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IS PERIOSTIN USEFUL AS A BIOMARKER FOR FIBROUS DYSPLASIA?

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Abstract

Fibrous dysplasia (FD) is an infrequent non-hereditary bone disease caused by a somatic mutation of the GNAS gene. Periostin is a novel marker that increases during tissue healing and fibrous or inflammatory diseases. We conducted an exploratory case-control study to evaluate sensitivity of periostin as a biomarker of FD. The study comprised 15 patients with FD, and healthy age- and sex-matched subjects (controls). Serum periostin levels were assessed and comparisons were established between FD patients and controls, and between patients with the monostotic and the polyostotic form of FD. No statistically significant differences in serum periostin levels were observed between the cohort of FD patients studied here and the control group (FD: 51.1±10ng/ml vs. control: 44.2±15ng/ml; p=0.15), or between the clinical forms of FD (polyostotic: 51.8±9.1ng/ml vs. monostotic: 49.6±13 ng/ml; p=0.66). A sub-analysis performed to compare serum levels of periostin in FD patients with and without a history of fractures showed no statistically significant differences [fracture patients (n=4): 41.2±17ng/ml vs. non-fracture patients (n=11): 49.9±11 ng/ml; p=0.47].Lastly, sensitivity of periostin as a biomarker of FD was analyzed, and was found to have low sensitivity to estimate disease activity [ROC curve; cut-off points: 39.625(0.867-0.467)]. To conclude, in the cohort of FD patients studied here, periostin serum levels did not differ significantly from those of the control group or between the two forms of the disease, and showed low sensitivity as a biomarker of the disease.

Key words: Fibrous dysplasia of bone, McCune-Albright, periostin.

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Resumen

¿ES ÚTIL PERIOSTINA COMO BIOMARCADOR DE DISPLASIA FIBROSA?

La displasia fibrosa (DF) es una enfermedad infrecuente del hueso, no hereditaria producida por una mutación somática del gen GNAS. Periostina (Postn) es un novedoso marcador, cuyos niveles séricos se encuentran elevados en los procesos de reparación tisular, enfermedades fibrosas o inflamatorias. Llevamos a cabo un estudio exploratorio caso-control para evaluar la sensibilidad de Postn como biomarcador de DF. Se incluyeron en el estudio 15 pacientes con DF apareados por edad y género con sujetos sanos (controles) en los cuales se evaluó los niveles séricos de Postn en pacientes con DF y controles y según forma de presentación clínica. No observamos diferencias estadísticamente significativas en los niveles séricos de Postn y el grupo control (DF: 51.1±10ng/ml vs. control: 44.2±15ng/ml; p=0.15) como así tampoco por forma clínica de DF (poliostótica: 51.8±9.1ng/ml vs. monostótica: 49.6±13 ng/ml; p=0.66). Posteriormente realizamos un sub-análisis para evaluar los niveles séricos de Postn en los pacientes con DF y antecedentes de fracturas no observando diferencias estadísticamente significativas [*fracturados (n=4): 41.2±17ng/ml vs. no fracturados (n=11): 49.9±11 ng/ml; p=0.47*]*. Por último analizamos la sensibilidad Postn como biomarcador de DF, mostrando este poseer escasa sensibilidad para estimar actividad de la enfermedad* [*curva ROC; puntos de corte: 39.625 (0.867-0.467)*]*. En conclusión, los niveles séricos de Postn en nuestra cohorte de pacientes con DF no mostraron diferencias estadísticamente significativas comparadas con el grupo control o por forma clínica de presentación, mostrando una baja sensibilidad como biomarcador de enfermedad.*

Palabras clave: *Displasia fibrosa del hueso, McCune-Albright, periostina.*

Introduction

Fibrous dysplasia (FD) is an infrequent non-hereditary bone disease caused by a somatic mutation of the GNAS gene, which is responsible for encoding for the α subunit of the stimulatory G-protein ($G_s\alpha$).[1] The clinical presentation of the disease varies greatly, with some patients being asymptomatic and others markedly symptomatic. Fibrous dysplasia can affect one bone (monostotic form) or multiple bones (polyostotic form), or can be associated with other conditions such as McCune-Albright syndrome (MAS), which is characterized by the combination of bone lesions, hyperfunctioning endocrinopathy (particularly precocious puberty, excess of growth hormone, or hyperthyroidism), and café-au-lait spots, or Mazabraud syndrome, a rare disorder characterized by the association of FD and intramuscular myxomas.[2]

Traditional bone turnover markers (BTMs) have been used in clinical practice as a surrogate marker of disease activity in FD.[3,4] Although it has recently been reported that BTMs progressively and persistently decline with age in patients with FD, a large number of FD patients have shown persistently BTM level higher than the normal range.[5] This interesting finding on the natural history of the disease poses the challenge of identifying a biomarker for the disease that reflects the intrinsic biological activity of the dysplastic bone lesions, and that, either alone or combined with other factors, allows estimating disease progression and prognosis.

Kashima *et al.* observed over expression of periostin in the fibrous component of dysplastic lesions in samples of bone tissue from FD patients.[6] Hence, it was hypothesized that periostin could be a potential marker

for disease activity in FD. In their study in a cohort of patients with FD, Guerin Lemaire *et al*. found serum periostin levels to be higher in FD patients than in controls, mainly in patients with a history of fracture, polyostotic forms, or MAS.[7]

The aim of the present study was to explore the sensitivity of periostin as a marker of FD activity in FD patients and in each of the forms of the disease separately, in a cohort of patients with FD.

Materials and Methods

Study design and population

An exploratory case-control study was conducted at the Laboratory of Osteoporosis and Bone Metabolic Diseases, Clinical Hospital, University of Buenos Aires, and the Mautalen Health and Research Center, both referral centers for FD, from August 2018 to June 2019. Patient recruitment was performed during follow-up medical visits to the Mautalen Health and Research Center. Only patients diagnosed with FD who had attained their peak bone mass (≥30 years) were included; patients presenting any of the following conditions were excluded: 1. presence of a fracture in the healing phase; 2. a history of cancer with or without bone involvement; 3. myocardial infarction within 12 months prior to the study; and 4. presence of mineral and bone metabolism disorders other than FD.

The study was approved by the independent ethics committee of the Metabolic Bone Diseases Center, and by the clinical research ethics committee of the Science and Technology Secretariat, School of Pharmacy and Biochemistry, University of Buenos Aires. The patients and healthy voluntary subjects (controls) were enrolled after signing the informed consent form. The following data were obtained from the clinical records of the FD patients: age at diagnosis, number of bones affected by the disease, clinical form, and treatments. The present study comprised a total of 30 subjects (15 patients with FD, and 15 controls).

Biochemical Determinations

Blood samples were collected from patients and controls at the Laboratory between 8 and 9:30 a.m. after 12-hour fasting. The samples were processed and fractioned into two aliquots, which were stored at -20°C and -80°C respectively.

The serum aliquot stored at -20°C was used to perform the following bone mineral metabolism determinations: serum calcium (Colorimetric (Arsenazo III), serum phosphorus [UV Endpoint (Ammonium Molybdate), 25-hydroxyvitamin D (25OHD) [Electrochemiluminescence (ECLIA)], intact parathyroid hormone (iPTH) [Electrochemiluminescence (ECLIA)], a bone resorption marker [serum crosslaps (sCTX)] [Electrochemiluminescence (ECLIA)],and a bone formation marker (BAP) [precipitation with wheat-germ lectin].

The aliquot stored at -80°C was used for determination of periostin by an ELISA method (Sigma-Aldrich; St. Louis, USA), which measures each of the periostin isoforms. The intra-assay coefficient of variation percentage (%CV) is below 10%, and inter-assay coefficient of variation percentage is less than 12%.

Assessment criteria

The first analysis consisted of evaluating serum periostin levels in FD patients and healthy controls to explore potential association between the marker and the disease. The second analysis involved comparing the concentration of periostin in the different forms of the disease [monostotic vs. polyostotic (including MAS)].The third analysis was the determination of the sensitivity of periostin as a marker of the disease.

Statistical analysis

Statistical analysis was performed using an R CoreTeam 2018 processor (R: A language and environment for statistical

computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org). Values obtained in FD patients were compared to those of healthy age-sex-matched subjects (control group). Comparisons between groups were performed using a non-parametric test (Mann-Whitney). Results are expressed as mean, standard deviation, and range. ROC curves were used to establish the cut-off point in order to analyze sensitivity of periostin (predictive value) as a marker of disease activity. A p value below 0.05 was considered statistically significant.

Results

The study comprised 15 patients with FD (60% women, 40% men), 6 of whom (40%) had the polyostotic form of the disease, 5 (33%) had the monostotic form, and 4 (27%) had MAS. Average age and standard deviation (X±SD) was 44.3±10 years (range: 33-67). Only 4 patients (27%) had a history of fractures, though none had suffered a fracture within the year prior to enrollment in the study. Most patients (87%) received pamidronate as treatment for FD (180mg every 6 months), and only 2 patients (13 %) received zoledronic acid, as shown in Table 1.

Table 1. Characteristics of fibrous dysplasia patients and the control group.

	Control Group (n=15)	FD Patients (n=15)
Age (years)	44.2±10.5	44.3±10.8
Men n (%)	6 (40)	6 (40)
Women n (%)	9 (60)	9 (60)
Clinical form		
Monostotic n (%)		5 (33)
Polyostotic n (%)		6 (40)
MAS n (%)		4 (27)
Treatment		
Pamidronate n (%)		13 (87)
Zoledronic Acid n (%)		2 (13)

MAS: McCune Albright Syndrome.

Values of biochemical variables of bone mineral metabolism of FD patients and controls are shown in Table 2. Serum periostin levels were similar in FD patients and controls [FD: 51.1±10ng/ml (range: 30-70) vs. control group: 44.2±15ng/ml (range: 24-67); p=0.15).

Comparison of serum BAP, sCTX, and periostin levels between the two forms of the disease showed a statistically significant difference in BAP levels [polyostotic form/MAS: 220.0±182IU/l (range: 47-613) vs. monostotic form: 56.6±5.02IU/l (range: 48-61); p=0.05]. However, no significant differences in serum sCTX [polyostotic/MAS: 649±494ng/l (range:120-1720) vs. monostotic: 340.8±109ng/l (range:216-448); p=0.37)] or periostin levels [polyostotic/MAS: 51.8±9ng/ml (range: 34-90) vs. monostotic: 49.6±13ng/ml (range: 30-112); p=0.66] were observed between the clinical forms of the disease.

Table 2. Biochemical determinations of bone mineral metabolism assessed in fibrous dysplasia patients and controls.

	sCa (8.5-10.5mg/dl)	sP (2.6-4.7mg/dl)	25OHD (≥30ng/ml)	iPTH (15-65pg/ml)	sCTX* (reference value)	BAP (31-95IU/l)
FD(n=15)	9.5±0.2	3.5±0.3	33.2±10	49.9±14	546±428	165±166
Control (n=15)	9.4±0.2	3.5±0.5	29.2±13	48.2±14	469±178	53.4±12
p	ns	ns	ns	ns	ns	0.01

sCa: serum calcemia; Ps: serum phosphatemia; 25OHD: 25-hydroxyvitamin D; iPTH: intact parathormone; CTXs: serum crosslaps; BAP: bone alkaline phosphatase. *Postmenopausal women: 80-590ng/l; premenopausal women: 40-450ng/l; men: 14-450ng/l.

Comparison of serum periostin levels of FD patients with and without a history of fracture showed no significant differences between fracture and non-fracture patients [fracture (n=4): 41.2±17ng/ml (range: 23-63) vs. non-fracture (n=11): 49.9±11 ng/ml (range: 33-70); p=0.47], nor as compared to controls [control group (n=15):44.2±15ng/ml (range: 24-67);p=0.23].

As regards the sensitivity of periostin as a marker of FD activity, the obtained ROC curve and optimum cut-off points showed low predictive value of periostin to estimate disease activity (Figure 1).

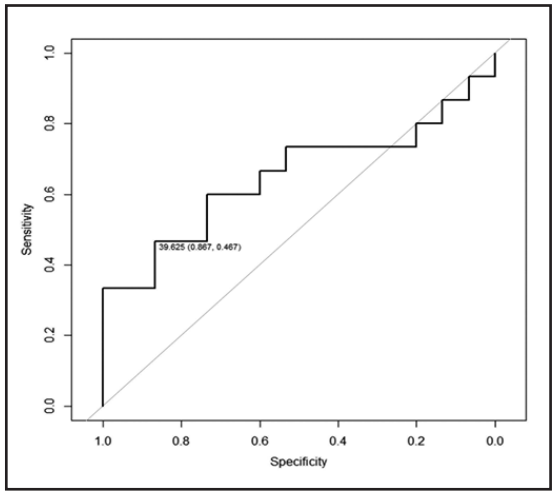

Figure 1: ROC curve: Sensitivity of periostin as a disease activity marker for fibrous dysplasia.

Discussion

At present, periostin is a novel marker associated with processes of tissue repair following injury (e.g. fracture healing, myocardial infarct, inflammatory diseases, and so forth).[8-11] However, little is known about its potential usefulness as a disease activity biomarker for a specific disease.

As to its implications in bone biology, periostin is a secreted extracellular matrix protein preferentially expressed in bone by osteocytes and periosteal osteoblasts [12]. With regard to FD, Kashima *et al.* found overexpression of periostin in the fibrous component of dysplastic lesions in bone tissue samples from patients with FD.[6] Given that periostin is a soluble factor, it can be detected in peripheral blood; hence, it could be inferred that serum periostin levels might reflect disease activity *in situ*.

However, our results showed no statistically significant increase in serum levels of periostin in the cohort of FD patients studied here as compared to healthy age and sex matched controls, and no differences between the clinical forms of the disease. The results obtained here are not biased by the bisphosphonate treatment the patients may have received prior to enrollment in the study, since periostin levels are not affected by this type of drugs.[13,14] With regard to the traditional bone resorption markers studied here, and

in line with reports in the literature, only BAP levels were significantly higher in FD patients (+68%; p=0.03) than in control subjects.

However, in their study on 64 patients with FD, Guerin Lemaire *et al.* found serum periostin levels to be higher in FD patients with a history of fracture, and in patients with the polyostotic form of the disease and MAS[5]. Of note, periostin was determined in both studies using an ELISA-like method, which equally measures all known forms of periostin with similar intra-assay and inter-assay coefficients of variation. Hence, the discrepancy between the authors' findings and the results of our study cannot be attributed to a methodological factor.

In addition, it must be taken into account that the study by Guerin Lemaire *et al*. included 3 patients who suffered a fracture within the year prior to periostin assessment. Although 3 patients account for a very small proportion of the total enrolled FD patients, their inclusion may have marginally affected results thus also contributing to the observed differences, though to a lesser degree. We found no statistically significant differences in serum periostin levels among FD patients with a history of fracture, non-fracture FD patients, and control subjects in the present study, possibly because the fractures occurred more than 3 years before periostin determination. There are reports showing that fractures can affect serum periostin levels, depending on the type of fracture and the time between fracture and periostin determination. As to type of fracture, non-vertebral fractures, but not vertebral fractures, have been found to be significantly associated with serum periostin levels. Regarding the time between fracture and periostin determination, high periostin levels can still be observed more than 1 year after the fracture event, though statistical significance is marginal.[15,16] Yet another factor to consider is that the currently available assessment methods measure all known isoforms of periostin, thus decreasing sensitivity of periostin to estimate disease activity, at least in FD.

To conclude, serum periostin levels did not differ significantly in our cohort of FD patients as compared to the control group, nor between the clinical forms of FD. Beyond the small sample size, the results obtained in our study may be affected by the limitation to the currently available immunoassays to measure the periostin isoforms of bone, decreasing its sensitivity as a disease activity marker for FD. Until assays that allow determining the bone fraction of periostin have been developed, and solely based on the data available to date, it cannot be confirmed whether periostin is or is not a reliable disease activity marker for FD.

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Conflict of interest: the authors declare that they have no conflict of interest.

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