Alpha-Klotho protein in systemic lupus erythematosus

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Abstract

Objective

Alpha-Klotho protein (α-Klotho) is an essential component of endocrine fibroblast growth factor receptor complexes that governs multiple metabolic processes including aging-related disorders, diabetes, cancer, arteriosclerosis, and chronic kidney disease. Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect almost any organ in the body and in which multiple pathophysiological abnormalities are observed. In the present work, our objective was to study whether the serum levels of α-Klotho differ between patients with SLE and controls, and how this protein is related to the clinical and laboratory characteristics of the disease.

Methods

Cross-sectional study that included 364 women, 195 of them diagnosed with SLE and 169 sex- and age-matched controls. Circulating α-Klotho was analysed in SLE patients and controls. A multivariable analysis was performed to assess whether α-Klotho differs between patients and controls, and to study its relationship with SLE features.

Results

No differences were found in α-Klotho levels between SLE patients and controls, both in univariable and multivariable analyses. Disease-related data like SLE duration, acute phase reactants, activity, severity and damage indices, and autoantibodies profile were not significantly associated with serum levels of α-Klotho. However, the use of prednisone and the presence of musculoskeletal manifestations were significantly related to higher α-Klotho serum levels.

Conclusion

α-Klotho protein serum levels do not differ between patients with SLE and controls. Nevertheless, SLE patients taking prednisone or those with musculoskeletal manifestations show significantly higher circulating levels of α-Klotho.

Key words

systemic lupus erythematosus, alpha-klotho protein

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Introduction

Alpha klotho (α-Klotho) is a membrane protein that functions as obligate co-receptor for fibroblast growth factor 23 (1). It has been recognised as an antiaging hormone (2), and acts as a humoral factor with strong anti-oxidant and anti-inflammatory effects (3). In this sense, α-Klotho induces the production of anti-inflammatory factors such as interleukin-10 (4), suppresses pro-inflammatory nuclear factor-κB activation, and decreases the expression of vascular cell adhesion and intercellular adhesion molecules (5, 6). Moreover, a deficiency in α-Klotho expression may generate renal and skeletal abnormalities, cardiovascular disease and lower response to inflammation and oxidative stress (7, 8). In this sense, several clinical studies have pointed that low serum levels of Klotho protein are associated with the prevalence and severity of cardiovascular disease and all-cause mortality, being associated with markers of vascular dysfunction and with the incidence of atherosclerosis (9). This cardioprotective effect is based on the fact that Klotho ameliorates vascular endothelial dysfunction, increases nitric oxide production, reduces elevated blood pressure, and prevents medial hypertrophy and perivascular fibrosis (10). Regarding the musculoskeletal system, Klotho deficiency has been associated with depleted numbers of osteoblasts, decreased alkaline phosphatase activities, potentially weakened bone formation, and a large non-mineral region in trabeculae and metaphysis of bone (11). Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown cause that can affect virtually any organ of the body. The aetiology of SLE remains unknown and is clearly multifactorial leading to many clinical symptoms and different pathophysiological abnormalities. Many observations suggest a role for genetic, hormonal, immunologic, and environmental factors. For all the aforementioned concepts, studies are warranted to investigate new pathophysiological metabolic pathways or biomarkers involved in this disease.

In the present work, our objective was to study whether circulating α-Klotho differs between SLE patients and matched controls. Besides, since α-Klotho is a pleiotropic molecule involved in several physiological processes, we set out to analyse possible links between this protein and different characteristics of the disease.

Material and methods

Study participants

This was a cross-sectional study that included 364 women, 195 of them diagnosed with SLE and 169 sex- and age-matched controls. All the patients were 18 years old or older, had a clinical diagnosis of SLE, and fulfilled ≥4 American College of Rheumatology (ACR) classification criteria for SLE (12). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. Controls were community-based, recruited by general practitioners in primary care centers. In addition, controls with a history of some inflammatory rheumatic disease were excluded. None of the controls were receiving glucocorticoids. However, as they are often used in the treatment of SLE, patients taking prednisone or an equivalent dose ≤10 mg/day were not excluded. Patients and controls were excluded if they had a history of cancer, or any other chronic disease, or evidence of active infection. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent (approval number. 2015_84).

Data collection and laboratory assessments

Individuals included in the study were interviewed for previous diagnoses and they underwent a medication use questionnaire and a physical examination. Weight, height, body-mass index (the weight in kilograms divided by the square of the height in meters), abdominal circumference, and systolic and diastolic blood pressure were assessed under standardised conditions. Medical records were reviewed to determine specific medications and diagnoses. Cholesterol, triglycerides, and

HDL cholesterol were measured using the enzymatic colorimetric assay. LDL cholesterol was calculated using the Friedewald formula. SLE disease activity and damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index -2000 (SLEDAI-2K) (13) and the SLICC/ACR Damage Index (SDI) (14), respectively. The SLEDAI-2k index was broken down into none (0 points), mild (1–5 points), moderate (6–10 points), high (11-19), and very high activity (>20) as previously described (15). Disease severity was measured as well, using the Katz Index (16). In addition, a carotid ultrasound was performed in patients with SLE to assess the carotid intima-media wall thickness (cIMT) in the common carotid artery and to identify focal plaques in the extracranial carotid according to the definitions of the Mannheim consensus (17, 18). Klotho levels were determined by ELISA (Elabscience, USA). Interassay and intraassay variation coefficients ranged from 3.49 to 5.77% and 4.90 to 5.29%, respectively

Statistical analysis

Demographic and clinical characteristics in patients with SLE and controls were described as mean (standard deviation) or percentages for categorical variables. For non-normally distributed continuous variables, data were expressed as median and interquartile range (IQR). Univariable differences between patients and controls were assessed through the Student's t, Mann-Whitney U, χ^2 or Fisher's exact tests according to normal distribution or number of subjects. Linear multivariable regression analysis was performed to study the relationship of clinical and laboratory data to α-Klotho. All the analyses used a 5% two-sided significance level and were performed using Stata software, version 17/SE (StataCorp, College Station, TX, USA). *p*-values <0.05 were considered statistically significant.

Results

Demographic and disease-related data

A total of 364 participants, 195 patients with SLE and 169 controls, were included in this study. Demographic- and

Table I. Characteristics of controls and SLE patients.

	Controls (n=169)	SLE patients (n=195)	*p*-value
Age, years	51 ± 17	50 ± 11	0.72
Women, n (%)	162 (96)	185 (95)	0.66
Body mass index, kg/m^2	30 ± 3	27 ± 5	**<0.001**
Abdominal circumference, cm	94 ± 7	92 ± 13	0.062
Cardiovascular co-morbidity			
Smoking, n (%)	30 (18)	46 (24)	0.17
Diabetes, n (%)	27 (16)	9 (5)	**<0.001**
Hypertension, n (%)	51 (30)	77 (40)	0.058
Obesity, n (%)	47 (28)	52 (27)	0.81
Statins, n (%)	41 (24)	52 (27)	0.60
Aspirin, n (%)	9 (12)	52 (27)	**0.007**
Antihypertensive treatment, n (%)	51 (30)	72 (37)	0.18
Lipid profile			
Cholesterol, mg/dl	201 ± 46	200 ± 38	0.81
Triglycerides, mg/dl	139 ± 64	194 ± 80	0.11
LDL cholesterol, mg/dl	118 ± 37	111 ± 29	**0.030**
HDL cholesterol, mg/dl	55 ± 15	63 ± 21	**<0.001**
Carotid intima media thickness, microns		630 ± 113	
Carotid plaque, n (%)		66 (34)	
SLE-related data			
Disease duration, years		14 (7-22)	
CRP, mg/dl	2.2 (1.3-5.5)	2.0 (0.9-4.9)	0.35
SLICC		1 (1-3)	
SLICC ≥1, n (%)		145 (74)	
Katz Index		2 (1-3)	
Katz ≥3, n (%)		75 (39)	
SLEDAI		2 (0-4)	
SLEDAI categories, n (%)			
No activity, n (%)		88 (45)	
Mild, n (%)		74 (38)	
Moderate, n (%)		24 (12)	
High or Very High, n (%)		9 (5)	
Auto-antibody profile			
Anti-DNA positive, n (%)		98 (70)	
ENA positive, n (%)		114 (64)	
Anti-Ro, n (%)		62 (39)	
Anti-La, n (%)		30 (19)	
Anti-RNP, n (%)		48 (29)	
Anti-Sm, n (%)		21 (12)	
Antiphospholipid autoantibodies, n (%)			
Lupus anticoagulant, n (%)		39 (25)	
ACA IgM, n (%)		20 (13)	
ACA IgG, n (%)		31 (20)	
Anti beta2 glycoprotein IgM, n (%)		13 (8)	
Anti beta2 glycoprotein IgG, n (%)		22 (14)	
C3, mg/dl		159 ± 47	
C4, mg/dl		29 ± 14	
Current prednisone, n (%)		99 (51)	
Prednisone, mg/day		5 (5-7.5)	
Hydroxychloroquine, n (%)		132 (69)	
Methotrexate, n (%)		23 (12)	
Mycophenolate mofetil, n (%)		15 (8)	
Azathioprine, n (%)		27 (14)	
Rituximab, n (%)		6 (3)	
Belimumab, n (%)		3 (2)	

Data represent mean ± SD or median (interquartile range) when data were not normally distributed.
BMI: body mass index; C3 C4: complement; CRP: C reactive protein; LDL: low-density lipoprotein; DMARD: disease-modifying anti-rheumatic drug; ACA: anticardiolipin; HDL: high-density lipoprotein; ANA: antinuclear antibodies; ENA: extractible nuclear antibodies; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics/ American Colleague of Rheumatology Damage Index.
SLEDAI categories were defined as: 0, no activity; 1-5 mild; 6-10 moderate; >10 high activity, >20 very high activity.

disease-related characteristics of the participants are shown in Table I. The patients and controls were matched for age (51±17 *vs*. 50±11 years, p=0.72) and gender (96 *vs*. 95% of women, p=0.66). Patients with SLE had a lower body mass index (p<0.001) and abdominal circumference (p=0.062) compared to controls and were less frequently diabetic (p<0.001). Regarding lipid pattern, patients had lower levels of LDL cholesterol but higher circulating HDL cholesterol. The cIMT in patients with SLE was 630±113 microns, and 34% (66) of them had carotid plaques.

The median duration of the disease in patients with SLE was 14 (IQR 7–22) years. Most SLE patients were in the no activity (45%) or mild activity (38%) categories as shown by the SLEDAI score. SLICC and Katz indexes were 1 (IQR 1–3) and 2 (IQR 1–3), respectively. Seventy-four percent of patients had a SLICC/ACR DI score equal to or greater than 1, and 39% had a Katz index equal to or greater than 3. At the time of recruitment, 70% patients were found to be positive for anti-DNA, and 64% were positive for ENA, being anti-Ro the most frequently found antibody (40%). Half of the patients (51%) were taking prednisone and 69% of the patients were taking hydroxychloroquine when the study was conducted. Additional information on the data related to SLE is shown in Table I.

Relationship of demographics and disease-related data to alpha-Klotho in patients with SLE

No differences were found in α-Klotho levels between SLE patients and controls, both in univariable and multivariable analyses (beta coefficient -0.10 [95% confidence interval CI -0.45–0.25-] ng/ml, p=0.57) (Table II and Fig. 1). Likewise, demographics, cardiovascular risk factors, lipid pattern, and subclinical atherosclerosis (cIMT and the presence of carotid plaque) were not significantly associated with serum levels of α-Klotho in patients with SLE. Only SLE patients with obesity had significantly higher levels of α-Klotho (beta coef. 0.66 [95%CI 0.20–1.11] ng/ml, p=0.005) compared to those without obesity. Regarding disease-related

Table II. Relation of demographics and disease-related data to alpha-Klotho serum levels in patients with SLE.

	alpha-Klotho, ng/ml			
	Univariable beta coef. 95%(CI)		Multivariable*	
SLE	-0.10 (-0.41-0.20)	0.50	-0.10 (-0.45-0.25)	0.57**
Demographics				
Age, years	0.01 (-0.01-0.02)	0.48		
Female	-0.04 (-1.01-0.94)	0.94		
Body mass index, kg/m^2	0.03 (-0.01-0.07)	0.13		
Abdominal circumference, cm	0.01 (-0.00-0.03)	0.15		
Cardiovascular co-morbidity				
Smoking	0.27 (-0.23-0.76)	0.29		
Diabetes	-0.18 (-1.10-0.74)	0.71		
Hypertension	0.13 (-0.30-0.55)	0.55		
Obesity	**0.66 (0.20-1.11)**	**0.005**		
Statins	0.38 (-0.08-0.84)	0.11		
Aspirin	0.07 (-0.42-0.56)	0.78		
Antihypertensive treatment	0.12 (-0.31-0.55)	0.57		
Lipid profile				
Cholesterol, mg/dl x10	0.03 (-0.02-0.09)	0.23		
Triglycerides, mg/dl x10	0.02 (-0.01-0.04)	0.28		
LDL cholesterol, mg/dl x10	0.00 (-0.08-0.08)	0.99		
HDL cholesterol, mg/dl x10	0.06 (-0.03-0.16)	0.21		
Carotid intima media thickness, microns	0.64 (-1.16-2.44)	0.48		
Carotid plaque	-0.16 (-0.59-0.28)	0.48		
SLE-related data				
Disease duration, years	0.02 (-0.01-0.04)	0.14	0.01 (-0.01-0.03)	0.34
CRP, mg/dl	0.01 (0.00-0.02)	0.13	0.01 (-0.00-0.02)	0.20
SLICC	0.09 (-0.02-0.2)	0.11	0.03 (-0.10-0.15)	0.65
SLICC ≥1, n (%)	0.42 (-0.06-0.90)	0.086	0.25 (-0.26-0.75)	0.34
Katz Index	-0.02 (-0.13-0.10)	0.79		
Katz ≥3	0.01 (-0.42-0.44)	0.97		
SLEDAI	0.02 (-0.04-0.07)	0.50		
SLEDAI categories				
No activity	ref.			
Mild	-0.07 (-0.52-0.38)	0.77		
Moderate	0.05 (-0.64-0.75)	0.88		
High or Very High	0.30 (-0.71-1.30)	0.56		
Auto-antibody profile				
Anti-DNA positive	0.12 (-0.36-0.61)	0.61		
ENA positive	0.03 (-0.40-0.47)	0.89		
Anti-Ro	0.00 (-0.48-0.48)	0.99		
Anti-La	-0.32 (-0.91-0.27)	0.29		
Anti-RNP	-0.17 (-0.66-0.33)	0.50		
Anti-Sm	0.08 (-0.55-0.72)	0.80		
Antiphospholipid autoantibodies				
Lupus anticoagulant	-0.35 (-0.86-0.16)	0.18	-0.34 (-0.84-0.17)	0.19
ACA IgM	-0.02 (-0.69-0.66)	0.96		
ACA IgG	-0.07 (-0.64-0.50)	0.81		
Anti beta2 glycoprotein IgM	-0.02 (-0.69-0.66)	0.96		
Anti beta2 glycoprotein IgG	-0.09 (-0.94-0.76)	0.83		
C3, mg/dl x10	0.00 (-0.05-0.04)	0.96		
C4, mg/dl x10	0.03 (-0.14-0.21)	0.71		
Current prednisone	**0.56 (0.16-0.97)**	**0.007**	**0.49 (0.08-0.90)**	**0.018**
Prednisone, mg/day	0.01 (-0.10-0.12)	0.92		
Hydroxychloroquine	-0.34 (-0.79-0.10)	0.13		
Methotrexate	-0.58 (-1.23-0.07)	0.079	-0.51 (-1.15-0.13)	0.12
Mycophenolate mofetil	0.29 (-0.54-1.13)	0.49		
Azathioprine	0.24 (-0.35-0.84)	0.41		
Rituximab	-1.11 (-2.32-0.10)	0.072	-0.88 (-2.09-0.34)	0.16
Belimumab	0.24 (-1.33-1.80)	0.77		

In the linear regression analysis alpha-Klotho is considered the dependent variable.
BMI: body mass index; C3 C4: complement; CRP: C reactive protein; LDL: low-density lipoprotein; DMARD: disease-modifying anti-rheumatic drug; ACA: anticardiolipin; HDL: high-density lipoprotein; ANA: antinuclear antibodies; ENA: extractible nuclear antibodies; SLE: Systemic lupus erythematosus; C3 and C4: Complement fractions C3 and C4; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index. SLEDAI categories were defined as: 0, no activity; 1-5 mild; 6-10 moderate; >10 high activity, >20 very high activity.
*Disease-related data association with alpha-Klotho are adjusted for BMI, abdominal circumference, obesity, and statins.
**Adjusted for BMI, abdominal circumference, smoking, diabetes, hypertension, and triglycerides and HDL serum levels (those differences between patients and controls in Table I with a p-value inferior to 0.20).

data, no associations were found between disease duration, acute phase reactants, activity, severity and damage indices, and autoantibodies profile and α-Klotho. Only the use of prednisone was significantly associated with higher serum levels of this protein after multivariable adjustment (beta coef. 0.49 [95%CI 0.08–0.13] ng/ml, p=0.018) (Table II).

Since SLICC showed a tendency to be significantly associated with α-Klotho, an additional analysis was performed breaking down this score into its different domains and items (Table III). However, after this univariable analysis, most of the domains and elements of SLICC were not found to be related to α-Klotho with some exceptions. In this sense, the muscle atrophy or weakness item, of the musculoskeletal domain was significant related to α-Klotho. Moreover, when the hole domain was considered as a binary variable (1 point or higher) a significant association with superior serum levels of α-Klotho was found (beta coef. 0.51 [95%CI 0.09–0.93] ng/ml, p=0.017) (Table III). Besides, seven patients underwent kidney biopsy in our series. No relation of α-Klotho was found to histologic class in this group of patients (data not shown). Similarly, the renal domain and items of the SLICC score was not associated with α-Klotho.

Table III. SLICC items and domains relation to alpha-Klotho serum levels.

			alpha-Klotho, ng/ml	
	n	(%)	Beta coef. (95%CI)	p-value
Ocular				
Any cataract ever	15	8%	0.14 (-0.59-0.87)	0.71
Retinal change or optic atrophy	18	9%	-0.54 (-1.26-0.18)	0.14
Points ≥1 in the domain	35	18%	0.02 (-0.52-0.55)	0.95
Neuropsychiatric				
Cognitive impairment	7	4%	0.05 (-1.07-1.18)	0.93
Seizures requiring therapy for 6 months	11	6%	0.38 (-0.59-1.36)	0.44
Cerebrovascular accident ever	9	5%	0.32 (-0.60-1.23)	0.49
Cranial or peripheral neuropathy	1	1%	-	
Transverse myelitis	0	0%	-	
Points ≥1 in the domain	24	12%	0.44 (-0.21-1.09)	0.19
Renal				
Estimated or measured glomerular filtration rate <50%	9	5%	0.39 (-0.74-1.51)	0.50
Proteinuria 3.5 gm/24 hours	7	4%	1.11 (-0.01-2.23)	0.052
End-stage renal disease	4	2%	0.19 (-0.68-1.05)	0.67
Points ≥1 in the domain	19	10%	0.61 (-0.11-1.33)	0.099
Pulmonary				
Pulmonary hypertension	2	1%	-0.78 (-2.70-1.13)	0.42
Pulmonary fibrosis	4	2%	0.31 (-1.05-1.67)	0.66
Shrinking lung	2	1%	-1.13 (-3.82-1.57)	0.41
Pleural fibrosis	1	1%	-1.37 (-4.06-1.33)	0.32
Pulmonary infarction	1	1%	-	
Points ≥1 in the domain	16	8%	-1.11 (-0.92-0.70)	0.79
Cardiovascular				
Angina or coronary artery bypass	4	2%	-0.20 (-1.56-1.16)	0.77
Myocardial infarction ever	2	1%	-0.89 (-2.80-1.02)	0.36
Cardiomyopathy	1	1%	-1.23 (-3.92-1.46)	0.37
Valvular disease	4	2%	-0.24 (-1.81-1.32)	0.76
Pericarditis for 6 months, or pericardiectomy	2	1%	1.11 (-0.80-3.01)	0.25
Points ≥1 in the domain	13	7%	0.12 (-0.73-0.96)	0.79
Peripheral vascular				
Claudication for 6 months	3	2%	-0.47 (-2.04-1.09)	0.55
Minor tissue loss (pulp space)	5	3%	0.07 (-1.50-1.64)	0.93
Significant tissue loss ever	0	0%	-	
Venous thrombosis	11	6%	0.17 (-0.86-1.21)	0.74
Points ≥1 in the domain	27	14%	0.34 (-0.30-0.99)	0.29
Gastrointestinal				
Infarction or resection of bowel	19	10%	-0.40 (-1.06-0.25)	0.23
Mesenteric insufficiency	1	1%	-	
Chronic peritonitis	1	1%	-1.09 (-3.73-1.54)	0.42
Stricture or upper gastrointestinal tract surgery ever	0	0%	-	
Points ≥1 in the domain	22	11%	-0.18 (-0.82-0.46)	0.58
Musculoskeletal				
Muscle atrophy or weakness	3	2%	**2.14 (0.26-4.03)**	**0.026**
Deforming or erosive arthritis	38	19%	0.52 (-0.01-1.04)	0.056
Osteoporosis with fracture or vertebral collapse	21	11%	0.55 (-0.10-1.20)	0.099
Avascular necrosis	5	3%	-0.36 (-1.72-1.00)	0.60
Osteomyelitis	1	1%	-0.45 (-3.15-2.25)	0.74
Points ≥1 in the domain	78	40%	**0.51 (0.09-0.93)**	**0.017**
Skin				
Scarring chronic alopecia	13	7%	-0.08 (-0.91-0.74)	0.84
Extensive scarring or panniculum	10	5%	0.60 (-0.35-1.55)	0.21
Skin ulceration	2	1%	-0.53 (-2.40-1.34)	0.58
Points ≥1 in the domain	30	15%	0.43 (-0.16-1.02)	0.16
Premature gonadal failure	14	7%	0.17 (-0.61-0.95)	0.67
Diabetes (regardless of treatment)	11	6%	-0.18 (-1.10-0.74)	0.71
Malignancy (exclude dysplasia)	8	4%	-0.56 (-1.54-0.41)	0.26

SLICC items and domains represent the independent variable.
SLICC: Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index.

Discussion

Patients with SLE present with variable clinical features ranging from mild joint and skin involvement to life-threatening renal, hematologic, or central nervous system involvement. The clinical heterogeneity of SLE and the lack of pathognomonic features or tests pose a diagnostic challenge for the clinician. For this reason, the search for biomarkers in this disease is necessary. According to the current work, α-Klotho did not differ between patients with SLE and matched controls. However, some features, such as the use of prednisone and musculoskeletal manifestations, were significantly associated with an upregulation in this protein in patients with SLE.

Several studies have studied Klotho protein in patients with autoimmune

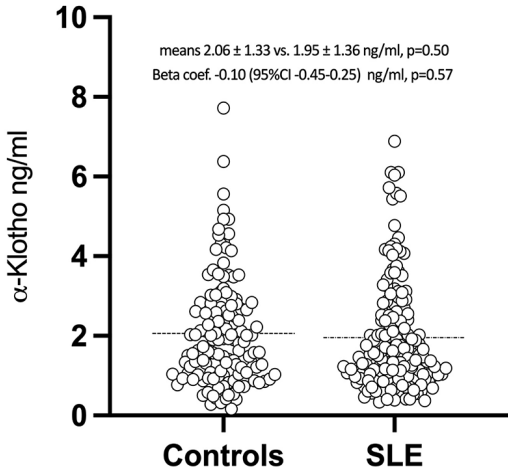

Fig. 1. Difference between systemic lupus erythematosus (SLE) patients and controls in alpha-Klotho serum levels. Beta coefficient (controls is the reference variable) is adjusted for body mass index, abdominal circumference, smoking, diabetes, hypertension, and triglycerides and HDL serum levels. Points represent unadjusted data.

diseases. In a study that aims to study the association between serum klotho levels and nailfold capillaroscopic abnormalities in systemic sclerosis it was found an inverse correlation between circulating klotho and the severity of the capillaroscopic pattern, which was not influenced by concomitant treatment (19). In other work of 60 scleroderma patients serum Klotho levels were lower compared to controls (20). In this study, the authors propose that Klotho may be associated with the pathogenesis of this disease and could be considered a future therapeutic target. Similarly, another study showed a lower concentration of klotho in the serum of 81 patients compared to that of 136 healthy controls, without any significant association with clinical manifestations and laboratory and instrumental findings (21). Patients with rheumatoid arthritis have been showed to have higher serum levels of α-Klotho than healthy controls (22). They were positively associated with the presence of anticitrullinated peptide antibody and rheumatic factor, and α-Klotho serum levels were higher in rheumatoid arthritis patients treated with biologic agents than in those undergoing conventional therapy. The association of the musculoskeletal domain of the damage score with α-Klotho found in our study in patients with SLE is in agreement with the results of this aforementioned study on rheumatoid arthritis. Likewise, as observed in our series of SLE, no association with carotid subclinical atherosclerosis was found in patients with rheumatoid arthritis (22). Other studies in rheumatoid arthritis have demonstrated a lower expression level of α-Klotho in synovial fibroblasts than in synovial membrane samples isolated from the knee joints of these patients (23), and that α-Klotho positively correlates with CD28 on $CD4^+$ cells in this population (24).

To our knowledge, only one study has evaluated α-Klotho in patients with SLE. In this work, α-Klotho was analysed in 34 patients with neuropsychiatric SLE. According to this study, low levels of this protein in the cerebrospinal fluid were a significant factor in predicting this manifestation (25). In our work a link between muscle atrophy and α-Klotho was found in the univariable analysis, but multivariable adjustment was not possible due to the fact that few SLE patients had muscle atrophy. For this reason, this association has to be taken with caution. Moreover, prednisone intake has been related to muscle atrophy and, due to this, we cannot discard that the relation between α-Klotho and muscle atrophy may be confused or mediated for the glucocorticoids used in the disease. Besides, in our series, patients were under a low prednisone dose since patients taking more than 10 mg were not allowed to participate. For this we believe that the confusion that prednisone may have exerted could be consider low.

We found a positive relationship between prednisone intake and α-Klotho after multivariable analysis. There are no previous studies that have evaluated the relationship between this drug and serum levels of α-Klotho. However, glucocorticoids have been found to strongly downregulate the fibroblast growth factor 23 plasma concentration (26). For this reason, we understand that the upregulation of α-Klotho in patients under prednisone treatment could be related to this decrease in fibroblast growth factor 23. On the other hand, since patients with musculoskeletal manifestations are frequently treated with glucocorticoids, it is possible that the higher levels of α-Klotho in these patients may be the result of glucocorticoid intake.

Besides, in our work we found a positive relation between α-Klotho and body mass index. This would be in accordance with previous reports in which this protein has been described to be related to body mass index through its relation to lean mass index but not to fat mass index (27).

We acknowledge the limitation that we did not record whether patients were taking calcium and vitamin D supplements. Moreover, vitamin D serum levels were not assessed in our series. Since α-Klotho is implicated in the calcium homeostasis regulation we cannot rule out this may have affected our results. Nevertheless, vitamin D supplementation is frequently use to achieve normal serum levels of this vitamin. For this reason, we also considered that the confusion that the lack of vitamin D in our series could be considered minor.

In conclusion, α-Klotho serum levels do not differ between patients with SLE and controls. However, the presence of musculoskeletal involvement is associated with higher levels of this protein. The pathogenic pathways related to the α-Klotho protein deserve further studies to clarify its role in this disease.

References

1. KURO-O M: The Klotho proteins in health and disease. *Nat Rev Nephrol* 2019; 15(1): 27-44. https://doi.org/10.1038/s41581-018-0078-3
2. BUCHANAN S, COMBET E, STENVINKEL P, SHIELS PG: Klotho, aging, and the failing kidney. *Front Endocrinol* (Lausanne) 2020; 11: 560. https://doi.org/10.3389/fendo.2020.00560
3. KIM SJ, CHERESH P, EREN M *et al*.: Klotho, an antiaging molecule, attenuates oxidant-induced alveolar epithelial cell mtDNA damage and apoptosis. *Am J Physiol Lung Cell*

Mol Physiol 2017; 313(1): L16-26. https://doi.org/10.1152/ajplung.00063.2017
4. MYTYCH J, ROMEROWICZ-MISIELAK M, KOZIOROWSKI M: Klotho protects human monocytes from LPS-induced immune impairment associated with immunosenescent-like phenotype. *Mol Cell Endocrinol* 2018; 470: 1-13. https://doi.org/10.1016/j.mce.2017.05.003
5. MAEKAWA Y, ISHIKAWA K, YASUDA O *et al*.: Klotho suppresses TNF-α-induced expression of adhesion molecules in the endothelium and attenuates NF-κB activation. *Endocrine* 2009; 35(3): 341-6. https://doi.org/10.1007/s12020-009-9181-3
6. TYPIAK M, PIWKOWSKA A: Antiinflammatory actions of klotho: implications for therapy of diabetic nephropathy. *Int J Mol Sci* 2021; 22(2): 1-15. https://doi.org/10.3390/ijms22020956
7. DONATE-CORREA J, MARTÍN-NÚÑEZ E, MORA-FERNÁNDEZ C, MUROS-DE-FUENTES M, PÉREZ-DELGADO N, NAVARRO-GONZÁLEZ JF. Klotho in cardiovascular disease: current and future perspectives. *World J Biol Chem* 2015; 6(4): 351. https://doi.org/10.4331/wjbc.v6.i4.351
8. ZOU D, WU W, HE Y, MA S, GAO J: The role of klotho in chronic kidney disease. *BMC Nephrol* 2018; 1981): 285. https://doi.org/10.1186/s12882-018-1094-z
9. DONATE-CORREA J, FERRI CM, MARTÍN-NÚÑEZ E *et al*.: Klotho as a biomarker of subclinical atherosclerosis in patients with moderate to severe chronic kidney disease. *Sci Rep* 2021; 11(1): 1-11. https://doi.org/10.1038/s41598-021-95488-4
10. SAITO Y, NAKAMURA T, OHYAMA Y *et al*.: *In vivo* klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. *Biochem Biophys Res Commun* 2000; 276(2): 767-72. https://doi.org/10.1006/bbrc.2000.3470
11. ZHENG S, CHEN Y, ZHENG Y, ZHOU Z, LI Z: Correlation of serum levels of fibroblast growth factor 23 and Klotho protein levels with bone mineral density in maintenance hemodialysis patients. *Eur J Med Res* 2018; 23(1): 1-7. https://doi.org/10.1186/s40001-018-0315-z
12. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40(9): 1725. https://doi.org/10.1002/art.1780400928
13. GLADMAN DD, IBAÑEZ D, UROWLTZ MB: Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29(2): 288-91.
14. GLADMAN D, GINZLER E, GOLDSMITH C *et al*.: The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996; 39(3): 363-9. https://doi.org/10.1002/art.1780390303
15. MOSCA M, BOMBARDIERI S: Assessing remission in systemic lupus erythematosus. *Clin Exp Rheumatol* 2006; 24(6 Suppl. 43): S99-104.
16. KATZ JD, SENEGAL J-L, RIVEST C, GOULET J-R, ROTHFIELD N: A simple severity of disease index for systemic lupus erythematosus. *Lupus* 1993; 2(2): 119-23. https://doi.org/10.1177/096120339300200210
17. CORRALES A, GONZÁLEZ-JUANATEY C, PEIRÓ ME, BLANCO R, LLORCA J, GONZÁLEZ-GAY MA: Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: Results of a population-based study. *Ann Rheum Dis* 2014; 73(4): 722-7. https://doi.org/10.1136/annrheumdis-2012-203101
18. TOUBOUL P-J, HENNERICI MG, MEAIRS S *et al*.: Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis* 2007; 23(1): 75-80. https://doi.org/10.1159/000097034
19. TALOTTA R, RIGAMONTI F, LETIZIA T *et al*.: Serum klotho concentrations inversely correlate with the severity of nailfold capillaroscopic patterns in patients with systemic sclerosis. *Reumatismo* 2019; 71(1): 19-23. https://doi.org/10.4081/reumatismo.2019.1129
20. AHMADI R, HAJIALILO M, GHORBANI-HAGHJO A *et al*.: FGF-23, klotho and vitamin D levels in scleroderma. *Iran J Public Health* 2017; 46(4): 530-6.
21. TALOTTA R, BONGIOVANNI S, LETIZIA T *et al*.: Measurement of serum klotho in systemic sclerosis. *Dis Markers* 2017; 2017. https://doi.org/10.1155/2017/9545930
22. ALVAREZ-CIENFUEGOS A, CANTERO-NIETO L, GARCIA-GOMEZ JA, ROBLEDO G, GONZÁLEZ-GAY MA, ORTEGO-CENTENO N: FGF23-Klotho axis in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2020; 38(1): 50-7.
23. WITKOWSKI JM, SOROCZYŃSKA-CYBULA M, BRYL E, SMOLEŃSKA Ż, JÓŹWIK A: Klotho – a common link in physiological and rheumatoid arthritis-related aging of human CD4 + lymphocytes. *J Immunol* 2007; 178(2): 771-7. https://doi.org/10.4049/jimmunol.178.2.771
24. SOROCZYŃSKA-CYBULA M, BRYL E, SMOLEŃSKA Z, WITKOWSKI JM: Varying expression of four genes sharing a common regulatory sequence may differentiate rheumatoid arthritis from ageing effects on the $CD4^+$ lymphocytes. *Immunology* 2011; 132(1): 78-86. https://doi.org/10.1111/j.1365-2567.2010.03341.x
25. USHIGUSA T, ICHINOSE K, SATO S *et al*.: Soluble α-klotho is a potential biomarker associated with neuropsychiatric systemic lupus erythematosus. *Clin Immunol* 2016; 165 29-34. https://doi.org/10.1016/j.clim.2016.03.001
26. FEGER M, EWENDT F, STROTMANN J *et al*.: Glucocorticoids dexamethasone and prednisolone suppress fibroblast growth factor 23 (FGF23). *J Mol Med* 2021; 99(5): 699-711. https://doi.org/10.1007/s00109-021-02036-8
27. AMARO-GAHETE FJ, DE-LA-O A, JURADO-FASOLI L *et al*.: Body composition and S-Klotho plasma levels in middle-aged adults: a cross-sectional study. *Rejuvenation Res* 2019; 22(6): 478-83. https://doi.org/10.1089/rej.2018.2092