Paget disease of bone (*n* = 764)	12 (1.6)
Antituberculosis therapy (*n* = 765)	11 (1.4)
Chronic granulomatous diseases (*n* = 766)	11 (1.4)

BMI, body mass index; HIV, human immunodeficiency virus. The number of patients assessed for each risk factor is shown in parentheses.

Table III. Risk factors associated with vitamin D deficiency

Risk factors	"Deficient-insufficient" categories (Vitamin D Test®)	
	Odds ratio (95 %CI)	*p* value
Hyperparathyroidism (yes vs no)	3.13 (1.21-8.09)	< 0.05
Low sun exposure (less than a few minutes per day without protection) (yes *vs* no)	2.43 (1.76-3.35)	< 0.05
Insufficient dietary intake (yes *vs* no)	1.89 (1.24-2.88)	< 0.05
Osteoporosis (yes *vs* no)	1.81 (1.30-2.53)	< 0.05
Chronic renal failure (yes *vs* no)	1.78 (1.09-2.93)	< 0.05
Obesity (BMI > 30 kg/m^2) (yes *vs* no)	1.52 (1.08-2.14)	< 0.05

95 %CI, 95 % confidence interval; BMI, body mass index.

Table IV. Results of the multivariate analysis: probability of vitamin D deficiency according to the presence of risk factors

Risk factor	Wald χ^2 test (degrees of freedom)	Odds ratio (95 %CI)	*p* value
Osteomalacia (yes *vs* no)	5.05 (1)	4.12 (1.20-14.18)	0.0246
Low sun exposure (less than a few minutes per day without protection) (yes *vs* no)	26.37 (1)	2.49 (1.76-3.52)	< 0.001
Osteoporosis (yes *vs* no)	13.38 (1)	1.97 (1.37-2.82)	0.0003
Obesity (BMI > 30 kg/m^2) (yes *vs* no)	6.60 (1)	1.65 (1.13-2.42)	0.010

95 %CI, 95 % confidence interval; BMI, body mass index. Hosmer-Lemeshow test, p = *0.8429, for overall model fit. The relationships among the factors included in the final model were analyzed, and no statistically significant associations were detected among them.*

this factor increased the odds of vitamin D deficiency by 2.49-fold. In most individuals, vitamin D production through exposure to solar ultraviolet B (UVB) radiation accounts for > 80 % of total vitamin D. In addition to the duration of sun exposure, skin color, geographical location, season, latitude, and altitude have a substantial impact on the cutaneous synthesis of vitamin D (22-24). Moreover, vitamin D may be involved in the potential preventive effect of sun exposure against certain types of cancer (prostate, breast, and colorectal cancer) (25). For the general Caucasian population, daily sun exposure of approximately 15 minutes to the face and arms between March and October, with a sun protection factor of 15-30 depending on latitude and radiation intensity, is recommended (26).

With regard to specific diseases, osteomalacia and osteoporosis showed a strong association with vitamin D deficiency, particularly osteomalacia (OR, 4.12), which is consistent with the robust evidence supporting the relationship between 25(OH)D levels and bone mineralization (27-32). Confinement due to age, cultural habits, or climate, as well as skin hyperpigmentation, have been associated with insufficient cutaneous synthesis and the development of osteomalacia. A study of Asian immigrants living in England reported an osteomalacia prevalence of 13.5 %, which was associated with strict vegetarian diets and the use of clothing covering most of the body, particularly among women (31). Vitamin D depletion is very common in celiac disease, and some case series have confirmed the development of osteomalacia in up to 50 % of these patients (32). Severe chronic liver disease, chronic renal failure, increased vitamin D catabolism, and decreased tubular phosphate reabsorption have also been described as causes of osteomalacia (33). Vitamin D levels should be measured in patients with osteomalacia, and appropriate treatment should be initiated.

Osteoporosis was present in 42 % of patients. Osteoporosis, bone fragility, and osteoporotic fractures represent a major global health problem, not only because of the multifactorial complexity of this condition and its association with an increased risk of death, disability, and health care costs, but also because of the high frequency of underdiagnosis and undertreatment. In fact, up to 75 % of women and 90 % of men with a high probability of osteoporosis do not undergo any diagnostic testing, and more than 75 % of patients with osteoporosis do not receive appropriate treatment, despite recommendations and evidence demonstrating the substantial safety and efficacy of bisphosphonates and other pharmacological treatments, either as monotherapy or in combination with vitamin D (35-37). In Spain, according to the latest epidemiological study of osteoporosis in the Spanish population conducted by the Sociedad Española de Reumatología (SER) in 1,522 individuals—the Osteo SER project—54.4 % of people older than 50 years had reduced bone mineral density (BMD), and 10.7 % had osteoporosis, which was more frequent in women (18.6 %) than in men (2.6 %) (38). Among women older than 65 years, who constitute the group at greatest risk of fracture, 24.9 % had osteoporosis. According to data updated to January 1, 2023, from the Spanish National Institute of Statistics, the absolute figure would amount to 1,300,021 Spanish women at high risk of fracture, which reinforces the importance of adopting healthy habits to prevent osteoporosis, such as engaging in physical exercise, following a Mediterranean diet that includes adequate calcium and vitamin D intake, and avoiding smoking and excessive alcohol consumption, among other factors (39). In a recent study using the Delphi methodology on the management of osteoporosis in Primary Care, panelists agreed on the need to appropriately identify patients according to their fracture risk and also emphasized the importance of exercise and nutrition (40).

Obesity (BMI > 30 kg/m^2) was present in 34 % of patients and was an independent risk factor statistically associated with vitamin D deficiency. The pathophysiological mechanisms underlying the association between hypovitaminosis D and obesity include se-

questration in adipose tissue, volumetric dilution, alterations in hepatic and renal hydroxylation, and limited sun exposure (41,42). In addition, hypovitaminosis D in patients with obesity is a risk factor for developing type 2 diabetes *mellitus* and insulin resistance (43). However, weight loss has little influence on improvements in vitamin D levels (42). An increased risk of vitamin D deficiency has also been reported in pediatric and adolescent populations with obesity (44).

The specialists who recruited the participants were distributed across Spain, predominantly worked in Primary Care, and had more than 10 years of professional experience, although their participation in vitamin D training programs, particularly research projects on the topic, was limited. Furthermore, emphasis should be placed on the need to extend systematic screening of plasma 25(OH)D concentrations to patients with symptoms or signs of deficiency, individuals with risk factors, and those with laboratory and/or radiographic abnormalities suggestive of vitamin D deficiency. Although vitamin D deficiency is highly prevalent, universal measurement of 25(OH)D is not cost-effective, and population-based screening is not recommended (45).

The Vitamin D Test® (PRIMA Home Test) is a simple and straightforward immunochromatographic assay capable of detecting 25(OH)D levels in a blood sample and indicating their concentration according to the intensity of the test line (T) obtained. The result, visible after 10 minutes, makes it possible to determine whether vitamin D levels are sufficient, deficient, insufficient, or excessive, which is of interest as a screening tool for vitamin D deficiency. However, the manufacturer of the Vitamin D Test® (PRIMA Home Test) does not provide detailed sensitivity or specificity data or concordance curves against LC-MS/MS or immunoassays; therefore, no clinical equivalence between the rapid test and standard analytical methods can be established. Consequently, an indicative analytical result obtained with the Vitamin D Test® should be confirmed using a secondary analytical method of greater accuracy. Furthermore, the test *per se* uses a fixed cutoff point of 30 ng/mL, with an estimated variability margin of ± 4 ng/mL, which may influence the classification of some participants, particularly those with concentrations close to the threshold. As with any diagnostic test, the results should be interpreted in conjunction with all other available clinical information. Therefore, when values are uncertain or considered inconsistent with the clinical context, additional measurements using standardized methods are recommended.

The results of the present study should be interpreted in light of its limitations, including its cross-sectional design, nonrandom patient selection, and use of a semiquantitative test that is not equivalent to the standard method for measuring 25(OH)D levels. Both the selection method (3 consecutive patients over 3 days), which was not free from bias, and the use of the Vitamin D Test® facilitated study logistics with the aim of obtaining an approximation of vitamin D deficiency detection using a screening test and risk factors under real-world conditions. Finally, the study was conducted between May 2023 and December 2024, but data on the regions and latitudes corresponding to the study population were not collected. Furthermore, the data were not adjusted for age and sex.

CONCLUSIONS

In conclusion, the findings highlight the high prevalence of vitamin D deficiency (71 %) detected in the Spanish population studied using a screening test, as well as the identification of osteomalacia, low sun exposure, osteoporosis, and obesity as independent risk factors statistically associated with deficient or insufficient vitamin D levels.

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Original

Cultural adaptation and validation of the Indonesian version of QUALEFFO-41 in patients with osteoporosis

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Abstract

Background: osteoporosis represents a growing public health concern in Indonesia and contributes substantially to morbidity through pain, functional decline, and vertebral and nonvertebral fractures. Despite the widespread international use of the Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41), a fully validated Indonesian version has not previously been available. This study aimed to conduct a rigorous cultural adaptation and psychometric validation of the Indonesian version of the QUALEFFO-41.

Methods: we conducted a cross-sectional study among 204 Indonesian-speaking patients diagnosed with osteoporosis or osteopenia. Cross-cultural adaptation followed the standardized guidelines proposed by Beaton et al., including forward and backward translation, expert panel review, and cognitive debriefing. Psychometric evaluation included internal consistency (Cronbach α), test-retest reliability (intraclass correlation coefficient [ICC]), convergent validity (average variance extracted [AVE] and composite reliability [CR]), and construct validity using confirmatory factor analysis (CFA).

Results: internal consistency was excellent for pain (α = 0.93) and physical function (α = 0.90), acceptable for social function (α = 0.73), moderate for mental function (α = 0.79), and marginal for general health perception (α = 0.65). Test-retest reliability demonstrated good to excellent ICC values across domains (0.66-0.87), with an exceptionally high ICC for the total score (0.999). Convergent validity was satisfactory across all domains (AVE > 0.50; CR > 0.70). CFA showed perfect model fit for general health perception, marginal fit for activities of daily living and household work, and poorer fit for pain, mobility, social function, and mental function.

Conclusions: the Indonesian QUALEFFO-41 demonstrates robust reliability and satisfactory validity, supporting its use as a culturally relevant instrument for assessing quality of life in Indonesian patients with osteoporosis. Although several domains showed suboptimal CFA fit, likely reflecting cultural and linguistic influences, the instrument remains psychometrically sound for clinical practice and research. Further longitudinal and multigroup analyses are recommended to refine the factor structure and evaluate responsiveness across diverse Indonesian populations.

Keywords:
Osteoporosis.
Quality of life.
QUALEFFO-41.
Reliability.
Validation.

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Consent to participate: Written informed consent was obtained from all participants before data collection.

Ethical approval: This study was approved by the Ethics Committee of the University.

Data availability: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest: The authors declare no conflict of interest.

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INTRODUCTION

Osteoporosis is a major and growing public health concern in Indonesia. The progressive loss of bone mass and deterioration of bone microarchitecture characteristic of osteoporosis lead to increased bone fragility and fracture risk, imposing substantial clinical, social, and economic burdens. Early Indonesian studies have reported osteoporosis prevalence rates of 28.8 % in men and 32.3 % in women (1). In addition, the prevalence of vertebral fractures among Indonesian women has been estimated at approximately 9 % (2). Given the rapid growth of the aging population and the projected increase in fracture incidence, with predictions that half of all osteoporotic hip fractures will occur in Asia by 2050 (3), the burden of osteoporosis in Indonesia is expected to continue increasing.

Within this context, measuring health-related quality of life (HRQoL) in patients with osteoporosis is critically important. Conventional clinical parameters such as bone mineral density (BMD) and fracture counts capture structural changes but often fail to reflect the broader impact of osteoporosis on pain, functional limitations, social participation, and psychological well-being. Disease-specific patient-reported outcome measures (PROMs) therefore play a crucial role in assessing the subjective burden of osteoporosis, evaluating treatment effects, and monitoring longitudinal changes. However, the use of PROMs in Indonesia has been constrained by the lack of validated, culturally adapted instruments. Although generic HRQoL instruments are available, disease-specific, locally validated measures remain limited in the Indonesian linguistic and cultural context.

One of the most widely used osteoporosis-specific PROMs is the Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41). Developed by the European Foundation for Osteoporosis (now the International Osteoporosis Foundation), the QUALEFFO-41 consists of 41 items across five domains: pain, physical function, social function, general health perception, and mental function (4,5). The questionnaire has been translated into numerous languages and has demonstrated clinical utility in populations with vertebral fractures and osteoporosis (6).

A recent systematic review assessing the measurement properties of the QUALEFFO-41 found that the quality of evidence supporting structural validity, reliability, responsiveness, and cross-cultural validity ranged from very low to moderate (6). Despite the availability of a Bahasa Indonesia version in certain instrument repositories, no peer-reviewed publication has documented a complete psychometric validation of the Indonesian QUALEFFO-41, including appropriate translation and adaptation procedures, evaluation of factor structure, reliability testing, construct validity, measurement error, and responsiveness. This methodological gap limits its confidence in the use of the QUALEFFO-41 in clinical practice and research in Indonesia.

Therefore, the aim of the present study was to conduct a comprehensive cultural adaptation and psychometric validation of the QUALEFFO-41 in Bahasa Indonesia for use among Indonesian patients with osteoporosis, with or without vertebral fractures. By adhering to internationally accepted methodological guidelines for cross-cultural adaptation (7) and applying rigorous psychometric evaluation, this study aimed to produce a validated Indonesian QUALEFFO-41 capable of reliably assessing HRQoL among Indonesian patients with osteoporosis.

METHODS

STUDY DESIGN

This cross-sectional study was designed to translate, culturally adapt, and psychometrically validate the Indonesian version of the Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41). The study followed the cross-cultural adaptation process recommended by Beaton et al. (2000) and the psychometric evaluation guidelines proposed by Nunnally and Bernstein (1994) and Hair et al. (2019).

PARTICIPANTS

A total of 204 participants diagnosed with osteoporosis or osteopenia were recruited from outpatient clinics and community health centers in Indonesia. Participants were aged 61-77 years and were able to read and understand Indonesian.

INSTRUMENT

The QUALEFFO-41 is a disease-specific quality-of-life questionnaire originally developed by the European Foundation for Osteoporosis to assess health-related quality of life in patients with vertebral fractures and osteoporosis. It comprises 41 items grouped into 5 domains:

1. Pain.
2. Physical function.
3. Social function.
4. Mental function.
5. General health perception.

Each item is scored on a 5-point Likert scale, with higher scores indicating poorer quality of life.

TRANSLATION AND CROSS-CULTURAL ADAPTATION

Translation and cultural adaptation of the QUALEFFO-41 into Indonesian were conducted according to the standard six-step process described by Beaton et al. (7):

1. *Forward translation:* Two independent bilingual translators translated the original English version into Indonesian.
2. *Synthesis:* The 2 translated versions were compared and synthesized into a single reconciled version following discussion among the translators and researchers.
3. *Backward translation:* Two additional translators who were blinded to the original instrument translated the Indonesian version back into English to assess conceptual equivalence.
4. *Expert committee review:* A multidisciplinary committee comprising methodologists, clinicians, and linguists reviewed all versions to evaluate semantic, idiomatic, experiential, and conceptual equivalence and produced a prefinal version.
5. *Pretesting:* The prefinal version was administered to patients to evaluate clarity, relevance, and cultural appropriateness through cognitive debriefing interviews.
6. *Final version:* Minor linguistic adjustments were made based on the feedback received, and the final Indonesian version was approved by the instrument developer, the International Osteoporosis Foundation (IOF).

CROSS-CULTURAL ADAPTATION AND LINGUISTIC VALIDATION

The cross-cultural adaptation of the QUALEFFO-41 questionnaire into Indonesian was conducted in accordance with internationally accepted guidelines for the adaptation of health-related quality-of-life instruments. Two independent bilingual translators whose native language was Indonesian performed forward translations of the original English version into Indonesian. One translator had a medical background, whereas the other had no clinical background, thereby ensuring both conceptual and lay-language equivalence. The 2 translated versions were then synthesized into a single reconciled Indonesian draft following discussion and consensus among the translators and research team. Subsequently, backward translation was performed by 2 independent professional translators who were blinded to the original instrument and had no prior knowledge of the QUALEFFO-41. The purpose of backward translation was to ensure semantic and conceptual equivalence with the original version.

The expert committee evaluated semantic, idiomatic, experiential, and conceptual equivalence and produced the prefinal Indonesian version. Cognitive debriefing (pilot testing) was conducted among patients with osteoporosis to assess clarity, comprehensibility, and cultural relevance. Minor wording adjustments were made based on participant feedback.

During the validation phase, participants completed only the Indonesian version of the QUALEFFO-41 questionnaire. No dual-language administration involving both the English and Indonesian versions was performed, and the same respondents did not complete both language versions. This approach was chosen to reflect real-world clinical use in the Indonesian population.

DATA COLLECTION PROCEDURE

Data were collected in Indonesia. Participants completed the Indonesian QUALEFFO-41 during routine clinical visits, either independently or with minimal assistance from trained research staff. To evaluate test-retest reliability, a subsample of 60 participants completed the questionnaire again after a 2-week interval, during which no clinical changes were expected. Sociodemographic data were also collected. The Indonesian-language version of the Qualeffo-41 questionnaire used in this study is publicly available through the International Osteoporosis Foundation (IOF) and can be accessed at: Indonesian version of the Qualeffo-41 questionnaire (https://www.osteoporosis.foundation/sites/iofbonehealth/files/2021-10/IOF-Qualeffo41-questionnaire-Indonesian.pdf).

STATISTICAL ANALYSIS

Psychometric analyses were conducted to assess the reliability and validity of the Indonesian QUALEFFO-41. Statistical analyses were performed using IBM SPSS Statistics, version 26.0; SmartPLS, version 4.0; and AMOS, version 24.0.

RELIABILITY

Internal consistency reliability was assessed using Cronbach α. Coefficients of $\alpha \geq 0.70$ were considered acceptable, $\alpha \geq 0.80$ were considered good, and $\alpha \geq 0.90$ were considered excellent. Test-retest reliability was assessed using the intraclass correlation coefficient (ICC) based on a 2-way mixed-effects model with absolute agreement. ICC values ≥ 0.75 indicated good reliability, whereas values ≥ 0.90 indicated excellent reliability.

CONVERGENT VALIDITY

Convergent validity was evaluated using factor loadings, average variance extracted (AVE), and composite reliability (CR). Values of AVE ≥ 0.50 and CR ≥ 0.70 were considered satisfactory evidence of convergent validity.

CONSTRUCT VALIDITY

Construct validity was examined using confirmatory factor analysis (CFA) with maximum likelihood estimation in AMOS. Model fit was assessed using χ^2/df, comparative fit index (CFI), Tucker-Lewis index (TLI), goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI), and root mean square error of approximation (RMSEA). Model fit was considered acceptable when χ^2/df < 3.0, CFI and TLI ≥ 0.90, GFI ≥ 0.85, and RMSEA ≤ 0.08.

RESULTS

SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PARTICIPANTS (TABLE I)

Table I. Sociodemographic and clinical characteristics of the participants

Characteristic	Category	Frequency (*n*)	Percentage (%)
Participant group	Prevalidation study	50	24.5
	Control	88	43.1
	Case	66	32.4
Current age (years)	60-64	13	6.4
	65-69	112	54.9
	70-74	57	27.9
	≥ 75	22	10.8
Age at osteoporosis onset (years)	≤ 59	7	3.4
	60-62	89	43.7
	63-65	81	39.7
	≥ 66	27	13.2
Body mass index (kg/m^2)	< 18.5 (underweight)	0	0.0
	18.5-22.9 (normal weight)	98	48.0
	23.0-24.9 (overweight)	77	37.7
	≥ 25 (obesity)	29	14.2
Educational level	No formal education	42	20.6
	Primary education	43	21.1
	Secondary education	71	34.8
	Tertiary education	48	23.5
Marital status	Married	98	48.0
	Widowed	106	52.0
Employment status	Employed	39	19.1
	Retired	165	80.9

RESULTS OF THE TRANSLATION AND ADAPTATION OF THE QUALEFFO-41 QUESTIONNAIRE INTO INDONESIAN

General information

The original QUALEFFO-41 (Quality of Life Questionnaire, 1997) was translated, culturally adapted, and validated into Indonesian by Z. N. and P. W. N. The translation was acknowledged and approved by the International Osteoporosis Foundation (IOF) on June 16, 2019.

Structure of the questionnaire

The Indonesian version of the QUALEFFO-41 comprises 41 items divided into 5 domains, identical to those of the original version. The pain domain includes 5 items assessing the frequency, duration, and intensity of back pain. The physical function domain is divided into three subdomains. The activities of daily living subdomain comprises 4 items evaluating respondents' ability to dress, bathe, use the toilet, and maintain sleep quality. The household work subdomain contains 5 items covering activities such as cleaning, cooking, washing dishes, shopping, and lifting objects. The mobility subdomain includes 8 items assessing the ability to stand, bend, climb stairs, walk, and maintain posture. The social function domain consists of 7 items assessing respondents' participation in hobbies, sports activities, visits to cinemas or theaters, and other social activities. The general health perception domain includes 3 items reflecting respondents' perceptions of their overall health and quality of life, whereas the mental function domain comprises 9 items assessing fatigue, hopelessness, loneliness, mood, and optimism.

Translation and cultural adaptation

The translation process emphasized semantic, idiomatic, experiential, and conceptual equivalence with the original instrument. Several linguistic and cultural adaptations were made to ensure clarity and relevance for respondents. Lexical adjustments and simplifications were introduced to improve readability, whereas unfamiliar or technical terms were replaced with more readily understood expressions. Cultural adaptations were made for items that might not fully correspond to the local context, such as activities involving cinema or theater attendance. Clarifying examples were added to improve comprehension, particularly for items involving specific weights or tasks. Semantic refinement was also undertaken to preserve the intended

psychological meaning of key terms, ensuring that the translated items accurately reflected the constructs assessed by the source version.

Scoring and interpretation

All response options were standardized according to the IOF scoring algorithm (2017):

1. Most items are rated from 1 (best quality of life) to 5 (worst quality of life).
2. Items 33, 34, 35, 37, 39, and 40 are reverse scored.
3. Domain scores are calculated as the mean of all items in the domain and then converted to a 0-100 scale.
4. If > 30 % of items are unanswered, the result is considered invalid.

Validation notes

1. The translation incorporated the official IOF scoring and conversion algorithm, ensuring methodological consistency.
2. The Indonesian version retains the original structure and psychometric intent while ensuring linguistic and cultural equivalence.
3. Official authorization was obtained from the International Osteoporosis Foundation, confirming approval of the adaptation.

The Indonesian version of the QUALEFFO-41 demonstrates semantic and conceptual equivalence with the original English instrument, with minor linguistic simplifications and contextual adjustments to accommodate Indonesian cultural norms. This version is suitable for assessing quality of life among Indonesian-speaking patients with osteoporosis.

Table II presents the internal consistency reliability of each domain as measured using Cronbach α. The pain domain, comprising 5 items, demonstrated excellent reliability (α = 0.930). The physical function domain, comprising 17 items, also showed a high level of reliability (α = 0.902). The social function domain demonstrated acceptable reliability (α = 0.725). The general health perception domain demonstrated marginal reliability (α = 0.653), indicating lower internal consistency than the other domains. The mental function domain demonstrated moderate reliability (α = 0.794). Overall, most domains demonstrated satisfactory internal consistency.

Test-retest reliability was assessed using the intraclass correlation coefficient (ICC) over a 14-day interval in a subsample of 60 participants. The pain domain showed good temporal stability (ICC, 0.8378), and the physical function domain also demonstrated good reliability (ICC, 0.8510). The social function domain

Table II. Integrated analysis of internal consistency, test–retest reliability, and construct validity across questionnaire domains

Domain			No. of items	Cronbach's Alpha (α)	Alpha interpretation	*n* (test-retest)	Time interval	ICC	ICC interpretation	AVE	CR	Validity interpretation
Pain			5	0.930	Highly reliable	60	14 days	0.8378	Good	0.721	0.928	Valid
Physical function	Activities of daily living		4	0.902	Highly reliable	60	14 days	N/A	N/A	0.831	0.980	Valid
		Household work	5					0.8510	Good			
		Mobility	8					N/A	N/A			
Social function			7	0.725	Acceptably reliable	60	14 days	0.6604	Moderate	0.584	0.787	Valid
General health perceived			3	0.653	Marginally reliable	60	14 days	0.7572	Good	0.695	0.830	Valid
Mental function			9	0.794	Moderately reliable	60	14 days	0.8664	Excellent	0.716	0.907	Valid
Total score			-	-	-	60	14 days	0.9994	Excellent	-	-	-

Note: Percentages are calculated based on valid responses (n = *204*).

showed moderate reliability (ICC, 0.6604). General health perception demonstrated good reliability (ICC, 0.7572), whereas the mental function domain showed excellent test-retest reliability (ICC, 0.8664). In addition, the total score exhibited an exceptionally high ICC of 0.9994, indicating nearly perfect temporal stability. The Bland-Altman plot used to assess agreement between the initial test and retest measurements and to provide an overview of agreement for the total QUALEFFO-41 score is shown in figure 1.

Convergent validity was assessed for each domain using average variance extracted (AVE) and composite reliability (CR). All domains met the conventional criteria for convergent validity, with AVE values > 0.50 and CR values > 0.70. Specifically, the pain domain demonstrated strong convergent validity (AVE, 0.721; CR, 0.928). Social function (AVE, 0.584; CR, 0.787), general health perception (AVE, 0.695; CR, 0.830), mental function (AVE, 0.716; CR, 0.907), and physical function (AVE, 0.831; CR, 0.980) also demonstrated satisfacto-

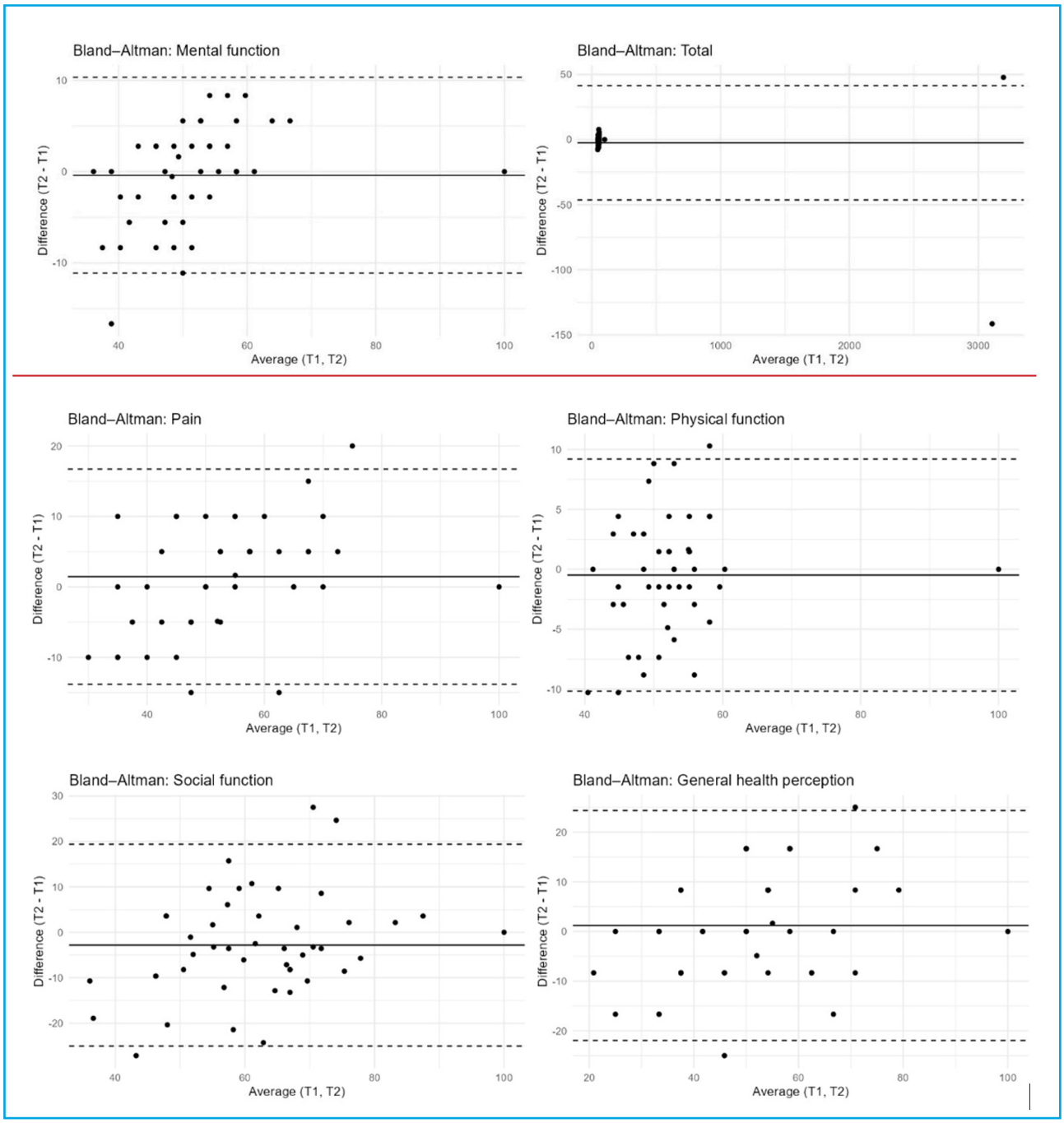

Figure 1. Bland-Altman plot for QUALEFFO-41 scores.

ry convergent validity. These results indicate that the items within each domain adequately represent their respective latent constructs.

Table III presents the results of the confirmatory factor analysis (CFA) for each construct. The pain construct showed poor model fit based on indices such as the goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI), comparative fit index (CFI), Tucker-Lewis index (TLI), and root mean square error of approximation (RMSEA). Within the physical function domain, the activities of daily living (ADL) and household work subconstructs demonstrated marginal fit, whereas mobility showed poor fit across all indices. The social function and mental function constructs also showed poor fit, suggesting substantial deviations from the assumptions of the proposed measurement model. In contrast, the general health perception construct exhibited perfect fit, indicating complete agreement between the measurement model and the observed data. Overall, the CFA results demonstrate variability in construct validity across domains, with some domains requiring further refinement. A structural equation modeling (SEM) path diagram depicting the relationships between latent constructs and the contribution of each indicator to the overall model is shown in figure 2. This diagram provides a visual representation of the direction and strength of the structural relationships examined in the study. A CFA model of the QUALEFFO-41 latent construct, showing the factor loading of each item and the fit of the model to the theoretical structure, is shown in figure 3. Together, these complementary figures support interpretation of the psychometric findings discussed below.

DISCUSSION

The validation results for the Indonesian version of the QUALEFFO-41 in this study demonstrate an overall satisfactory psychometric profile. The high internal consistency observed in domains such as pain (α = 0.93) and physical function (α = 0.90) indicates that the items within these domains are highly cohesive and consistently measure the intended constructs. This finding is consistent with the Korean adaptation, in which Cronbach α values for the QUALEFFO-41 ranged from 0.733 to 0.942, indicating adequate reliability in the Korean population (8).

Although internal consistency was strong in several domains, the CFA results showed that not all domains conformed closely to the original factor structure. The general health perception domain demonstrated very good model fit, whereas pain, social function, mobility, and mental function showed poor to marginal fit based on indices such as CFI, TLI, and RMSEA. This finding indicates that, although the items may be internally consistent, the theoretically proposed latent structure may not fully correspond to the pattern observed among Indonesian respondents. The discrepancy between high internal consistency and suboptimal CFA fit can be explained by the fact that Cronbach α assesses item homogeneity, whereas CFI, TLI, and RMSEA assess how well the hypothesized factor structure corresponds to the empirical data. In other words, items may correlate strongly with one another without necessarily fitting a proposed unidimensional structure. A similar issue was reported in a recent systematic review, which found relatively strong evidence for construct validity but limitations in cross-cultural validity and factor structure (6).

Cross-cultural comparisons are particularly relevant when interpreting these findings. For example, in the Hebrew-language adaptation for postmenopausal women in Israel, the QUALEFFO-41 demonstrated good internal consistency (α = 0.88) and adequate construct validity, although several domains, particularly cognitive/mental and social domains, required refinement to improve their relevance within the Israeli sociocultural context (9). Similarly, the Serbian adaptation of the QUALEFFO-41 showed challenges related to translation and cultural adaptation despite the use of methodologically rigorous forward-backward translation procedures (10). In Malaysia, the Malay version of the QUALEFFO-41 demonstrated good internal consistency (0.752-0.925), although the social activity do-

Table III. Construct validity (Confirmatory Factor Analysis - CFA)

Construct		χ^2/df	GFI	AGFI	CFI	TLI	RMSEA	RMR	Fit conclusion
Pain		32.13	0.771	0.314	0.840	0.680	0.392	0.083	Poor fit
Physical function	Activities of daily living	8.19	0.963	0.815	0.969	0.906	0.188	0.059	Marginal fit
	Household work	7.49	0.941	0.822	0.935	0.871	0.179	0.101	Marginal fit
	Mobility	11.00	0.783	0.609	0.693	0.570	0.222	0.222	Poor fit
Social function		11.236	0.821	0.642	0.662	0.493	0.225	0.173	Poor fit
General health perceived		–	1.000	–	1.000	–	–	0.000	Perfect fit
Mental function		34.434	0.585	0.308	0.462	0.283	0.406	0.242	Poor fit

Source: Authors' primary data (2025).

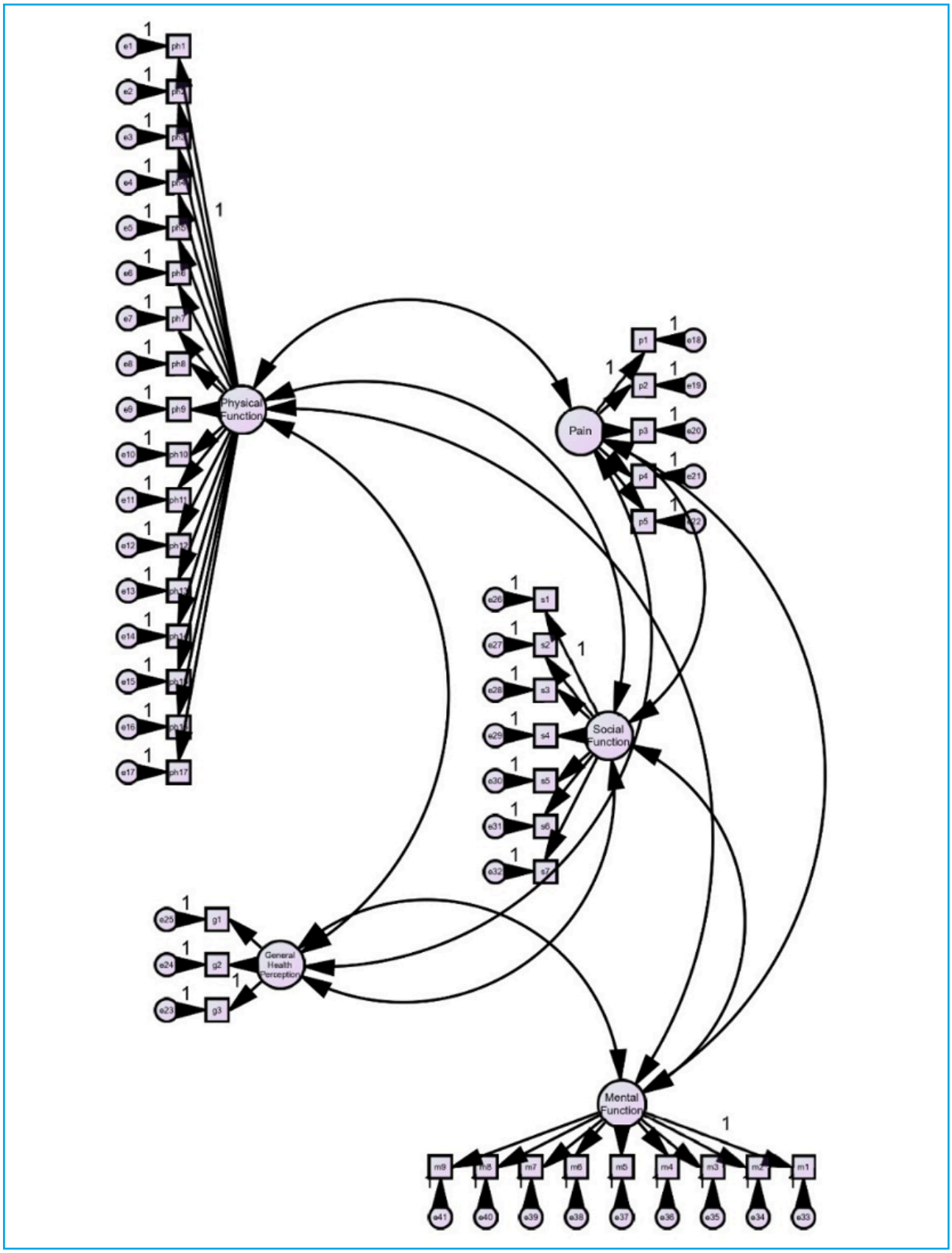

Figure 2. Structural equation modeling (SEM) path diagram.

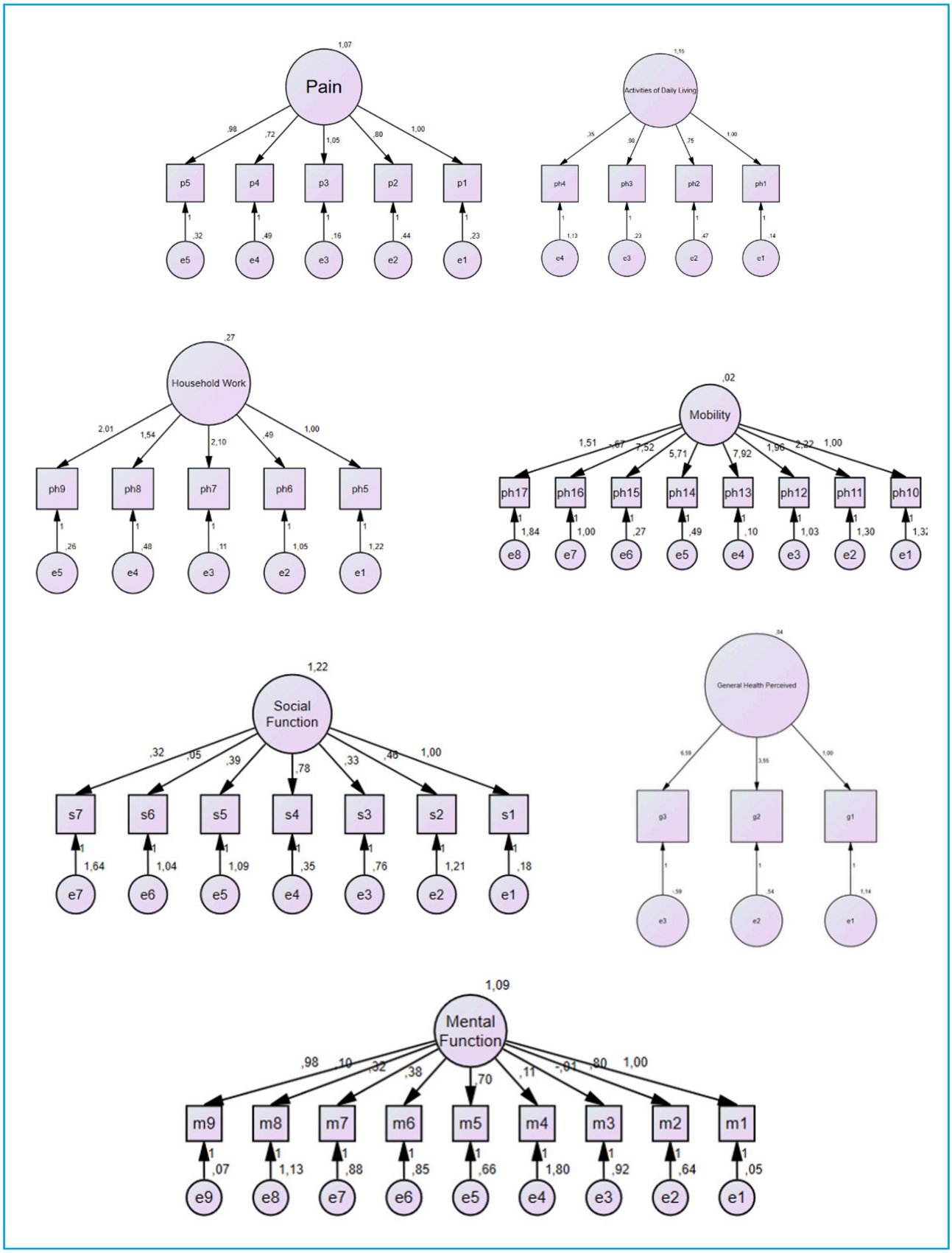

Figure 3. Confirmatory factor analysis (CFA) model for the QUALEFFO-41 latent construct.

main had a lower α value (0.692), possibly reflecting differences in the perception of social activity within the Malay cultural context (11).

Cultural and linguistic factors are likely to be major contributors to these differences. For example, the interpretation of household tasks in Indonesia may differ substantially from that in European or other Western contexts because Indonesian households often distribute such tasks among extended family members. This may lead respondents to rate the burden of household activities differently from how it was conceptualized in the original QUALEFFO framework. In addition, expressions related to "social function" and "mental function" may have different connotations in Indonesian; idiomatic expressions, emotional communication, and social roles, including family roles and social support, are strongly influenced by local cultural values. Linguistic adaptation that does not adequately account for these nuances may result in item cross-loading or high error covariance, which can adversely affect CFA model fit.

The high reliability and satisfactory convergent validity, including adequate AVE and composite reliability values across the major domains, indicate that the Indonesian version of the QUALEFFO-41 remains a useful disease-specific quality-of-life instrument for the Indonesian population with osteoporosis. Good internal consistency indicates coherent response patterns within domains, whereas convergent validity suggests that the items adequately reflect the constructs they are intended to measure. Collectively, these findings support the use of the Indonesian QUALEFFO-41 in clinical research, epidemiological surveys, and outcome monitoring in patients with osteoporosis in Indonesia.

The importance of the Indonesian version of the QUALEFFO-41 lies in its role as a locally adapted and validated instrument. It allows a more sensitive and contextually appropriate assessment of quality of life than generic instruments such as the EQ-5D in an Indonesian population that may differ in its perceptions of pain, mobility, social activities, and mental health. This provides practical benefits for clinical practice, including rehabilitation management and therapeutic interventions; research, including evaluation of treatment and rehabilitation effects; and health policy, such as assessment of the burden of osteoporosis-related impairment in quality of life in developing countries.

This study reinforces the view that health-related quality of life (HRQoL) is a critical clinical outcome in the management of osteoporosis, particularly among patients with vertebral fractures. Osteoporosis should not be conceptualized solely as a reduction in bone mineral density (BMD), but rather as a chronic, multifactorial condition with substantial physical, psychological, and social consequences. From a global epidemiological perspective, osteoporotic fractures represent a substantial and growing public health burden, particularly in aging populations (12). Vertebral fractures, although frequently underdiagnosed, are associated with chronic back pain, progressive kyphotic deformity, impaired mobility, reduced pulmonary function, and increased risk of falls. Collectively, these sequelae contribute to long-term deterioration in HRQoL that may not be adequately captured by radiological findings or densitometric parameters alone (13).

The development of the Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO) represented a paradigm shift in osteoporosis outcome assessment by emphasizing patient-reported outcomes (4). Unlike generic instruments, the QUALEFFO was specifically designed to detect the multidimensional impact of vertebral fractures, including pain severity, physical functioning, social participation, mental health, and general health perception. Subsequent validation studies confirmed its internal consistency, construct validity, and discriminative ability in distinguishing patients with and without vertebral fractures (14). The shortened version, QUALEFFO-41, further improved feasibility in clinical and research settings without compromising psychometric robustness.

Former studies have consistently demonstrated that vertebral fractures are a major determinant of impaired HRQoL. Oleksik et al. (15) reported that postmenopausal women with low BMD and prevalent vertebral fractures experienced significantly poorer HRQoL than women without fractures. Similarly, Jahelka et al. (16) found that patients with osteopenia or osteoporosis without fractures had relatively preserved quality of life compared with patients with fractures. These findings suggest that vertebral fracture represents an important clinical turning point in the course of osteoporosis and underscore the importance of fracture prevention strategies.

Disease-specific instruments such as the QUALEFFO demonstrate greater sensitivity than generic measures for detecting clinically meaningful differences and changes after treatment. Badia et al. (17) showed that the QUALEFFO had strong discriminative ability in women with vertebral fractures when compared with the Osteoporosis Quality of Life Questionnaire (OQLQ). Furthermore, Yilmaz et al. (18) demonstrated that disease-specific instruments had higher responsiveness indices than generic instruments when evaluating therapeutic interventions. This is particularly relevant in clinical trials assessing pharmacological treatments, physical rehabilitation, or combined interventions.

The association between HRQoL and functional parameters further strengthens the clinical relevance of disease-specific assessment. Bergland, Thorsen, and Kåresen (19) demonstrated significant correlations between HRQoL scores and measures of mobility and balance among women with osteoporosis and vertebral fractures. Spinal deformity and chronic pain not only impair physical performance but also increase the risk of falls, creating a vicious cycle of disability and further fracture risk. Therefore, integrating HRQoL assessment with functional mobility evaluation provides a more comprehensive framework for patient-centered osteoporosis management.

Moreover, rehabilitation guidelines emphasize the importance of monitoring quality of life as part of therapeutic evaluation (20). Treatment success should not be restricted to improvements in BMD but should also encompass functional recovery and psychosocial well-being. This reinforces the clinical utility of the QUALEFFO beyond research settings and supports its use as a valuable decision-support tool in routine care. HRQoL in osteoporosis is inherently multidimensional. Physical domains, particularly pain and activity limitation, often exert the greatest influence on total scores; however, psychological and social domains also contribute substantially to overall health perception (14,15). Chronic pain, fear of falling, reduced autonomy, and social withdrawal collectively shape the lived experience of osteoporosis. Consequently, a biopsychosocial framework is essential when interpreting HRQoL outcomes.

Several limitations should be acknowledged. First, the study sample may have been predominantly female, limiting the generalizability of the findings to men with osteoporosis. Sex-related differences in social roles, household responsibilities, and pain perception may produce different response patterns. Second, the cross-sectional design precludes assessment of changes in quality of life over time and causal inference. Third, although test-retest reliability was assessed, long-term temporal stability over months or years and measurement invariance across subpopulations, including age, sex, and geographic region, were not evaluated. Without measurement invariance analyses, it is difficult to determine whether the QUALEFFO-41 factor structure remains consistent across different segments of the Indonesian population. Subgroup analyses according to T-score severity and fracture type were not performed because of limited statistical power. Future studies with larger samples are warranted to assess the discriminative ability of the Indonesian QUALEFFO-41 across clinical severity groups.

Recommendations for future research include the following: (1) conducting item-level analyses, such as item response theory or communality analyses, to identify problematic items, including cross-loading, redundant, or culturally irrelevant items; (2) conducting cognitive interviews with respondents from diverse cultural and social backgrounds to ensure that item meaning is understood as intended; (3) considering modifications to the factor structure, including potential division of domains or subscales, based on local empirical data and cultural theory; (4) conducting multigroup CFA to assess measurement invariance across sex, geographic region, and age groups; and (5) implementing longitudinal studies to evaluate responsiveness to change and temporal stability of the Indonesian QUALEFFO-41.

Overall, although not all domains demonstrated optimal model fit in CFA, the strengths in internal consistency and convergent validity support the Indonesian version of the QUALEFFO-41 as a valuable, relevant, and contextually appropriate instrument for assessing quality of life in Indonesian patients with osteoporosis. Further adaptation and validation studies will help strengthen its utility in clinical research, population-based studies, and osteoporosis management interventions in Indonesia.

CONCLUSIONS

This study demonstrates that the Indonesian version of the QUALEFFO-41 is a valid and reliable instrument for assessing health-related quality of life in patients with osteoporosis, with satisfactory internal consistency across domains and evidence supporting several aspects of its construct validity. Although some CFA domains showed suboptimal model fit, the overall psychometric findings support use of the instrument within the Indonesian cultural context. Careful linguistic and cultural adaptation helped ensure that the instrument captures patients' lived experiences more appropriately than nonlocalized measures. The study is limited by its cross-sectional design, predominantly female sample, and absence of long-term longitudinal evaluation. Future research should assess longitudinal responsiveness, measurement invariance, and applicability across more diverse populations with osteoporosis.

AUTHORS' CONTRIBUTIONS

P. W. N. conceptualized the study, coordinated data collection, and performed the statistical analyses. Z. N. supervised the overall study design, provided methodological oversight, contributed to data interpretation, and critically revised the manuscript for important intellectual content. Both authors have read and approved the final version of the manuscript and are accountable for the accuracy and integrity of the work.

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Original

Osteogenic modulation through electrical stimulation: an *in vitro* study in human osteoblasts

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Abstract

Introduction: electrical stimulation (ES) has become established as a promising tool in regenerative medicine because of its ability to modulate key cellular processes. In bone tissue, ES may promote proliferation, differentiation, and extracellular matrix synthesis.

Hypothesis and objective: the application of low-frequency alternating current modulates, in a "voltage-dependent manner," the proliferation, maturation, and morphology of hOB obtained from human biopsies. The aim of this study was to evaluate the effect of alternating ES on proliferation, osteogenic activity (ALP and OCN), and morphological characteristics of cultured hOB.

Material and methods: we obtained primary hOB cultures from the tibial biopsies of 4 patients. After expansion, they were subjected to ES (0, 500, or 750 mV) for 10 days, 3 h/day. Proliferation was assessed by nuclear counting, ALP activity, osteocalcin (OCN) synthesis by immunofluorescence, and cellular morphology after pmCherry transfection. Statistical analysis included normality analysis followed by Student's t test and ANOVA for parametric variables, and Kruskal-Wallis ANOVA on ranks for nonparametric variables.

Results: proliferation increased significantly at 500 mV, whereas 750 mV showed values similar to the control group. ALP activity progressively increased at both voltages, reaching maximum values on day 10. OCN expression was significantly higher at 500 mV. Morphologically, 500 mV increased cell area without relevant changes in the number or length of pseudofilopodia.

Conclusion: alternating ES at 10 Hz and 500 mV promotes osteoblastic proliferation and maturation. The results indicate that 500 mV constitutes the most suitable condition to promote a balanced osteogenic response in hOB.

Keywords:
Human primary osteoblasts.
Electrical stimulation.
Alternating current.
Tissue engineering.

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INTRODUCTION

Regenerative medicine is an interdisciplinary field aimed at repairing or replacing damaged tissues and organs to restore their physiological functions. Within this field, electrical stimulation (ES) has emerged as a significant therapeutic modality, recognized for its ability to modulate different biological processes at both the cellular and tissue levels (1,2).

The generation of functional tissue through tissue engineering and the application of electrical stimulation has a major impact on several areas of regenerative medicine, as well as on the correction of bone defects through the production of functional osteoblasts (OBs) (3). Osteoblastic cells, widely recognized for their role in bone tissue formation, originate from mesenchymal stem cells (MSCs) present in the bone marrow stroma (4).

Throughout the osteoblastic differentiation process, these cells express different genes, markers that indicate the stage of osteoblast maturation. Initially, there is a proliferation stage in which pre-OBs express, among other genes, RUNX2. Subsequently, the transition from pre-OBs to mature OBs is characterized by the expression of genes such as osterix (OSX) and osteocalcin (OCN), together with an increase in alkaline phosphatase (ALP) activity for subsequent synthesis of bone matrix proteins, such as OCN (5). The process of extracellular bone matrix synthesis by mature OBs is carried out through deposition of the inorganic matrix and subsequent mineralization, in which ALP and OCN play a fundamental role (6). ALP is an enzyme produced by OBs involved in bone mineralization by providing the inorganic phosphate necessary for hydroxyapatite formation, the principal mineral component of bone (7). On the other hand, OCN is considered a specific marker of bone formation because it is synthesized exclusively by OBs; furthermore, its blood concentration reflects osteoblastic activity (8).

Currently, there are several conditions associated with bone disease that require solutions in clinical practice. These include significant bone tissue defects, traumatic fractures with poor consolidation, tumor resections, and debridement of necrotic tissues, both septic and aseptic. Similarly, the increase in prosthetic replacements due to degenerative or metabolic bone diseases is becoming increasingly common, especially in older individuals who exhibit lower osteoregenerative capacity, thereby failing to guarantee adequate future biomechanics (3).

It has been demonstrated that the application of electrical currents to bone produces an electrical effect capable of stimulating osteogenesis (3). Current research is focused on the development of conductive systems for localized ES, the application of ES in stem cells, the use of different biomaterials, optimization of ES parameters (frequency, amplitude, duration) for different applications, and the study of their mechanisms of action at the cellular and molecular levels (9). Overall, ES shows great potential for advancing bone regeneration, particularly in challenging cases such as large bone defects and prosthesis integration, because of its ability to improve cellular proliferation, differentiation, and tissue remodeling, especially in bone tissue (10).

Various experiments have demonstrated that ES has a positive effect on key processes involved in bone regeneration, such as vascularization, chondrogenesis, cell adhesion, and modulation of inflammation (11).

At cellular level, values in the range of tens or hundreds of millivolts (mV) are considered adequate to induce excitation and optimal for activating proteins sensitive to the electrical field, mainly membrane channels and receptors.

The general objective of this study was to investigate the role of electrical stimulation as a modulator of differentiation and maturation in osteoblastic cells obtained from human bone biopsies from knee arthrodesis procedures.

MATERIAL AND METHODS

STUDY DESIGN

Experimental *in vitro* study of primary human osteoblasts (hOBs) obtained from bone biopsies and subjected to electrical stimulation using alternating current (Fig. 1A).

STUDY SUBJECTS

All hOBs were obtained from tibial plateau explants. These explants were collected during arthroplastic procedures from four patients attending the Traumatology Clinical Management Unit of Hospital Universitario Virgen Macarena (HUVM) (Sevilla, Spain). The study was approved by the Provincial Research Ethics Committee of Sevilla (SICEIA-2024-001743).

All patients included in the study were informed and signed the informed consent form. No patients were excluded during the procedure.

Patients were selected according to the exclusion and inclusion criteria defined in the study. Included in the study were patients older than 50 years who had been admitted for knee joint prosthesis implantation for mechanical reasons and had signed informed consent.

Excluded were those receiving chronic oral corticosteroid treatment for a period longer than 6 months at a dose ≥ 7.5 mg/day of prednisone or equivalent corticosteroid. Also excluded were patients who had received osteoporosis treatment within the previous 5 years, had a past medical history of prolonged immobilization for > 6 months, or suffered from hepatic, renal, endocrine, or hematologic diseases affecting bone metabolism, or neoplastic disease.

CELL CULTURE

From the bone biopsies of 4 patients, 4 explant-derived cell cultures were obtained through mechanical disaggregation of trabecular bone, constituting 4 independent biological replicates for each experimental condition.

After washing with PBS, they were cultured to obtain human osteoblasts in DMEM medium (Minimum Essential Medium - PanBiotec, Germany) supplemented with 15 % heat-inactivated fetal bovine serum, 2 mM GlutaMAX i, 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco, USA), and 2.5 µg/mL amphotericin-B (Cytiva, United States). After 4 days in culture, this growth medium was replaced with osteogenic medium, which included: 50 µg/mL β-glycerophosphate (Merck, Germany), 10 mM ascorbic acid (StemCell Technologies, Canada), and 10 nM dexamethasone (Sigma-Aldrich, Madrid). Cultures were maintained in an incubator with a humidified atmosphere at 95 % humidity and 5 % CO_2. When the cells reached 80-85 % confluence, cell passaging was performed to increase cell number and subject them to the different electrostimulation conditions.

ELECTROSTIMULATION SYSTEM

Electrostimulation of the 4 hOB cultures was performed using a system that generates electrical fields while adapting and applying them to the cells. To perform these functions, the system contains 3 main components: monitoring, electrostimulation, and control functions such as energy supply. All components are coupled to a circuit board and interact through a bidirectional wireless connection, which allows monitoring and modification of operating parameters at any time during the experimental procedure (10).

The electrostimulation system used housed four 8W10E+ electrode plates (Applied Biophysics, Troy, NY, United States), each containing 8 wells of 1 cm^2 with 20 gold electrodes of 250 µm diameter (10). The applied frequency was 10 Hz. The electrostimulation system was designed at the Instituto de Microelectrónica de la Universidad de Sevilla (Sevilla, Spain) (TIC-178 group) (Fig. 1B).

In this study, alternating current with a frequency of 10 Hz was applied at voltages of 500 mV/mm or 750 mV/mm for 10 days, 3 h daily, in addition to a control condition (0 mV). This voltage and frequency range was defined based on previous unpublished experiments conducted by our research group.

The electrostimulation system used allows operation under conditions compatible with standard cell culture procedures, maintaining the culture plates in an incubator (37 °C, 5 % CO_2, and controlled humidity). The system is designed to maintain sterility during stimulation.

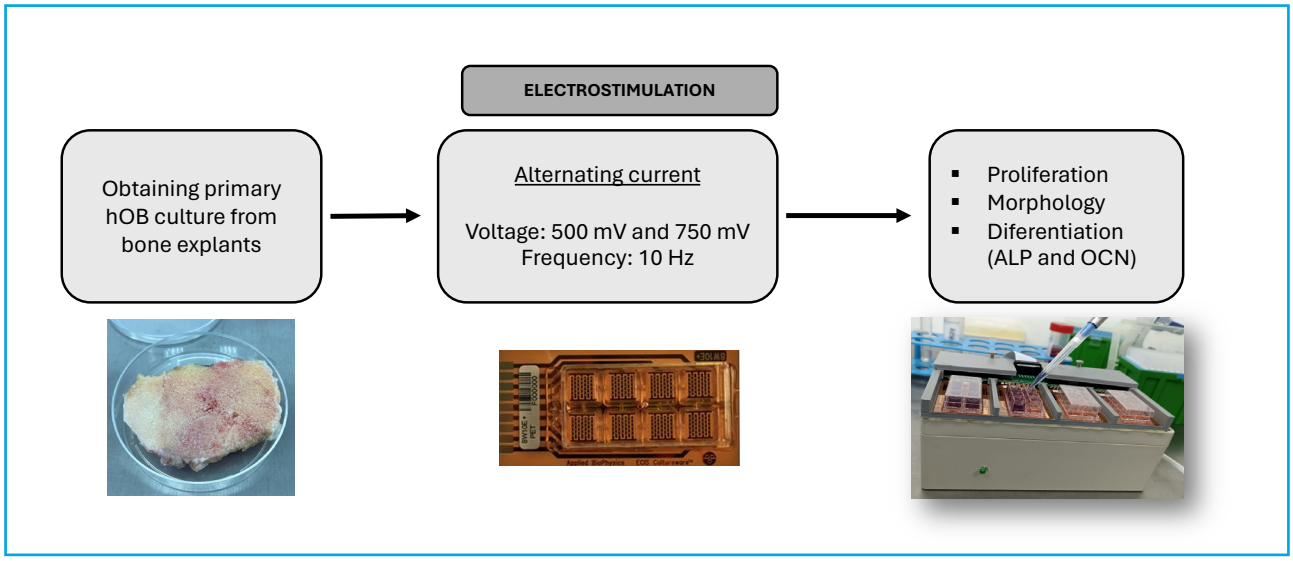

Figure 1. General schematic of the performed process, created with BioRender.

ALKALINE PHOSPHATASE ACTIVITY ASSAY

During stimulation, supernatant (SN) samples were collected on days 4, 7, and 10 and stored at -80 °C for subsequent analysis.

ALP activity was determined using the commercial Alkaline Phosphatase Assay Kit (Abcam, Cambridge, United Kingdom) and measured through a colorimetric assay based on the conversion of p-nitrophenyl phosphate (pNPP) to p-nitrophenol.

Absorbance was determined using the Infinity 200 Pro plate reader, and the obtained results were analyzed and represented using GraphPad Prism.

OSTEOCALCIN DETERMINATION

To study hOB maturation, the presence of OCN was determined by immunofluorescence (IF).

Cells were fixed with 4 % paraformaldehyde. The primary antibody used was anti-human OCN (R&D Systems, United States), and the secondary antibody was anti-mouse Alexa Fluor 488 (Invitrogen, Spain).

Cells were visualized using a fluorescence microscope (Hamamatsu ORCA-03G), and fluorescence was measured using ImageJ software (Softonic, Spain).

CELL TRANSFECTION AND IMMUNOFLUORESCENCE

hOBs must be cultured at high density to ensure survival. Cell expansion complicates individual morphological analysis. To avoid this problem, hOBs were transfected after 10 days of culture with fluorescent exogenous protein expression vectors. The low transfection efficiency allowed the analysis of individual cells under the electron microscope.

Transfection was performed by lipofection using the pmCherry-N1 vector (Clontech Laboratories, Takara BioCompany, Canada) and Lipofectamine 2000 reagent (Invitrogen Life Technologies, United States) in OptiMEM medium (Invitrogen).

Subsequently, to evaluate proliferation, morphology, and cellular differentiation, IF was performed on hOBs after 10 days of electrostimulation. Cells were fixed with 4 % paraformaldehyde, permeabilized with 0.5 % Triton-PBS, and immediately blocked with 1 % bovine serum albumin in PBT (0.1 % Triton in PBS). Cells were incubated with the rabbit anti-mCherry primary antibody 1:500 (Rabbit, ab167453, Abcam) for morphological analysis. After washing with PBS-T, incubation was carried out with the corresponding secondary antibody in blocking solution for 1 hour at a 1:500 dilution, Cy3 AffiniPure Donkey (H+L) (Rabbit, AB_2340612, Jackson ImmunoResearch). Finally, plates were washed with PBS and incubated with DAPI 1:500 for proliferation analysis. Mounting and visualization were performed using a fluorescence microscope (Olympus BX50, PA, United States).

Immunofluorescence was also performed on all cells as an independent study using anti-human osteocalcin 1:500 (Mouse, MAB1419, R&D Systems) as a marker of cellular differentiation. The same protocol used for IF of transfected cells was followed, using the secondary antibody at a 1:500 dilution: anti-mouse Alexa Fluor 488 (Goat, A11001, Invitrogen) and DAPI at 1:500.

ANALYSIS OF PROLIFERATION, MORPHOLOGY, AND OSTEOBLASTIC DIFFERENTIATION STUDIES

Morphological analysis of the cells was performed using fluorescence microscopy, and 10 images per well were captured using a fluorescence microscope (Olympus BX50) equipped with a digital camera (Hamamatsu ORCA-03G).

For the proliferation study, nuclei from 40 random fields per condition were counted using the Cell Counter tool of the ImageJ software. For morphological analysis, cell area, number of pseudofilopodia, and their maximum and mean length were quantified in pmCherry+ cells using ImageJ software. For each image, specific calibration was performed on a pixel-µm scale (1 µm = 12 pixels).

STATISTICAL ANALYSIS

Experiments were performed in triplicate, and each condition in duplicate.

SPSS 23.0 statistical software (IBM, España) was used for data analysis. An initial normality analysis was performed using the Shapiro-Wilk test. Means for independent data were compared using Student's *t* test or one-way ANOVA, depending on whether ≥ 2 experimental conditions were compared, respectively. For the ALP activity study, paired Student's *t* test analysis was performed because results were obtained over time (4, 7, and 10 days). Data related to the OCN ICC signal showed normal distribution. However, proliferation and morphological analyses (area, number of pseudofilopodia, mean and maximum length) did not show normality. For normally distributed variables,

statistical analysis was performed using Student's *t* test to compare 2 independent groups, followed by Tukey *post-hoc* analysis. Nonparametric variables were analyzed using Kruskal-Wallis analysis.

Patient age data are presented as mean ± standard deviation (SD), whereas experimental analysis data are shown as mean ± standard error of the mean (SEM). In all cases, $p < 0.05$ was considered the level of statistical significance.

RESULTS

We obtained 4 primary hOB cultures from 4 patients who underwent arthrodesis. The mean age of the patients was 55 ± 2 years; there were 2 women and 2 men, and in all 4 cases optimal cell confluence was reached at 22 ± 2 days. Data are organized according to the different functional and morphological parameters evaluated, allowing observation of how the cellular response varied in relation to the applied voltage.

PROLIFERATION

We analyzed hOB proliferation according to the electrostimulation voltage applied (0, 500, or 750 mV) at a frequency of 10 Hz for 10 days. The analyzed variable corresponded to cell density (cells/cm²).

In the control condition, without electrical stimulus, a mean cell density of 90.26 ± 7.70 cells/cm² was recorded. In cells stimulated at 500 mV, a significant increase in cell proliferation was observed ($p < 0.001$), reaching a density of 112.25 ± 11.03 cells/cm², whereas under the 750 mV condition, proliferation was similar to the control condition (75.90 ± 7.82 cells/cm²) (Fig. 2).

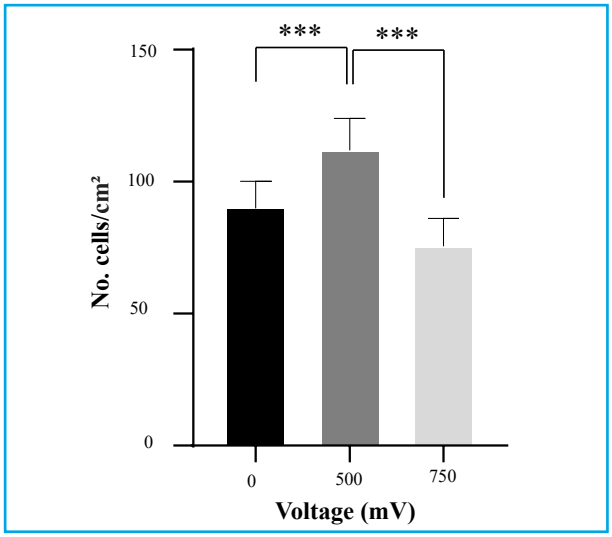

Figure 2. Effect of alternating current stimulation on proliferation in primary hOB cultures after 10 days of culture. Cellular responses were evaluated at a frequency of 10 Hz under different voltages: 500 mV and 750 mV. Values are expressed as percentage viability relative to the unstimulated control (0 Hz). Statistical differences: ***$p < 0.001$.

OSTEOBLASTIC MATURATION

Cell maturation was analyzed by quantifying ALP activity values at 4, 7, and 10 days of culture in the cellular supernatant. Values are shown in figure 3.

Both in the 500 mV and 750 mV conditions, a progressive increase in ALP activity was observed throughout the culture days; in both cases, maximum activity was obtained at day 10.

On day 7, significant increases in ALP activity were observed vs the control condition vs 750 mV, with values of 0.72 U/mL ± 0.02 vs 0.76 U/mL ± 0.01. After 10 days of culture, ALP levels at 500 mV and 750 mV increased significantly vs unstimulated control cells, with values of 0.74 U/mL ± 0.006 at 500 mV ($p < 0.01$) and 0.82 U/mL ± 0.02 at 750 mV ($p < 0.001$) vs control 0.70 U/mL ± 0.01.

Regarding OCN synthesis, figure 4 shows the OCN IF signal in hOBs exposed to the study voltages. The recorded variable corresponded to IF signal intensity measured in arbitrary units.

The OCN signal was significantly higher ($p < 0.001$) at 500 mV vs the control condition. At 750 mV, an increasing trend was observed without reaching statistical significance.

MORPHOLOGICAL ANALYSIS

We aimed to study the cellular morphology of hOBs to determine whether electrostimulation induced changes in shape, size, number of cytoplasmic extensions (pseudofilopodia), or their extension. Since observing morphology in culture is complex, the cells were transfected, with a transfection rate of 10 % to observe isolated cells and quantify and evaluate all parameters (Fig. 5).

Regarding the study of the mean cellular area of hOBs (µm²), the control culture showed a mean area of 167.03 µm² ± 12.56. Stimulation at 500 mV produced a notable increase in area, reaching 181.78 µm² ± 17.50. At 750 mV, the mean area was 200 µm² ± 15.89, which

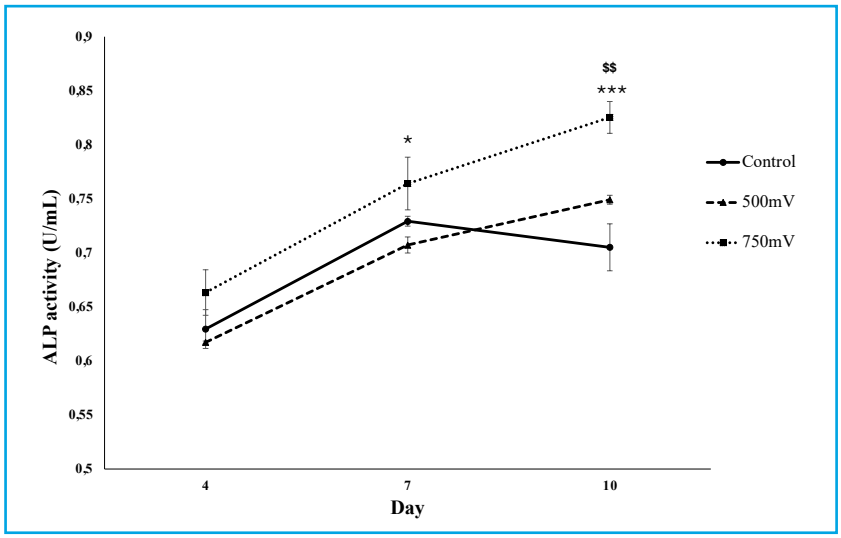

Figure 3. Study of ALP activity in hOBs during electrostimulation measured on days 4, 7, and 10 using alternating current at 10 Hz applying voltages of 500 mV and 750 mV. Data are expressed in U/mL, and statistical comparison was performed with the unstimulated osteogenic control (0 Hz) on the corresponding study day. $*p < 0.05$; $***p < 0.001$.

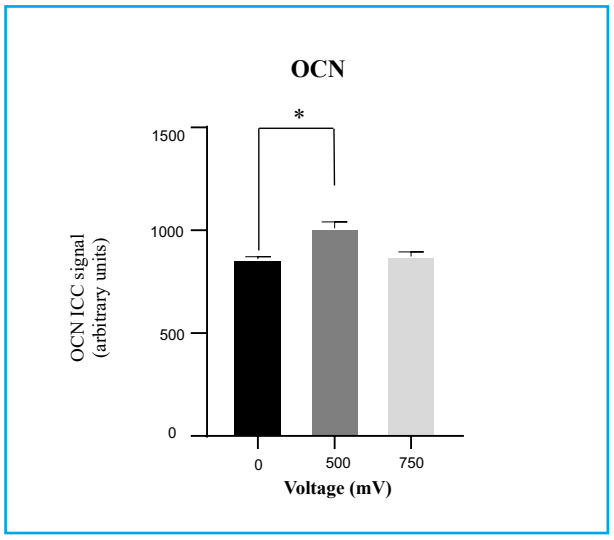

Figure 4. Study of the OCN IF signal in hOBs after electrostimulation with alternating current at 10 Hz applying voltages of 500 mV and 750 mV for 10 days ($*p < 0.05$).

was higher than the value observed in the control group, although without reaching statistical significance.

Regarding the total number of pseudofilopodia per hOB, no significant differences were observed either in stimulated cells or in the control culture, since OBs stimulated at 500 mV, at 750 mV, and under the control condition all presented 6.32 ± 0.25 pseudofilopodia per cell.

Values reached for maximum pseudofilopodia length were similar in all hOB cultures, reaching values close to 14.55 µm ± 1.40 under each condition. Regarding mean pseudofilopodia length, no significant differences were observed between the control group and cells stimulated at 500 mV, since both reached a mean of 7.1 µm ± 0.40. In contrast, electrical stimulation at 750 mV resulted in a nonsignificant decrease, reaching values of 5.19 µm ± 0.63.

DISCUSSION

The results demonstrate that hOB cultures stimulated at 500 mV exhibit increased proliferation. These findings are consistent with those reported by other authors, who also observed increases in both metabolic activity and cellular proliferation under electrical stimulation. This suggests that electrical stimulation enhances cellular growth under specific conditions, with capacitively coupled electrical fields significantly increasing osteoblastic cell proliferation, supporting the notion that electrical stimulation may favor cellular expansion during early phases of the osteogenic process (12,13). Other studies have described that electrical stimulation produces an increase in hOB metabolic activity and in the expression of markers involved in bone remodeling (14). However, several studies have not observed significant differences in bone regeneration after applying alternating electrical stimulation at 0.2 V and 1.4 V, suggesting that the response depends on electrical stimulation conditions (15). This increase in proliferation could be explained by the fact that direct current may alter the cellular membrane potential, activating voltage-dependent ionic channels, especially calcium channels. Increased Ca^{2+} influx may activate intracellular signaling cascades that promote proliferation through activation of protein kinases A and C (PKA and PKC). On the other hand, increased vinculin expression has been described to induce im-

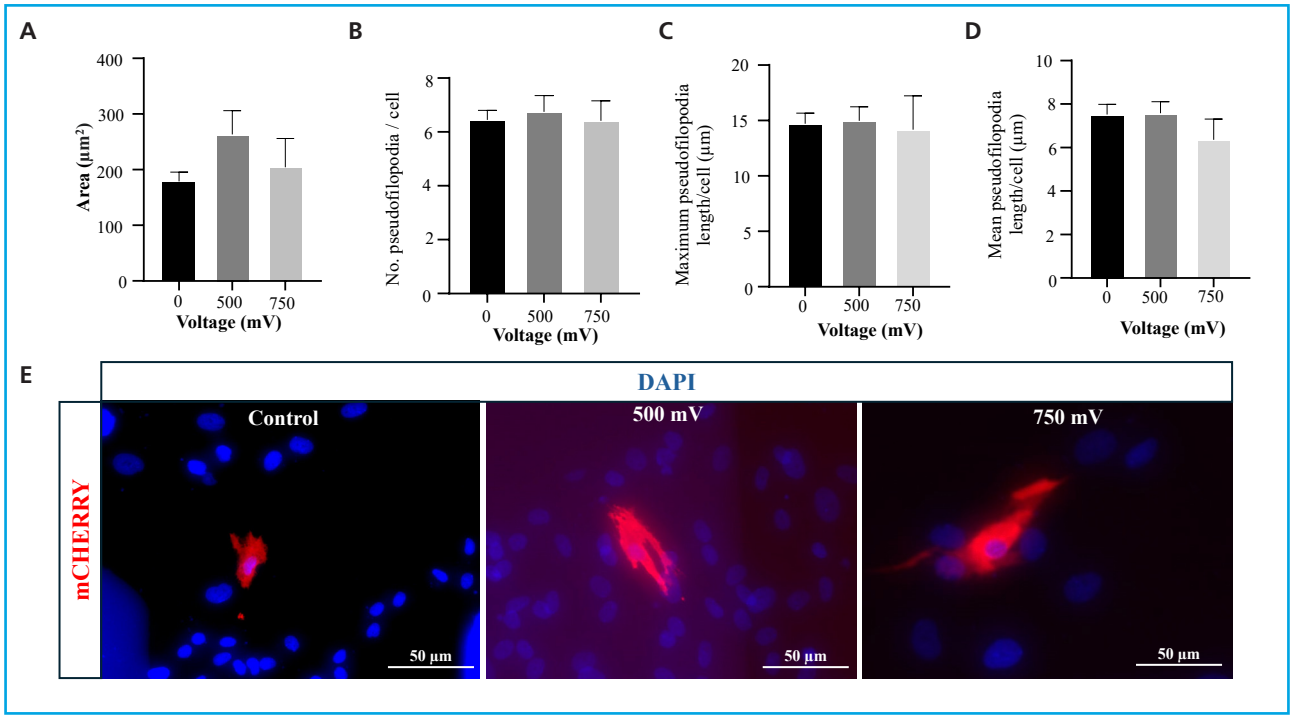

Figure 5. Morphological study in hOBs after electrostimulation with alternating current at 10 Hz applying voltages of 500 mV and 750 mV. A. Area (µm^2). B. Number of pseudofilopodia/cell. C. Maximum pseudofilopodia length/cell. D. Mean pseudofilopodia length/cell. E. Immunofluorescence of hOBs after electrostimulation at 10 Hz with voltages of 500 mV and 750 mV vs the control condition (0 mV). Cells transfected with the mCherry plasmid (red); cell nuclei with DAPI (blue).

proved cellular adhesion, which may activate integrin-mediated signaling and promote proliferation through mechanotransduction signals (14-16).

Cell maturation was evaluated by quantifying ALP and OCN activity as key biomarkers in the bone formation process, since they are enzymes produced by osteoblasts that participate in bone mineralization.

Based on the obtained data, a progressive increase between days 4 and 10 was observed in both stimulated cultures, being more evident in cells stimulated at 750 mV. ALP is a marker of early osteoblastic maturation and matrix preparation for mineralization; this temporal pattern is consistent with the transition from pre-OBs to mature OBs, where ALP activity precedes the expression of matrix proteins (such as OCN) and hydroxyapatite deposition. On the other hand, the highest OCN expression was observed in hOB cultures stimulated at 500 mV.

Several authors have described that ALP and/or OCN expression increased markedly after the application of electromagnetic fields together with a traditional alternating electrical field, both in human alveolar bone-derived stem cells and in MC3T3 cell cultures (17-19). Similarly, it has been shown that combining electrostimulation with hydroxyapatite hydrogel increases the expression of osteogenic markers and promotes hOB differentiation, favoring their maturation and mineralization (20).

The morphological changes observed in osteoblasts after exposure to different voltages reflect relevant variations in their biological behavior. Based on the study results, an increase in cellular area was observed in cells stimulated at 500 mV, possibly related to increased extracellular matrix production (15). Regarding the number of observed pseudofilopodia, no significant differences were found among the different groups of stimulated OBs. Although these results are inconclusive, former studies have demonstrated that electrical stimulation can modulate tissue organization and increase cellular activity, which is indirectly related to pseudofilopodia formation. Specifically, studies in neurons have shown that electrical stimulation modulates structural parameters such as cellular length and orientation (10), suggesting that this phenomenon could also occur in OBs, since pseudofilopodia formation is related to migration and bone regeneration processes. Similarly, previous studies have described that electrical stimulation induces general morphological changes in hOBs, such as alterations in cell shape and cytoskeletal reorganization, reinforcing the concept that osteoblast morphology responds to electrical stimuli (21).

Based on former studies, the hypothesis has been proposed that direct current stimulation possibly increases extracellular calcium concentration and promotes cellular signaling, as well as the expression of different cellular adhesion proteins and other extracellular matrix markers (such as vinculin, alkaline phosphatase, osteocalcin, etc.), thereby accelerating osteoblast growth and progressive maturation.

From former studies, it has been proposed that direct current stimulation could elevate calcium levels in the extracellular space and activate various cellular signaling pathways (16). This stimulus would also favor the production of proteins involved in cellular adhesion and the extracellular matrix, alkaline phosphatase, and osteocalcin, thus contributing to acceleration of both proliferation and progressive osteoblast maturation (22).

The strength of this study is the use of primary hOBs as a research model, avoiding the genetic changes present in conventional cell lines. Furthermore, the stimulation system is fully controlled and regulated by integrated analyses. In bone fracture models, electrical stimulation has been shown to improve bone quality, evidenced by increased mineral density and mechanical resistance of fractured bone, supporting the applicability of electrical stimulation in bone regeneration studies (23). The main limitations are, on the one hand, the small sample size and the fact that the sample included 2 men and 2 women, which did not allow sex-specific analyses, and on the other hand, the limited study duration (10 days), determined by the difficulty of maintaining hOB cultures and the absence of late mineralization analyses.

The results show three main conclusions: a) improved proliferation and expansion of cellular area at 500 mV (but reduced density at 750 mV); b) increased ALP activity over time, particularly significant on day 10 at both voltages vs the control; and c) a significant increase in osteocalcin (OCN) signal at 500 mV, without relevant changes in the number or maximum length of pseudofilopodia vs the control.

It is concluded that alternating stimulation at 10 Hz and 500 mV improves proliferation, expands cellular area, and enhances osteoblastic maturation (OCN), representing the optimal and recommended condition for subsequent studies in animal and human models.

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Original

Failure of the mechanical response as a cell-intrinsic effect in osteoblastic cell lines with *GBA1* mutations used as models of Gaucher bone disease

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Abstract

Background: Gaucher disease (GD) is caused by mutations in the *GBA1* gene that result in lysosomal accumulation of glucosylceramides. Bone manifestations associated with GD include Erlenmeyer flask deformity, osteopenia, osteosclerosis, osteonecrosis, and bone pain. Although osteoblast function has previously been evaluated in GD, no studies have assessed osteoblast function under mechanical stimulation.

Objectives: we aimed to generate an *in vitro* osteoblastic cellular model of GD and to evaluate the effect of mechanical stimulation on the function of this model.

Material and methods: *GBA1* was knocked out using CRISPR/Cas9, and two ROS17/2.8 clones with no detectable GBA1 enzymatic activity were selected. ROS wild-type (wt) and ROS-*Gba1*KO cells were plated and subjected to mechanical stress using laminar fluid flow at a shear stress of 10 dynes/cm^2 and 8 Hz for 10 minutes in a Flexcell® Streamer® Shear Stress Device. The remaining cells were maintained under static conditions. Cells were then cultured in fresh medium for an additional 18 hours before RNA extraction and quantitative polymerase chain reaction (qPCR) analysis of osteoblastic biomarkers. Cell viability and proliferation were assessed throughout the experiment, with no significant differences observed among the experimental conditions.

Results: as expected, mechanical stimulation induced osteoblast differentiation in all cell lines tested, as demonstrated by increased mRNA expression of *Runx2* and *Bglap* (osteocalcin). However, *Opg* (osteoprotegerin) expression was significantly reduced in KO cells compared with control cells.

Conclusions: loss of *GBA1* activity reduces osteoprotegerin expression in mechanically stimulated osteoblast models, suggesting disruption of osteoblast-osteoclast crosstalk and consequent impairment of bone remodeling in GD.

Keywords: Gaucher disease. *GBA1* mutations. Osteoblasts.

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INTRODUCTION

Gaucher disease (GD; OMIM #230800; ORPHA355) is a rare autosomal recessive genetic disorder caused by biallelic pathogenic variants in the *GBA1* gene (GRCh37/hg19 chromosome 1: 155,204,239-155,214,653), resulting in deficient glucocerebrosidase activity (GCase; EC 3.2.1.45; *GBA1*) and accumulation of glucosylceramide (GlcCer) and its deacylated metabolite glucosylsphingosine (GlcSph) in lysosomes of cells of the monocyte/macrophage system (1).

The characteristic signs of the disease, including splenomegaly, hepatomegaly, cytopenias, and bone lesions, are attributable to infiltration by Gaucher cells in the spleen, liver, and bone marrow, respectively (2). Gaucher cells are enlarged macrophages containing expanded lysosomes caused by accumulation of the undegraded substrate glucosylceramide. These cells damage the tissues in which they accumulate and exhibit increased activity of several lysosomal acid hydrolases, including acid phosphatase, angiotensin-converting enzyme (ACE), and lysozyme. In addition, one of the most characteristic features of Gaucher cells is their ability to release the enzyme chitotriosidase and numerous cytokines (3). GlcCer can be metabolized by acid ceramidase to glucosylsphingosine (GlcSph), accumulation of which is a characteristic consequence of GCase deficiency. GlcSph is subsequently metabolized to sphingosine, and high concentrations of this metabolite are toxic to bone and may contribute to neuronal dysfunction and death (4). The Gaucher disease phenotype is heterogeneous, but three clinical forms have traditionally been distinguished according to the severity of neuronopathic involvement: nonneuronopathic GD (GD1; OMIM 230800), acute neuronopathic GD (GD2; OMIM 230900), and chronic or subacute neuronopathic GD (GD3; OMIM 231000) (5-7).

Type 1 Gaucher disease (GD1) encompasses a broad spectrum of clinical severity, ranging from affected infants to asymptomatic adults. GD1 is also associated with numerous musculoskeletal signs, including bone marrow infiltration by Gaucher cells, bone infarction, recurrent avascular osteonecrosis, microvascular occlusion within bone, cortical thinning, impaired bone remodeling, osteolytic lesions, and reduced bone mineral density. These abnormalities can result in bone pain, increased susceptibility to fractures, joint damage with secondary osteoarthritis, bone deformity, and disability, thereby impairing patients' quality of life and occasionally necessitating orthopedic surgery. Imaging studies have demonstrated bone involvement in up to 90 % of patients with Gaucher disease. One of the most characteristic skeletal findings is Erlenmeyer flask deformity, with the femur being among the most frequently affected bones (8,9).

GD1 is the most common form of the disease and is associated with skeletal signs in up to 80 % of patients, substantially reducing quality of life. Bone involvement may be limited to asymptomatic defective remodeling, such as Erlenmeyer flask deformity. Bone remodeling depends on crosstalk between bone-forming osteoblasts and bone-resorbing osteoclasts and is essential for maintaining bone homeostasis. Mechanical stimulation plays an important role by activating osteoblasts to produce osteoprotegerin (*Opg*), which protects against osteoclast-mediated bone resorption (10,11). Bone involvement in GD may also include medullary and corticocancellous infarcts, pathological fractures, osteonecrosis of the humeral or femoral heads, and vertebral collapse (12-14). However, the precise mechanisms underlying bone pathophysiology in GD remain unclear.

Lysosomes, as components of the autophagy pathway, play important roles in regulating osteoblast differentiation and osteoclast maturation (15). Autophagy protects osteoblasts from apoptosis and may be critical for osteogenic differentiation and bone formation.

In this study, we aimed to generate osteoblast-like cells harboring loss-of-function mutations in the *Gba1* gene using CRISPR/Cas9 and to evaluate their intrinsic response to mechanical stimulation to broaden our understanding of the skeletal phenotype in GD.

MATERIALS AND METHODS

GENERATION OF *Gba1*KO MODELS USING CRISPR/Cas

The rat osteosarcoma cell line ROS17/2.8 (RRID: CVCL_0508) was used as a model of osteoblast-like cells in its wild-type and *Gba1* knockout (ROS-*Gba1*KO) forms. *Gba1* editing was performed at the Organelle Homeostasis Laboratory of TIGEM (Naples, Italy) using the pSpCas9(BB)-2A-GFP (PX458) plasmid and a Sigma-Aldrich synthetic guide RNA (RN00064-0684536; sgRNA) targeting exon 3 of the *Gba1* gene (Supplementary Table I - https://www.revistadeosteoporosisymetabolismomineral.com/files/533/ADMA1-00122-02.pdf). For guide selection, off-target site prediction was performed using Benchling (https://www.benchling.com/) (Supplementary Table II - https://www.revistadeosteoporosisymetabolismomineral.com/files/533/ADMA1-00122-02.pdf). The sgRNA was cloned into the pSpCas9(BB)-2A-GFP vector according to the protocol described by Ran et al. (16) After the sgRNA had been cloned and tested, cells were seeded 1 day before transfection in 6-well plates (3.5×10^5 cells/well). Reverse transfection was performed using 2500 ng of pSpCas9 plasmid containing sgRNA, 7.5 µL of Lipofectamine™ 3000, and 5 µL of Lipofectamine™ P3000 reagent (Invitrogen-Thermo Fisher Scientific, United States), according to the manufacturer's in-

structions for use. After 48-72 hours, the edited cell pool was subjected to single-cell sorting by flow cytometry (BD Biosciences LSR II flow cytometer, San Jose, CA, United States). Cells derived from individual clones were expanded to obtain sufficient numbers for DNA extraction and identification of edited clones by Sanger sequencing. Edited clones predicted to generate KO proteins were analyzed by western blotting to confirm loss of GCase expression, and GCase activity was measured in selected clones.

CELL CULTURES

ROS wt and ROS-*Gba1*KO cells were cultured and maintained in DMEM/F-12 medium (Gibco) containing 10 % fetal bovine serum (Gibco; origin: Brazil) and 1 % penicillin/streptomycin (Gibco) as complete medium, in a humidified atmosphere containing 5 % CO_2 at 37 °C. Osteoblast viability was assessed using the MTT assay (Sigma). Briefly, 80,000 cells were cultured in 24-well plates for 3 days, after which MTT reagent was added at a final concentration of 0.45 mg/mL. Absorbance was measured at 560 nm after incubation at 37 °C for 4 hours. Three independent replicates were performed.

WESTERN BLOT

To evaluate GCase expression levels, cells were washed once with phosphate-buffered saline (PBS) and lysed in RIPA buffer using cell scrapers. Protein extracts were quantified using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, United States). Proteins (20 µg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a hydrophobic polyvinylidene fluoride (PVDF) membrane (Merck-Millipore, Burlington, MA, USA). A primary antibody against GCase/GBA (2 µg/mL; GBA 2E2 WH0002629M1, Sigma-Aldrich, St. Louis, MO, United States) was used, and an antibody against actin (1:200; A2066, Sigma-Aldrich, St. Louis, MO, United States) served as the loading control. Membranes were developed using a peroxidase-conjugated anti-rabbit IgG secondary antibody (A0545), diluted 1:10,000, for GCase/GBA and actin detection. Each quantification was obtained from an independent experiment, for a total of 3 experiments per biological replicate.

GLUCOCEREBROSIDASE ENZYMATIC ACTIVITY

Glucocerebrosidase (GCase) enzymatic activity was measured using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucopyranoside (Sigma-Aldrich, St. Louis, MO, United States). Total protein concentration in cell lysates was determined using the Bradford assay (Bio-Rad, Hercules, CA, United States), according to the manufacturer's instructions for use. In each well of a 96-well plate, 24 µL containing 24 µg of protein was incubated at 37 °C for 30 minutes. Subsequently, 100 µL of McIlvaine buffer (150 mM citrate-Na_2HPO_4 solution; 0.2 % taurocholate; 0.1 % Triton X-100; 0.1 % BSA; 1.25 mg/mL substrate; pH 5.2) was added, and the plate was incubated again at 37 °C for 30 minutes. The reaction was stopped with 0.3 M NaOH-glycine buffer, pH 10.4, and the fluorescent product was quantified using a fluorimeter (Thermo Scientific Fluoroskan Ascent FL Microplate Fluorimeter and Luminometer) at an excitation wavelength of 365 nm and an emission wavelength of 495 nm. Quantification was performed relative to readings obtained without substrate addition (blank). Values below the blank were reported as 0. This assay was performed once for each selected clone.

FLUID-FLOW ASSAY

ROS wt and ROS-*Gba1*KO cells were plated at 2×10^4 cells/cm^2 on collagen-pretreated glass slides (FlexCell, Hillsborough, NC, United States) and cultured in complete DMEM/F-12 medium. After 24 hours of incubation, half of the cells were subjected to mechanical stress by laminar fluid flow (FF) at a shear stress of 10 dynes/cm^2 and 8 Hz for 10 minutes using a Flexcell® Streamer® Shear Stress Device. The remaining cells were maintained under static conditions (SC). Cells were subsequently cultured in fresh medium for an additional 18 hours before RNA extraction and qPCR analysis (Supplementary Table I - https://www.revistadeosteoporosisymetabolismomineral.com/files/533/ADMA1-00122-02.pdf). During the procedure, cell viability and proliferation were assessed, with no significant differences observed among the experimental conditions. Briefly, non-adherent cells were collected and pooled with trypsinized adherent cells and counted using a Neubauer chamber. The number of viable cells and cell viability were determined using the trypan blue exclusion assay, in which refractile cells were considered viable, whereas cells showing intracellular blue staining were considered nonviable. Three independent replicates were performed.

RT-qPCR

Total RNA was extracted from ROS wt and KO cells subjected to FF or SC using the High Pure RNA Isolation Kit (Roche, Basel, Switzerland), according to

the manufacturer's recommendations. Two micrograms of RNA were reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, United States), followed by RT-qPCR using LightCycler® 480 Probes Master (Roche, Basel, Switzerland) in a LightCycler® 480 system (Roche, Basel, Switzerland), according to the manufacturer's protocol. Primers were designed from available mouse and rat sequences using Primer3 software (Supplementary Table I - https://www.revistadeosteoporosisymetabolismomineral.com/files/533/ADMA1-00122-02.pdf). Mouse and rat *Gapdh* genes were used as internal controls. The comparative threshold cycle (Ct) method was used for data analysis, with results expressed as 2-ΔCt. Three independent experiments were performed for each biomarker.

STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism, version 8 (GraphPad Software, San Diego, CA, United States). Unpaired and paired *t* tests, the Mann-Whitney *U* test, or analysis of variance (ANOVA) were used, as appropriate. Data are expressed as mean ± SD and are representative of three or more independent experiments.

RESULTS

GENERATION OF *Gba1* GENE KNOCKOUT IN OSTEOBLAST-LIKE CELL LINES

We generated and characterized *Gba1* knockout rat osteosarcoma cell lines (ROS-*Gba1*KO) as putative osteoblast-like cellular models of GD. Two KO clones were obtained from ROS cells. The first clone (ROS-*Gba1*KO1) carried a homozygous 2-nucleotide frameshift deletion (NM_001127639.1:c.256_257del; p.Leu86AsnfsTer44), whereas the second clone (ROS-*Gba1*KO2) carried a homozygous 1-nucleotide frameshift deletion (NM_001127639.1:c.258del; p.Leu87ProfsTer11) (Fig. 1A). These deletions were validated by Sanger sequencing (Supplementary Fig. 1 - https://www.revistadeosteoporosisymetabolismomineral.com/files/533/ADMA1-00122-02.pdf).

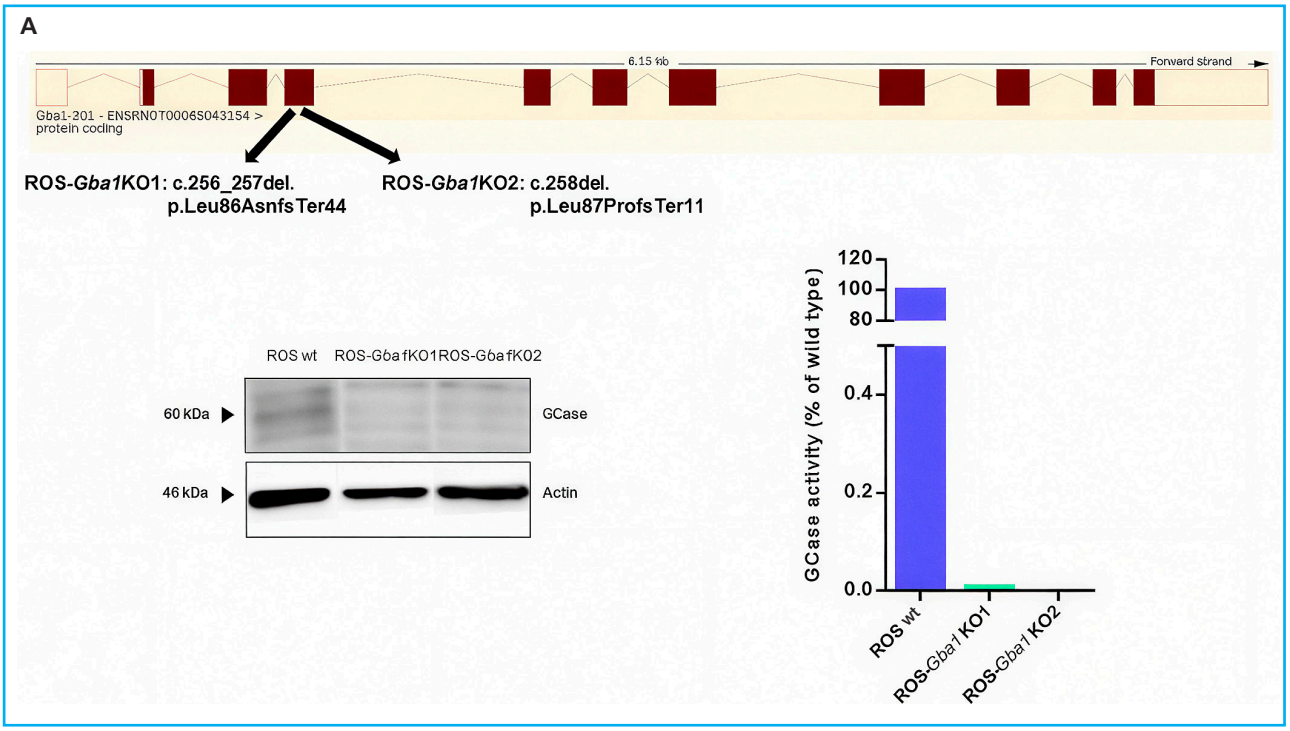

Figure 1. Generation and characterization of Gaucher osteoblastic models. A. Schematic representation of the *Rattus norvegicus Gba1* gene showing the locations of the mutations. The ROS-*Gba1*KO1 clone carries a homozygous 2-nucleotide deletion (256-257), whereas the ROS-*Gba1*KO2 clone carries a homozygous 1-nucleotide deletion (258). B. Western blot analysis of GCase expression in ROS cells (wt and KO). The KO clones showed markedly reduced GCase expression compared with wt cells. C. GCase enzymatic activity in ROS cells (wt and KO). GCase enzymatic activity was markedly reduced in the selected KO clones compared with wt cells. Results correspond to one biological replicate for each cell type.

ROS-*Gba1*KO cells showed no detectable GCase protein expression, as determined by western blotting (Fig. 1B), and exhibited < 1 % residual GCase enzymatic activity (Fig. 1C).

FUNCTIONAL CHARACTERIZATION OF GENERATED KO OSTEOBLASTS UNDER MECHANICAL STIMULATION

First, we assessed cell viability and proliferation and observed no significant differences between the KO clones and the wild-type cell line (Fig. 2).

Given the well-established anabolic effects of mechanical stimuli on bone, we analyzed the response of KO cells to mechanical stimulation and compared it with that of wt cells. Mechanical stimulation induced a significant increase in *Runx2* and *Bglap* mRNA expression in all cell types, with no significant differences between KO and wt cells. However, for *Runx2*, a statistically significant difference was detected between wt cells and one KO line (ROS-*Gba1*KO2) under static conditions. In contrast, *Opg* expression showed a distinct pattern: wt cells exhibited a 3-fold increase after mechanical stimulation, whereas this stimulatory effect was significantly attenuated or absent in KO cells (Fig. 2).

DISCUSSION

In this study, we aimed to generate and characterize an osteoblast-like cell model of GD to investigate mechanisms underlying the skeletal manifestations associated with the disease. We used CRISPR/Cas9 gene editing to generate *Gba1*knockouts in rodent bone cell lines because rodents do not harbor the *GBA* pseudogene. After validating the models, we evaluated the response of these *Gba1* knockout cells to mechanical stimulation by measuring the expression of osteoblastic biomarkers.

To assess osteoblast function in ROS-*Gba1*KO cells, we analyzed the expression of *Runx2*, *Bglap*, and *Opg*, which are specifically involved in osteoblast differentiation and function. In our mechanical loading experiments, no consistent differences were observed in either *Runx2* or *Bglap* expression compared with wt cells, either before or after mechanical stimulation. These markers represent distinct aspects of osteoblast differentiation and function. *Runx2* is a transcription factor that plays an essential role in osteoblast differentiation and expression of osteoblast-specific genes. It is crucial for commitment of mesenchymal stem cells to the osteoblast lineage and positively regulates early stages of osteoblast differentiation; accordingly, it is considered an early marker of osteoblast differentiation. Osteocalcin (*Bglap*) is a hormone secreted primarily by osteoblasts and is thought to contribute to systemic metabolic regulation. It is expressed by mature osteoblasts and therefore represents a marker of later-stage differentiation. Finally, osteoprotegerin (*Opg*) is secreted by osteoblasts and acts as a decoy receptor that binds specifically to *Rankl*. Binding of *Rankl* to its receptor *Rank* on the membrane of osteoclast precursors is essential for osteoclast formation and activation. Osteoprotegerin regulates bone remodeling by reducing osteoclast formation and is therefore used as a marker of osteoblast function (10,17-19).

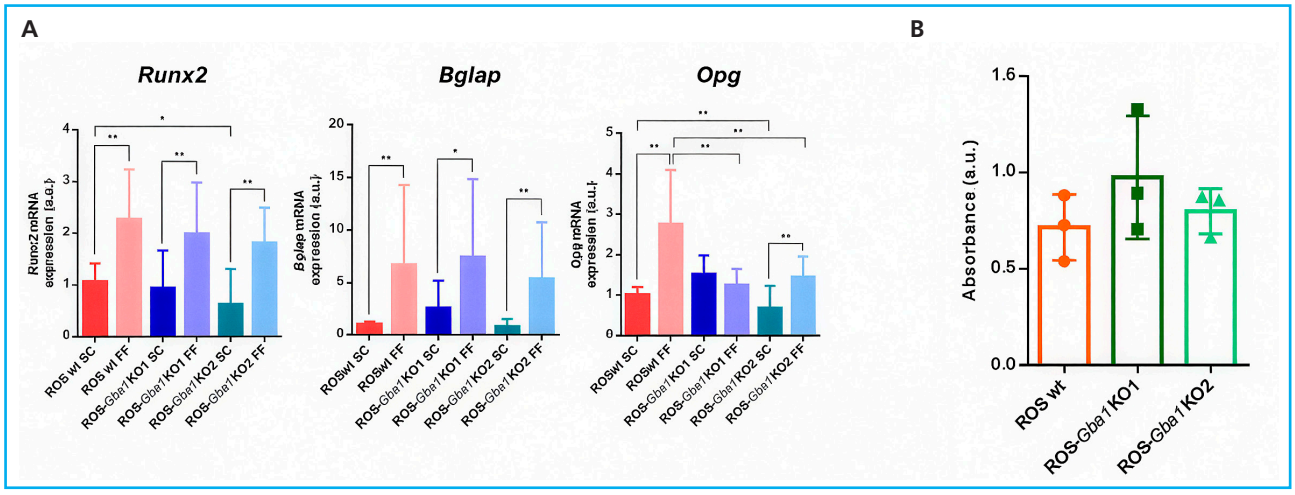

Figure 2. Mechanical stimulation assay (fluid flow) in ROS-*Gba1*KO cell models. A. mRNA expression of osteoblastic markers after mechanical stimulation. Results are expressed as mean ± SD of 3 independent experiments. B. Cell viability. MTT assay results are expressed as mean ± SD of three independent experiments. *$p < 0.05$; **$p < 0.001$. SC, static control; FF, fluid flow (mechanically stimulated sample).

Former *in vitro* studies investigating osteoblast function in GD have reported conflicting results. Several studies, including those using induced pluripotent stem cell-derived osteoblasts, osteoblast-like cell lines treated with the glucocerebrosidase inhibitor conduritol-B-epoxide (CBE), or cells obtained directly from patients with GD, have reported impaired osteoblast function, with significantly reduced expression of biomarkers such as *Runx2*, *Alp*, *Col1*, and *Bglap* (20-22). In contrast, Lecourt et al. (23), using an *in vitro* model of CBE-treated and untreated mesenchymal stem cells (MSCs), observed impaired proliferation in treated compared with untreated MSCs. However, when osteoblast differentiation was assessed by measuring *Runx2* and Osterix mRNA expression, no significant differences were found between treated and untreated cells after seven days of culture in osteogenic medium. Similarly, studies of samples from cohorts of untreated patients with type 1 GD found no statistically significant differences in bone biomarkers such as osteocalcin and bone alkaline phosphatase isoenzyme (24,25), or osteocalcin, bone-specific alkaline phosphatase, N-telopeptide cross-links, and deoxypyridinoline (D-PYD) (26), which remained within the normal range compared with the respective controls. Our findings showing no differences in osteoblastic mRNA biomarkers between disease-model and control cells are consistent with these observations.

With regard to osteoblast proliferation defects, several studies have consistently reported decreased proliferation in both animal and cellular models of Gaucher disease (21-23,27). Given the essential role of the Wnt signaling pathway in osteoblastogenesis (28), it is not surprising that abnormalities in this pathway have been reported in several models (21,23,29), potentially accounting for the observed proliferation defects. In the present study, we did not observe clear alterations in proliferation in our ROS KO cell lines, possibly because of their osteosarcoma origin, immortalized phenotype, and *in vitro* culture conditions.

Our Gaucher bone cell models have several limitations, primarily related to their origin as transformed cell lines and their maintenance as isolated monocultures. These characteristics preclude assessment of cell-cell interactions and do not fully reproduce the physiological processes observed in animal models, potentially limiting their usefulness as comprehensive models of Gaucher disease. Nevertheless, these systems are easy to maintain and, importantly, allow investigation of intrinsic cell type-specific mechanisms contributing to the skeletal phenotype. Future studies combining these cell lines in coculture systems to more closely reproduce the bone microenvironment may enable more accurate cellular characterization and facilitate drug testing.

Our principal finding was that osteoblasts used as Gaucher disease models failed to upregulate osteoprotegerin in response to mechanical stimulation, whereas wild-type osteoblasts significantly increased expression of this marker. To our knowledge, this represents a novel finding. We hypothesize that the absence of *Opg* activation may partly explain the impaired crosstalk described between Gaucher bone cells, in which osteoblasts affected by Gaucher disease promote greater osteoclast differentiation (30,31). Reduced *Opg* expression would decrease competitive inhibition of the *Rank-Rankl* interaction required for osteoclast differentiation, ultimately disrupting bone homeostasis (32).

CONCLUSIONS

Within the context of our cellular model, these findings suggest impaired osteoblast-osteoclast crosstalk mediated by osteoprotegerin, which may disrupt bone remodeling and partly explain the skeletal phenotype observed in Gaucher disease.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

J. D. P., S. B., N. M. G., N. G. G., D. G., and R. R. contributed to study conceptualization and design. J. D. P., L. C., M. C., and E. M. G. performed the main experiments. J. A. A., A. R. G., S. H. J., and E. M. G. performed the fluid-flow assays. X. N. and D. O. contributed to project administration and funding acquisition. S. B., D. G., R. R., and N. G. G. contributed to validation and supervision. The first draft of the manuscript was written by J. D. P., S. B., N. G. G., and R. R. All authors contributed to, read, and approved the final version of the manuscript.

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DATA AVAILABILITY

Data are contained within the article or supplementary material. Additional raw data are available from the corresponding author upon reasonable request.

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ARÁN

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Revisión

Narrative literature review of ectopic parathyroid adenoma in the submandibular region following a case detected by ^{18}F-FCH PET/CT

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Abstract

Ectopic parathyroid glands pose a significant challenge in the diagnosis and management of primary hyperparathyroidism, particularly when minimally invasive parathyroidectomy is planned, because its success depends heavily on accurate preoperative localization. Ectopic parathyroid adenomas in the submandibular region are exceptionally rare, and their surgical management may require conversion to bilateral open exploration or subsequent reoperation because of persistent disease.

Prompted by a complex clinical case, this narrative review examines the current literature on ectopic submandibular parathyroid adenomas, with particular emphasis on diagnostic strategies, imaging modalities, and surgical considerations. The findings underscore the critical importance of precise preoperative localization for successful targeted resection, even in challenging ectopic presentations.

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is caused by increased activity of the parathyroid glands resulting in excessive secretion of parathyroid hormone (PTH). Most cases are caused by a single adenoma (85 %), of which 5 %-20 % are ectopic (1-4), and, less frequently, by hyperplasia of all 4 glands (10 %-15 %) (2,5-7). PHPT has an approximate prevalence of 1 % in the general population, with a female-to-male ratio of 3-4:1, and typically affects middle-aged women between 40 and 60 years of age (3,6-8). It is the third most common endocrine disorder after diabetes *mellitus* and thyroid disease (2,3,5,7).

The diagnosis of PHPT is biochemical and is often established incidentally after hypercalcemia is detected on repeated laboratory testing, regardless of the presence of clinical symptoms suggestive of the disease. Simultaneous elevation of serum calcium and PTH levels is required for diagnosis.

The curative treatment for PHPT is surgical removal of the affected parathyroid gland or glands. Once PHPT has been diagnosed, surgery is considered in patients who meet the criteria established by European and US guidelines (9-11). Preoperative imaging to localize hyperfunctioning parathyroid glands is indicated only when surgical treatment is planned.

Traditionally, surgery consisted of standard bilateral cervical exploration (BCE), with the aim of identifying all 4 parathyroid glands. Surgical management has now evolved toward a more targeted approach, namely minimally invasive parathyroidectomy (MIP), for which precise preoperative localization plays a fundamental role. MIP reduces pain and complications, shortens operative time and hospital stay, lowers healthcare costs, and provides better cosmetic outcomes (2,6).

The combination of ^{99m}Tc-sestamibi scintigraphy and ultrasonography is currently considered a standard first-line approach for localization of parathyroid adenomas (6). However, when findings are negative, uncertain, or discordant, guidelines recommend additional imaging techniques according to local availability, including single-photon emission computed tomography/computed tomography (SPECT/CT), 4-dimensional computed tomography (4D-CT), or magnetic resonance imaging (4D-MRI) (9,10). More recently, PET/CT with ^{18}F-fluorocholine (^{18}F-FCH PET/CT) has been increasingly used for localization of parathyroid adenomas.

Ectopic localization of a parathyroid adenoma in the submandibular triangle is uncommon and represents a diagnostic and surgical challenge (12). Despite its complexity, adequate preoperative radiologic localization and intraoperative tools such as intraoperative PTH (ioPTH) monitoring can enable effective MIP even in these atypical ectopic cases. We present the case of a patient with persistent PHPT after previous BCE, without postoperative normalization of calcium or PTH levels, in whom an occult ectopic adenoma was suspected.

MATERIAL AND METHODS

STUDY DESIGN

We conducted a narrative literature review to analyze the available scientific evidence on ectopic parathyroid adenomas in the submandibular region.

INFORMATION SOURCES

The literature search was performed in PubMed/MEDLINE. Articles published up to July 2025 were included.

SELECTION CRITERIA

Included: Original articles, reviews, meta-analyses, and case reports.

Excluded: Articles for which the full text was unavailable and studies not relevant to the topic.

SEARCH STRATEGY

The following MeSH terms and keywords were used: primary hyperparathyroidism; submandibular ectopic parathyroid adenoma; ultrasound; ^{99m}Tc-sestamibi scintigraphy; SPECT/CT; and ^{18}F-fluorocholine PET/CT. The Boolean operators AND and OR were used to combine search terms.

DATA EXTRACTION

The following data were collected from each article:
- Article title, first author, city, year of publication, and reference.
- Study design and sample size.
- Main results relevant to the topic, including number of surgical procedures, preoperative imaging, surgical technique, time to case resolution, complications, and conclusions.

DATA SYNTHESIS

The extracted data were organized descriptively in tabular form to summarize and compare the findings of the different studies (Table I).

Table I. Literature review of ectopic parathyroid adenomas in the submandibular region

Title	Author, year, city/country	*n*	Design	Imaging	No. of surgical procedures/ technique	Location	Complications	Time to resolution	Conclusion
SPECT and subtraction imaging of an ectopic parathyroid adenoma (23)	LS Serchuk, 1997, United States	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT and subtraction imaging	2 (BCE)	In relation to the right submandibular salivary gland	-	-	SPECT localized the ectopic parathyroid adenoma in relation to the right submandibular salivary gland, whereas subtraction imaging confirmed that the focus represented a parathyroid lesion rather than an anatomical variant of a normal salivary gland
Localization of abnormal parathyroid tissue with use of technetium-99m-sestamibi (14)	VG Calcutt, 1997, United States	24 (1 submandibular)	Case series	^{99m}Tc-sestamibi	3 (BCE)	Medial to the right carotid artery, below the angle of the mandible	No	-	Precise localization of an adenoma facilitates surgical exploration. ^{99m}Tc-sestamibi has become a preferred noninvasive method for localizing ectopic parathyroid glands
Appearance of ectopic undescended inferior parathyroid adenomas on technetium-99m sestamibi scintigraphy: a lesson from reoperative parathyroidectomy (6)	D Axelrod, 2003, United States	3 (3 submandibular)	Case series	^{99m}Tc-sestamibi	3 (BCE + thymectomy + subtotal thyroidectomy)	In the left carotid sheath, inferior to the angle of the mandible	-	-	Attention to the contour of radioactivity in the region of the submandibular salivary gland may alert surgeons to the presence of an undescended inferior adenoma and facilitate selective surgery
65-year-old female patient with persistent hypercalcemia (21)	Wiedmann, 2007, Germany	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT + CT + US	2 (BCE + thyroidectomy)	Left submandibular region	Transient hypocalcemia	—	If an adenoma is not identified during exploratory surgery, an ectopic location should be considered; surgery may be discontinued and further localization studies performed before reoperation
Para-hyoid ectopic parathyroid adenoma localized by ^{99m}Tc-MIBI SPECT (22)	Rajagopalan, 2008, United States	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT + CT + US	2 (BCE)	Posteroinferior parahyoid adenoma of the right submandibular gland	-	2 years	Attention should be paid to an uptake focus in the "submandibular tail," which may indicate an ectopic parathyroid adenoma in the submandibular region
Adenomas of cervical maldescended parathyroid glands: pearls and pitfalls (2)	James C Lee, 2012, Australia	5241 (1 submandibular)	Case series	^{99m}Tc-sestamibi + US	1 (MIP)	Submandibular triangle	-	-	High surgical success rates can be achieved with MIP when knowledge of embryology is combined with reliable preoperative localization
Ectopic undescended parathyroid adenoma-SPECT/CT avoids false-negative interpretation on ^{99m}Tc-MIBI dual-phase scintigraphy (4)	S Mahajan, 2018, United States	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT	-	Ectopic adenoma inferior to the left submandibular gland	-	-	Ectopic adenomas adjacent to the submandibular gland may be overlooked on scintigraphy because of overlapping salivary gland activity. This case demonstrates the potential advantage of SPECT/CT over sestamibi scintigraphy alone

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Table I (cont). Literature review of ectopic parathyroid adenomas in the submandibular region

Title	Author, year, city/country	*n*	Design	Imaging	No. of surgical procedures/ technique	Location	Complications	Time to resolution	Conclusion
Ectopic parathyroid adenoma in the submandibular region: a case report (24)	Y Kong, 2019, China	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT	1 (MIP)	Posteromedial to the left submandibular gland	No	-	Ectopic parathyroid adenoma should be considered in the differential diagnosis of tumors of the submandibular region
Acute hyperparathyroid crisis: ectopic submandibular parathyroid gland the culprit (13)	M Unais, 2020, India	1 (1 submandibular)	Case report	US + CT	2 (MIP)	Right submandibular region	No	1 week	Precise localization of the adenoma is essential, particularly in life-threatening situations such as hyperparathyroid crisis. Imaging has limitations, and further surgical exploration may be necessary
Ectopic submandibular parathyroid adenoma by ^{99m}Tc-sestamibi SPECT/CT localization (1)	Fung Him Ng, 2020, China	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT	-	Inferior to the right submandibular gland	-	-	Ectopic adenomas require a multimodal approach to operative planning. SPECT/CT provides useful anatomical information
^{99m}Tc-MIBI SPECT/ CT-negative ectopic parathyroid adenoma in the submandibular region: a case report (18)	Y Lai, 2023, China	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/ CT + US + ^{11}C-choline PET/CT	2 (BCE + right hemithyroidectomy)	Submandibular region, posterior to the right submandibular gland	No	6 years	Ectopic adenoma in the submandibular region is exceptionally rare. When conventional imaging is negative, clinical suspicion should be maintained, and ^{11}C-choline PET/CT may accurately localize the lesion and facilitate successful surgery
Submandibular ectopic parathyroid adenoma: a case report (26)	JA H Tawil, 2023, Colombia	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT + US + ^{18}F-FCH PET/CT	1 (MIP)	Right submandibular region	No	6 months	Surgical management of PHPT reduces morbidity and mortality. Ectopic locations should be considered. ^{18}F-FCH PET/CT can precisely localize the adenoma and facilitate successful surgery
Multimodality evaluation of persistent hyperparathyroidism in a rare case of ectopic submandibular parathyroid adenoma (25)	Edamadaka, 2024, India	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT + US + 4D-CT	1 (MIP)	Submandibular region	-	-	^{99m}Tc-MIBI SPECT/CT and 4D-CT may improve preoperative detection of parathyroid adenomas in rare ectopic locations, as illustrated by this case of persistent hyperparathyroidism
Management of primary hyperparathyroidism with rare localization of ectopic parathyroid adenoma (7)	Aboisheva, 2024, Russia	1 (1 submandibular)	Case report	^{99m}Tc-sestamibi + SPECT/CT + US + CT	Conservative therapy (high surgical risk)	Posterior to the internal jugular vein, adjacent to the right submandibular gland	No	10 years	Ectopic adenomas complicate topographic diagnosis and may require advanced imaging methods. Management should be individualized according to lesion location and patient comorbidities

4D-CT, four-dimensional computed tomography; BCE, bilateral cervical exploration; CT, computed tomography; ^{18}F-FCH, ^{18}F-fluorocholine; MIP, minimally invasive parathyroidectomy; PET, positron emission tomography; PHPT, primary hyperparathyroidism; SPECT, single-photon emission computed tomography; SPECT/CT, single-photon emission computed tomography/computed tomography; US, ultrasonography.

CASE REPORT

A 42-year-old White woman presented to her primary care physician with dizziness and generalized weakness of 3 days' duration. Her past medical history included hypertension treated with hydrochlorothiazide. Physical examination findings and vital signs were unremarkable. Laboratory testing revealed hypercalcemia, with a protein-corrected calcium level of 10.3 mg/dL.

Further evaluation showed phosphorus, 2.5 mg/dL; PTH, 156 pg/mL; vitamin D, 43 ng/mL; and thyroid-stimulating hormone, 1.6 µIU/mL. A 24-hour urine collection showed hypercalciuria (486 mg/24 h). The patient was referred to the Department of Endocrinology.

Targeted history taking confirmed symptoms compatible with PHPT, including arthralgia, asthenia, and dizziness. The patient had no history of pathological fractures but reported 2 episodes of nephrolithiasis 2 years earlier. Abdominal ultrasonography and radiography ruled out current nephrolithiasis. Bone densitometry showed lumbar and femoral *z*-scores within the normal range for her age and sex.

INVESTIGATIONS AND DIFFERENTIAL DIAGNOSIS

The patient's evaluation began after the incidental detection of hypercalcemia. The differential diagnosis included PHPT, malignancy-associated hypercalcemia, vitamin D intoxication, chronic kidney disease, medications such as thiazides and lithium, familial hypocalciuric hypercalcemia, and granulomatous diseases such as sarcoidosis and tuberculosis (11).

After correction of concomitant vitamin D deficiency with monthly calcifediol and discontinuation of hydrochlorothiazide, repeated laboratory testing showed persistent abnormalities: calcium, 10.4 mg/dL; PTH, 128 pg/mL; and urinary calcium, 594 mg/24 h. These findings confirmed PHPT after secondary causes had been excluded.

The patient met European and US criteria for surgery because of her age and persistent hypercalcemia (9-11). Surgical treatment was proposed and accepted. Initial imaging with cervical ultrasonography and ^{99m}Tc-sestamibi SPECT/CT was negative. Endoscopic ultrasonography identified a possible 4-mm left inferior parathyroid adenoma adjacent to the vascular bundle.

The patient underwent bilateral neck exploration. The left side was explored first without identification of a lesion. Exploration then proceeded to the right side, where a 2-cm nodule was excised and sent for histopathological examination. Intraoperative PTH monitoring showed no decrease (baseline PTH, 135 pg/mL; ioPTH, 165 pg/mL; percentage decrease, –1.85 %), prompting extended exploration of the retroesophageal, thymic, thyroid capsular, and vascular compartments without further findings. Because a mediastinal ectopic adenoma was suspected, the procedure was concluded. Postoperative calcium remained elevated at 10.7 mg/dL. Histopathological examination identified the excised tissue as a reactive lymph node.

The patient was followed for 4 years. Laboratory abnormalities persisted: calcium, 11.2 mg/dL; PTH, 197 pg/mL; and urinary calcium, 172 mg/24 h. Repeated imaging studies remained negative. During this period, the patient experienced a new and more severe episode of renal colic. Abdominal ultrasonography confirmed a 2-mm calculus in the lower calyceal group of the left kidney.

Given the need for definitive treatment, a newer preoperative imaging modality that had recently been introduced in parathyroid surgery, ^{18}F-FCH PET/CT, was performed. This study identified a hypermetabolic lesion measuring 6 × 6 × 14 mm at left cervical level IIA in the submandibular region, adjacent to the submandibular gland (Fig. 1).

In the clinical context of this patient, visualization of a hypermetabolic focus in the submandibular region suggested an ectopic parathyroid adenoma. The differential diagnosis included reactive lymphadenopathy, salivary gland tumors, and superior thyroid lesions. These alternatives were excluded by fine-needle aspiration and PTH measurement in the aspirate, supporting the diagnosis of an ectopic parathyroid adenoma.

TREATMENT

Revision surgery was performed. Intraoperatively, a brownish nodule located posterior and caudal to the left submandibular gland and corresponding to the imaging findings was excised. ioPTH monitoring showed a > 50 % decrease (baseline PTH, 225 pg/mL; ioPTH, 87.6 pg/mL). Histopathological examination confirmed a 17-mm, 0.7-g parathyroid adenoma.

OUTCOME AND FOLLOW-UP

At 2 years postoperatively, the patient remains under follow-up. Her clinical symptoms have improved, with no further episodes of nephrolithiasis. Calcium and urinary calcium levels are within the normal range (8.5 mg/dL and 138 mg/24 h, respectively).

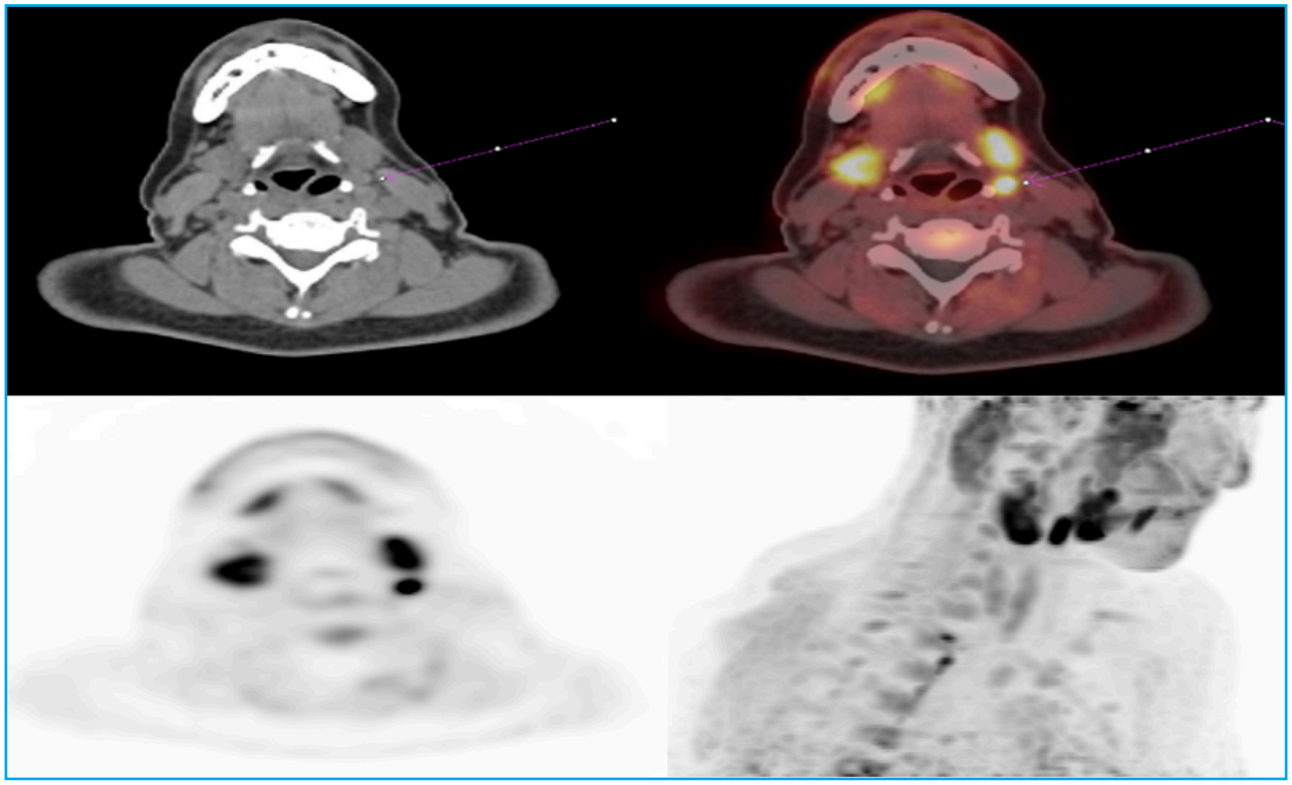

Figure 1. An elongated nodular lesion measuring approximately 6 mm × 6 mm × 14 mm (AP × T × CC) is identified in the left submandibular region at level IIA. The lesion is located posterior and inferior to the left submandibular gland, anterior to the vascular bundle, and medial to the sternocleidomastoid muscle. It appears hyperintense in the arterial phase, with intense ^{18}F-choline uptake, suggestive of an ectopic parathyroid adenoma. No other lesions showing radiotracer uptake are identified. Physiological tracer uptake is observed in the bone marrow, salivary glands, and thyroid gland. SUVmax values were 20.9 immediately after injection, 15.8 at 20 minutes, and 15.9 at 60 minutes.

DISCUSSION

The parathyroid glands usually consist of 4 small oval structures located on the posterior surface of the thyroid gland. However, their position may vary, making localization difficult in some patients, as occurred in the present case. Ectopic localization has been reported in up to 20 % of cases (2-4). The most frequent ectopic locations include the retroesophageal, thymic, carotid, mediastinal, and intrathyroidal regions, whereas the submandibular region is among the rarest sites (2,4,7,8).

This atypical high cervical or submandibular location (< 1 %) can be explained by abnormalities in embryological migration of the parathyroid glands. Such lesions are often referred to as high cervical or undescended parathyroid adenomas and may resemble lymph nodes or salivary gland lesions (2,4,12). Lee et al. reported 16 high cervical adenomas among 5241 patients (0.3 %), only 1 of which was located in the submandibular region. Fourteen occurred during primary parathyroidectomy and 2 were identified at reoperation. In this group, scintigraphy had a sensitivity of 67 %, and 9 patients required BCE (2).

Treatment of PHPT is surgical, and resection of the hyperfunctioning parathyroid gland or glands is curative. Reoperation is relatively common in patients with ectopic adenomas after failed initial surgery (2,13). Axelrod et al. reported 3 ectopic submandibular adenomas identified only during revision surgery (6). Calcutt et al. described a case requiring 3 surgical procedures before the submandibular adenoma was identified (14). Accurate preoperative localization is therefore crucial to minimize unnecessary surgical exploration. Aboisheva et al. reported the case of an 84-year-old woman who underwent years of conservative medical management without normalization of calcium levels because the pathological focus could not be identified (7).

Conventional imaging modalities, including scintigraphy and ultrasonography, have reported sensitivity and specificity rates of approximately 70 %-80 % for adenoma detection (3-5,15-18). In the 20 %-30 % of patients with negative findings, more advanced imaging techniques may be required. ^{18}F-FCH PET/CT combines functional and anatomical imaging and provides greater spatial resolution with shorter acqui-

sition times and potentially lower radiation exposure. Despite its greater cost and limited availability, it has shown promising diagnostic performance. A 2019 meta-analysis reported a sensitivity of 95 %, positive predictive value of 97 %, and detection rate of 91 % for ^{18}F-FCH PET/CT (19), potentially avoiding unnecessary bilateral exploration in 75 %-87 % of cases (20).

The present case illustrates a rare ectopic submandibular adenoma diagnosed after years of persistent PHPT and unsuccessful surgery. Conventional imaging was nondiagnostic, whereas ^{18}F-FCH PET/CT provided precise localization and enabled curative surgery.

Few cases of parathyroid adenoma in the submandibular region have been published. Our literature review identified 14 articles indexed in PubMed, the earliest dating from 1997 (Table I). In most reported cases, the adenoma was detected using conventional imaging or SPECT/CT (1,4,21-26). Only 2 published cases described identification of a submandibular adenoma using PET/CT: 1 with an ^{11}C-choline tracer (18) and another with ^{18}F-choline (26). We report an additional case of a submandibular parathyroid adenoma localized using ^{18}F-FCH PET/CT after neither ^{99m}Tc-sestamibi scintigraphy with SPECT/CT nor cervical ultrasonography was able to detect the lesion.

Identification of an ectopic submandibular adenoma represents an exceptionally uncommon anatomical presentation and can pose a substantial challenge for the head and neck surgeon. Many of these patients have already undergone unsuccessful bilateral exploratory surgery and have persistent hyperparathyroidism, requiring revision surgery and thereby increasing the risk of postoperative complications. More accurate imaging modalities are therefore needed for preoperative localization to enable targeted resection, even in atypical ectopic cases such as the present one.

The use of ^{18}F-FCH PET/CT for preoperative localization in the surgical management of PHPT represents a significant advance in patient safety and clinical care. Its greater sensitivity and diagnostic accuracy allow more targeted and less invasive surgical planning. This may result in shorter operative times, a lower risk of complications such as recurrent laryngeal nerve injury and hypocalcemia, and faster recovery. Moreover, by increasing the likelihood of successful initial surgery, it may reduce the need for reoperation and thereby improve clinical outcomes and patients' quality of life.

CONCLUSIONS

The present case emphasizes the importance of surgical management of PHPT to reduce associated morbidity and potential complications. Rigorous clinical, biochemical, and radiological correlation is essential, together with the surgeon's awareness of the different embryological migration patterns of the parathyroid glands and their possible anatomical variants. This case also highlights the value of advanced preoperative imaging techniques in optimizing surgical outcomes, particularly in the context of minimally invasive approaches. ^{18}F-FCH PET/CT is a promising modality for preoperative localization of ectopic parathyroid adenomas. In the present case, it enabled accurate localization and curative surgical treatment after conventional imaging modalities had failed.

Finally, multidisciplinary clinical committees bringing together specialists from different fields, including endocrinology, otorhinolaryngology, radiology, and nuclear medicine, are important for the discussion and management of complex clinical cases.

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Case Report

Sacral insufficiency fractures: a forgotten diagnosis in low back pain in older adults

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Abstract

Introduction: sacral insufficiency fractures represent an underrecognized cause of low back pain in patients with risk factors for bone fragility. Their insidious clinical presentation, absence of prior trauma, and the low sensitivity of plain radiographs make diagnosis difficult.

Case report: we present the case of a 61-year-old woman with premature menopause and a history of anorexia nervosa, who presented with progressive low back pain without prior trauma. She was diagnosed by computed tomography (CT), magnetic resonance imaging (MRI), and bone scintigraphy, and was treated conservatively, including osteoanabolic treatment for osteoporosis, with good clinical outcome.

Discussion: this case illustrates the need to include this entity in the differential diagnosis of persistent low back pain and to consider more effective therapeutic options, such as fixation by osteosynthesis or sacroplasty, in refractory cases.

Keywords:
Osteoporosis.
Sacral fracture.
Bone insufficiency.
Low back pain.
Sacroplasty.

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INTRODUCTION

Sacral insufficiency fractures, included among fragility fractures, predominantly affect patients with osteoporosis or predisposing factors such as premature menopause, corticosteroid therapy, or nutritional disorders (1). Their nonspecific clinical presentation, without an evident traumatic history, causes these fractures to go unnoticed in the initial stages. Delayed diagnosis compromises functional prognosis; therefore, maintaining a high index of suspicion and using appropriate diagnostic tools are essential (2).

CASE REPORT

A 61-year-old woman with a past medical history of premature menopause (41 years), anorexia nervosa (BMI 17.6), and psychoaffective disorder treated with subcutaneous aripiprazole was evaluated. She had no previous fractures.

She presented with a 2-week history of bilateral sacroiliac radicular pain radiating to the left leg, exacerbated by standing, movement, and weight-bearing, developing urinary retention and distal weakness with left foot drop while in the emergency department.

There was no history of falls or significant exertion. Symptoms progressed to severe functional limitation, without response to conventional analgesia.

Physical examination revealed pain on lumbosacral and sacroiliac joint palpation, with absent Achilles reflex in the left lower limb, without superficial or deep sensory abnormalities, and preserved strength except for slight reduction in hip flexion-extension and knee flexion due to poor cooperation due to pain in the lower back. The remaining reflexes were normal. Plantar cutaneous reflex was flexor on the right and indifferent on the left.

Plain radiographs were unremarkable. Computed tomography (CT) revealed a fracture in the left sacral ala (Figs. 1 and 2), and magnetic resonance imaging (MRI) of the spine was requested (Fig. 3), showing bilateral sacral ala insufficiency fractures with anterior angulation and apparent S2-S3 foraminal involvement with associated bone edema, without evidence of myelopathy (type IVb fracture according to the Rommens-Hofmann classification). Bone scintigraphy demonstrated the typical "H" uptake pattern of this entity (Fig. 4).

The diagnosis of osteoporosis was confirmed by bone mineral density (BMD) measurement using densitometry: lumbar spine 0.554 g/cm^2 (T-score -4.5); femoral neck 0.385 g/cm^2 (T-score -4.2); and total femur 0.391 g/cm^2 (T-score -4.5).

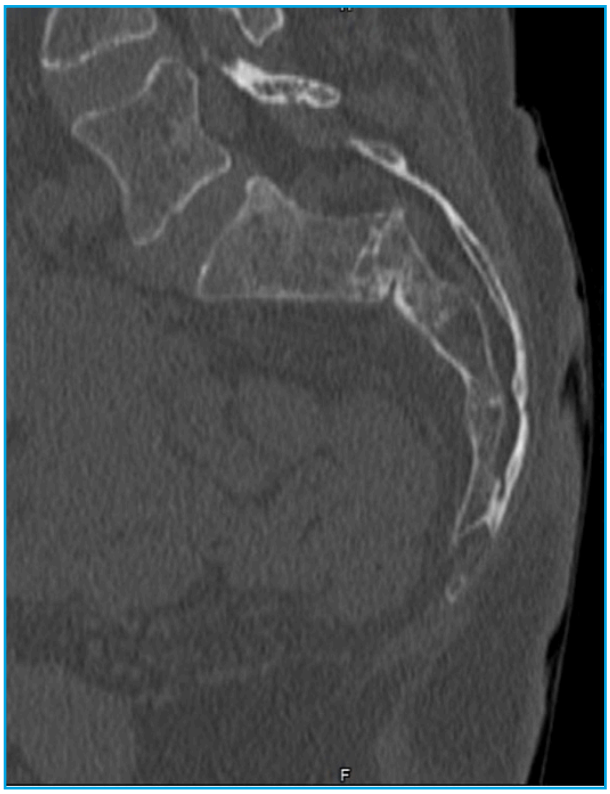

Figure 1. CT images in a sagittal plane.

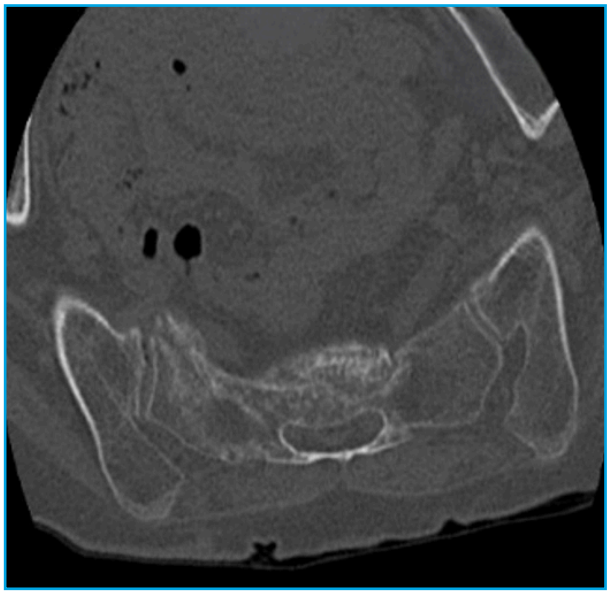

Figure 2. CT images in an oblique coronal plane.

Surgery was ruled out by the Orthopedic Surgery Department, in agreement with the patient and her family, taking into consideration the patient's frailty. Relative rest and adjustment of analgesic therapy were recommended. Osteoporosis treatment with an

osteoanabolic agent (daily subcutaneous teriparatide) was initiated to facilitate consolidation, together with prophylactic anticoagulation to prevent thrombotic complications.

During follow-up, the patient showed good pain control, with CT at 1 month demonstrating a more sclerotic fracture line appearance as a sign of consolidation. Initially, active rehabilitation exercises were not started to avoid displacement; however, she is currently undergoing rehabilitation with good functional recovery and adequate pain control, and therefore no interventional or surgical treatment is currently considered necessary. At the 18-month follow-up, the patient showed marked clinical improvement, was pain-free, and had fully recovered mobility, with no functional limitation for activities of daily living. Densitometry also improved, with a significant increase in BMD in the lumbar spine (0.792 g/cm^2, T-score -3.3); femoral neck (0.550 g/cm^2, T-score -3.6); and total femur (0.513 g/cm^2, T-score -4.1).

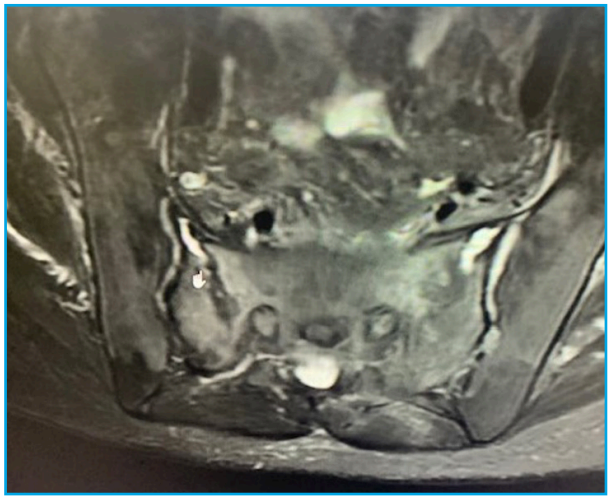

Figure 3. MRI image in a T2 fat-suppressed sequence.

DISCUSSION

Chronic and progressive low back pain, in the absence of a clear traumatic history, should raise suspicion for sacral insufficiency fractures, especially in patients with predisposing factors such as advanced osteoporosis, osteopenia, anorexia, premature menopause, prolonged corticosteroid treatment, or rheumatologic diseases such as rheumatoid arthritis.[1,2] Due to the increase in the elderly population, with a higher prevalence of osteoporosis, sacral insufficiency fractures may increase in incidence. However, this condition continues to be underdiagnosed because the clinical presentation is usually nonspecific and physical examination does not provide pathognomonic findings (3,4).

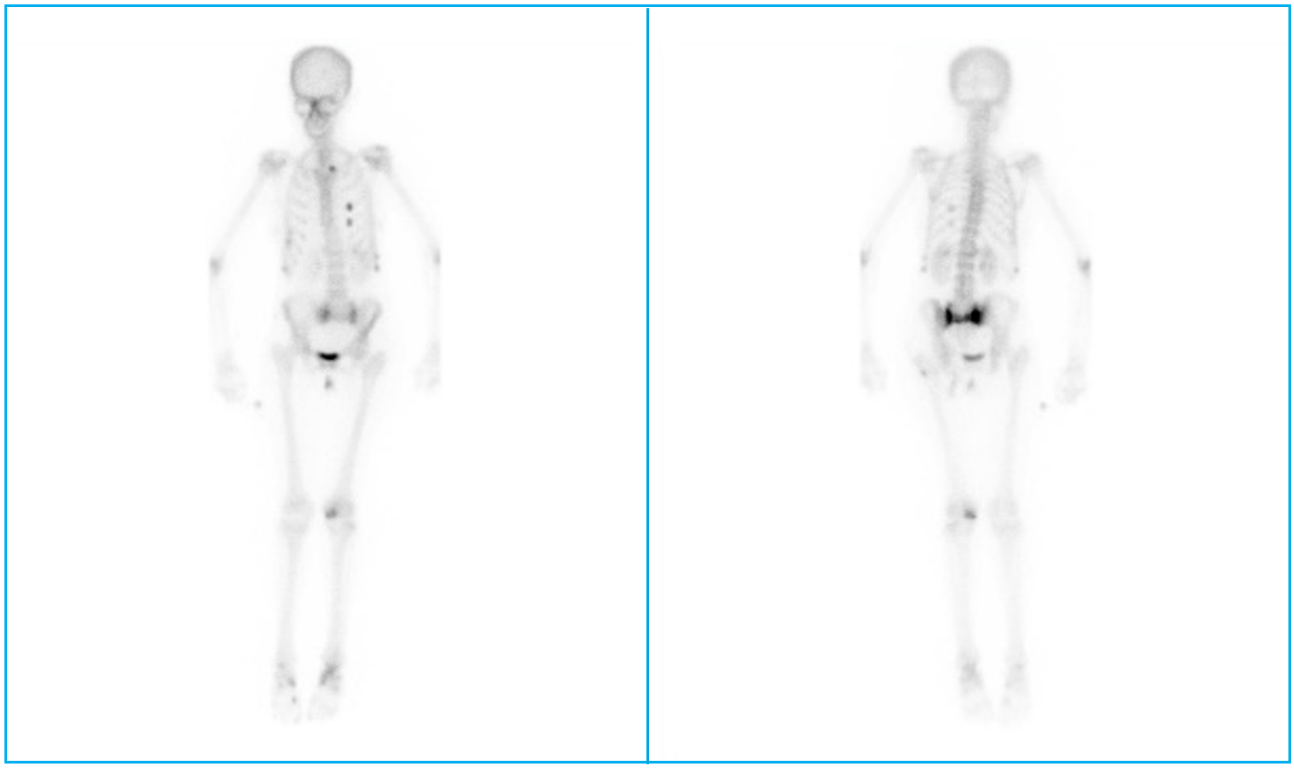

Figure 4. Bone scintigraphy image.

In the initial stages, plain radiography has limited diagnostic yield, since it frequently does not allow visualization of the fracture line. Bone scintigraphy constitutes a highly sensitive although poorly specific tool, with the characteristic "H" or "H-sign" finding, secondary to bilateral uptake in the sacral alae with transverse involvement of the body (1,4). Nevertheless, the imaging modality of choice is MRI, which allows early identification of bone edema and precise delineation of the fracture line, making it the diagnostic gold standard (3). Computed tomography, in turn, provides excellent detail of bone morphology and can confirm the presence of cortical fractures (2).

Regarding the therapeutic approach, conservative treatment—based on relative rest, multimodal analgesia, and early rehabilitation—may be effective in stable cases without neurological complications.

In cases of refractory pain, marked functional limitation, spinopelvic dissociation, neurological involvement, or high risk of prolonged immobility, surgical treatment with osteosynthesis fixation with or without neurological decompression should be considered, with the aim of improving sacral stabilization and neurological symptoms, thereby facilitating early mobilization. However, it is important to consider whether the patient's general condition and bone fragility allow such intervention, since the anatomy of the sacrum itself and the fracture lines in patients with osteoporotic bone make fixation of osteosynthesis material difficult, resulting in mechanical failure of implants, in addition to the risk of local complications such as infections or iatrogenic nerve injuries. Therefore, some authors advocate conservative treatment in this type of fracture except in cases of neurological compromise or spinal instability (6-8).

On the other hand, sacroplasty (percutaneous injection of polymethylmethacrylate into the fracture site, guided by fluoroscopy or CT) has proven to be a safe and effective therapeutic alternative (6,9). Various studies and meta-analyses have documented significant pain improvement (reduction in the VAS scale), accompanied by earlier functional recovery and a lower complication rate compared with conservative management (4,9,10). However, in our case it would not be indicated because of involvement of the neural foramina.

Possible complications of sacroplasty include leakage of bone cement into the sacral foramina or soft tissues and, less frequently, embolization phenomena. Nevertheless, its incidence is low, and most reports agree that it is a procedure with a favorable safety profile (6-8).

In this context, surgical osteosynthesis and sacroplasty should be considered therapeutic tools of increasing importance, particularly in patients with disabling pain, neurological involvement, and poor response to conservative treatment (11).

With the progressive increase in life expectancy and the higher prevalence of osteoporosis in the general population, it is foreseeable that the incidence of these fractures will continue to rise, making it essential to reinforce clinical suspicion, optimize early diagnosis using advanced imaging techniques, and appropriately select the most beneficial therapeutic strategy for each patient (1-12).

CONCLUSIONS

Sacral insufficiency fractures should be included in the differential diagnosis of persistent low back pain in patients with osteoporosis or risk factors for fragility fractures.

Diagnosis requires advanced imaging, with MRI being the gold standard. Surgical osteosynthesis or interventional sacroplasty are valid options in cases refractory to conservative treatment or with neurological compromise.

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