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Original article

Relationship between vitamin D level and left atrial fibrosis in patients with lone paroxysmal atrial fibrillation undergoing cryoballoon-based catheter ablation

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ABSTRACT

Background: Left atrial (LA) fibrosis is known as the hallmark for arrhythmogenic substrate in atrial fibrillation (AF). Quantification of LA fibrosis by using delayed-enhanced magnetic resonance imaging (DE-MRI) in AF patients is a pioneering noninvasive technique. Vitamin D (vitD) negatively regulates the renin–angiotensin system, binds to vitD receptors on cardiac myocytes, and has antioxidant properties that may ameliorate the inflammation and proarrhythmic substrate formation. However, its role in LA fibrosis is unclear. We aimed to investigate the association of serum 25(OH)D level with the extent of LA fibrosis by using DE-MRI and also predictors for AF recurrence after cryoablation was assessed in patients with paroxysmal AF.

Methods: A total of 48 patients with lone paroxysmal AF (41.7% female; age: 48.5 ± 8.4 years) who underwent DE-MRI at 1.5 T and initial cryoballoon-based catheter ablation along with 48 healthy control subjects were enrolled. Fibrosis degree was categorized according to Utah class defined in the DECAAF study.

Results: Serum 25(OH)D levels were significantly lower in AF group compared to control group (25.8 ± 7.6 ng/ml vs. 31.0 ± 9.5 ng/ml, $p = 0.004$). Serum 25(OH)D levels were associated with moderate–severe LA fibrosis independent of other measures (OR: 0.72, 95% CI: 0.54–0.97, $p = 0.028$). At a mean 16.5 ± 2.6 months follow-up, late recurrence was observed in 10 (20.8%) patients. In multivariable Cox regression analysis, LA volume index (HR: 1.42, 95% CI: 1.01–2.01, $p = 0.045$) and the extent of LA fibrosis (HR: 1.14, 95% CI: 1.01–1.28, $p = 0.034$) were found as independently associated with late AF recurrence during follow-up.

Conclusion: Lower levels of serum 25(OH)D are significantly associated with more extensive LA fibrosis in patients with lone paroxysmal AF and may be implicated in the pathophysiology of AF recurrence after cryoablation. Further large-scale studies are needed to elucidate the exact role of vitD deficiency and replacement on LA fibrosis.

Introduction

The exact pathogenesis of atrial fibrillation (AF) has not been clarified. AF develops and persists as a complex interaction between triggers and atrial substrate, which are essential components for disease process [1]. As a surrogate of atrial substrate, atrial fibrosis is associated with electrical, contractile, and structural remodeling of atrial tissue. Enhanced oxidative stress, inflammation and activation of renin–angiotensin system (RAS) are shown to be involved in the pathogenesis of atrial fibrosis [2,3]. Currently, catheter ablation techniques primarily aim to eliminate triggers such as pulmonary veins for patients with paroxysmal AF [4]. However, a significant amount of patients fail to remain in sinus rhythm during follow-up [5,6]. Thus, the role of atrial substrate should be considered among these patients [7]. Cardiac magnetic resonance imaging with delayed enhancement technique (DE-MRI) has emerged as an effective method to noninvasively

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assess and quantify the extent of left atrial (LA) fibrosis for selection of appropriate patients before catheter ablation [8].

Besides its essential role in healthy bone metabolism, vitamin D (VitD) is also important for physiological functioning of various extraskeletal tissues and organs including heart [9]. Serum 25-hydroxyvitamin D [25(OH)D] level is usually measured as a circulating indicator of VitD status [10]. It has been proposed that vitD exhibits antioxidant properties, and regulates RAS and inflammatory pathways [11,12]. Furthermore, vitD receptors are also found in myocytes and fibroblasts in the heart, which mediate cardiac remodeling [13]. Given the associations of vitD status with several AF risk factors and the potential link between vitD, RAS, and inflammation, low vitD levels may be involved in occurrence of atrial fibrosis and AF. However, previous studies regarding the association of serum vitD level with the risk of AF revealed conflictory data [14–16]. Also, to the best of our knowledge, there was no study in the literature evaluating the association of serum vitD level with the severity of LA fibrosis and success of cryoballoon-based catheter ablation among patients with lone paroxymal AF.

Therefore, in this study, we aimed to assess the relationship between serum vitD level and quantity of LA fibrosis using DE-MRI. Also, the prognostic value of serum vitD level was evaluated after catheter ablation for paroxysmal AF.

Methods

Study population

In this prospective study, a total of 53 symptomatic lone paroxysmal AF patients who underwent preablation DE-MRI and subsequent initial first-generation cryoballoon-based catheter ablation at our University Hospital between October 2010 and August 2011 were enrolled. Of these patients, catheter ablation was postponed in one patient because of the development of cardiac tamponade during transseptal puncture. Four patients were excluded due to poor-image quality MRI characterized as blurring due to patient motion and significant gating artifacts. Thus, a total of 48 patients with AF were eligible for the final analysis. Additionally, a total of 48 healthy subjects who were admitted to our outpatient clinic for check-up and had no cardiovascular or any other organ system disease were randomly enrolled as a control group.

Subjects who were aged $\geq$60 years, had persistent or permanent AF, structural heart disease, moderate–severe valvular disease, thrombus in LA, uncontrolled thyroid or parathyroid disease, and individuals using calcium or vitD supplementation or therapies that interfere with vitD metabolism, coronary artery disease, stroke, hypertension, diabetes mellitus, chronic liver or kidney disease, systemic/local inflammatory or infectious disease, contraindication for anticoagulation, pregnancy, malignancy, gastrointestinal dysfunction (inflammatory bowel disease and malabsorption), LA anteroposterior diameter $>$55 mm, previous history of catheter ablation, and history of claustrophobia (for MRI) were excluded from the study.

Severity of clinical symptoms was recorded according to European Heart Rhythm Association (EHRA) score [17]. Lone AF was defined in patients who were $<$60 years old, without structural heart disease based on patient history, physical examination, and imaging methods including chest X-ray and echocardiography, and no history of coronary artery disease, diabetes mellitus, or hypertension [18]. Paroxysmal AF is defined as self-terminating episode, usually within 48 h, and may continue for up to 7 days [17].

Demographic and clinical information was recorded on the day of echocardiographic evaluation. Body weight (kg) and height (m) were determined, and body mass index (BMI; kg/m^2) was calculated. Alcohol intake is defined as having up to 1 drink per day for women and up to 2 drinks per day for men, in which heavy drinkers and abusers were excluded. Additionally, in order to properly demonstrate other factors that may have an impact on vitD status, all subjects were enquired about age, weight, height, clothing style, physical activity (by using subject responses in regard to hours spent on exercise, at work or leisure time), current smoking habits, and sunlight exposure (by calculating the average time spent in the sun per day) by a questionnaire.

Preablation evaluation

All AF patients underwent preablation transthoracic and transesophageal echocardiographic examination and cardiac MRI as a study protocol. All echocardiographic measurements were calculated according to the criteria proposed by the American Society of Echocardiography [19]. Echocardiographic examination was performed by using a VIVID 5 Dimension Cardiovascular Ultrasound System (Vingmed-General Electric, Horten, Norway) with a 3.5 MHz transducer.

Informed consent was taken from each patient before enrollment. The study was in compliance with the principles outlined in the Declaration of Helsinki and approved by local ethics committee.

Laboratory tests

Venous blood samples were obtained without venostasis by the venipuncture of the large antecubital veins of the patients at least 24 h before cryoablation. Serum was stored at -80 °C. The 25-OH-D concentration was measured with direct enzyme-linked immunosorbent assay (ImmunDiagnostiK, Bensheim, Germany), and intraassay and interassay coefficient of variations were $\leq$3.8% and $\leq$4.1%, respectively. The concentration of intact-parathyroid hormone (iPTH) was determined by use of immunoradiometric assay Immulite 2000 (Siemens Healthcare Diagnostics Products, Erlangen, Germany) with a reference range of 9.5–75 pg/ml. The average intra- and interassay CV [coefficient of variation] for iPTH were $\leq$4.2% and $\leq$3.5%, respectively.

Cardiac magnetic resonance imaging

Scanning and quantification of LA fibrosis was performed using similar techniques that have been previously described by the Utah group [8]. All the patients were in normal sinus rhythm during DE-MRI acquisition. Studies were performed by a General Electric 1.5-T High Definition scanner 2.0 $\pm$ 0.8 days prior to ablation (Signa Excite HD; GE Medical Systems, Waukesha, WI, USA) using an 8-channel phased-array receiver coil. In all patients, there was only one DE-MRI study performed at our center prior to cryoablation. A contrast-enhanced three-dimensional (3D) fast low-angle shot (FLASH) MR angiography sequence and a cine true-fast imaging with steady-state precession (FISP) sequence were used to define the anatomy of the LA and the PVs. The scan was acquired $\sim$18 (range: 17–20) min after contrast agent injection [0.15 mmol/kg i.v., meglumin gadoterad (Dotarem, Guerbet, Aulnay, France)] using a 3D inversion recovery, respiration navigated, electrocardiogram (ECG)-gated, gradient echo pulse sequence. Typical acquisition parameters were as follows: free breathing using navigator gating; a transverse imaging volume with voxel size 1.25 mm $\times$ 1.25 mm $\times$ 4 mm, which was then reconstructed to 0.625 mm $\times$ 0.625 mm $\times$ 2 mm; repetition time (TR)/echo time (TE) = 4.8/2.3 ms; parallel imaging using the generalized autocalibrating partially parallel acquisitions (GRAPPA) technique with reduction factor R = 2 and 32 reference lines; field of view (FOV): 300–340 mm; flip angle: 20°; matrix size:

272 × 272; slice thickness: 4 mm; spacing gap: 0; inversion time (TI) = 280–350 ms; bandwidth: 224 Hz/pixel; number of excitations (NEX): 1; and phase FOV: 0.75. ECG gating was used to acquire a small subset of phase-encoding views during the diastolic phase of the LA cardiac cycle. The time interval between the R-peak of the ECG and the start of data acquisition was defined using the cine images of the LA. The typical value of the interval was 60% of the mean RR interval for patients in sinus rhythm. Fat saturation was used to suppress the fat signal. The TE of the scan was chosen such that the signal intensity of partial volume fat-tissue voxels was reduced allowing improved definition of the LA wall boundary. The TI value for the DE-MRI scan was identified using a scout scan. Typical scan time for the DE-MRI study was between 8–12 min depending on the subject's respiration and heart rate.

All MRIs were analyzed at our Cardiovascular Imaging Unit by an experienced radiologist (T.H.) who has been blinded to patient clinical data. Raw images were transferred to a workstation for storage and quantitative analysis. LA regions were defined as superior, inferior, anterior (annular), posterior, septal, and lateral walls. In all DE-MRI images, epicardial and endocardial contours were manually drawn around LA myocardium with image display. Threshold for fibrosis identification was determined for each patient individually by using a dynamic threshold algorithm based partly on work in left ventricle [20]. The relative extent of contrast enhancement was quantified within the LA wall using a threshold-based algorithm based on pixel intensity distribution of healthy myocardium and nonviable myocardium [21]. Patients were assigned to one of four stages (stage I–IV) based on the percentage of LA wall enhancement as mentioned in The DECAAF trial 12. Stage I was defined as <10% LA wall enhancement, stage II as ≥10% and <20%, stage III as ≥20% and <30%, and stage IV as ≥30% [22].

Furthermore, to assess the potential effects of intraobserver variability in measurement of delayed enhancement, we randomly selected a subset of 16 patients (4 patients for each stage) in whom the responsible user repeated MRI segmentation in a separate session (median duration between 2 segmentations was 24 h). The intraclass correlations for intrareader variability of detected LA wall enhancement were 0.93 for reliability of observations (95% CI: 0.76–0.96) and 0.95 for reliability of the mean (95% CI: 0.83–0.97). The average difference in Bland–Altman analyses for repeated measurement of detected LA wall enhancement was 0.44% (limits of agreement = −4.78–4.57%) for intrareader variability.

Cryoballoon-based catheter ablation

Antiarrhythmic drugs were discontinued for five half-lives before the procedure. All procedures were performed under conscious sedation using boluses of midazolam. The procedural details were described previously [5]. Also, treating physicians were blinded to the data of fibrosis quantification during ablation procedure.

Postablation follow-up

The patients remained under continuous hemodynamic and ECG monitoring for 24 h. Immediately after the procedure and 24 h following the procedure, transthoracic echocardiography was performed to ascertain the absence of pericardial effusion. Oral anticoagulation with warfarin was initiated after 4–6 h of the procedure and also concomitant enaxoparin 1 mg/kg was administered until target international normalized ratio (INR) of 2.0–3.0. For the following 3 months, the patients remained on the antiarrhythmic drug regimen they were prescribed before the ablation procedure.

Follow-up visits were performed at 3, 6, and 12 months and for every 6 months thereafter or earlier if they developed symptoms consistent with recurrent AF. A clinical assessment and a 12-lead ECG were routinely performed in all patients at each follow-up visit. A 24-h Holter ECG was recorded 3 months after the procedure, usually on antiarrhythmic therapy. In the absence of arrhythmia, all antiarrhythmic drugs were discontinued. In addition, all the patients underwent a 24-h Holter ECG monitorization at each follow-up visit and patients who experienced arrhythmia symptoms also transmitted cardiac event recordings for a week.

Acute procedural success is defined as electrical isolation of all PVs. The first 3 months after AF ablation was defined as blanking period. Early recurrence of AF was defined as detection of AF (at least 30 s duration when assessed with ECG monitoring) within 3 months of ablation. Recurrence of AF was defined as detection of AF (at least 30 s duration when assessed with ECG monitoring) >3 months following AF ablation [4].

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL, USA). Normal distribution of variables was evaluated with Kolmogorov–Smirnov test. Numerical variables with a normal distribution were presented as the mean ± standard deviation and numerical variables with a skewed distribution were presented as the median, and categorical variables were presented as percentages. For numerical variables, an independent sample t test and Mann–Whitney *U* test were used for intergroup comparisons. Chi-square test and Fisher's exact tests were used for comparisons of categorical variables. Tukey's honestly significant difference test was used to compare vitD levels in all Utah stages of LA fibrosis. To determine independent predictors of ≥20% LA fibrosis and late AF recurrence, binominal logistic regression and Cox regression analyses were performed, respectively. Spearman correlation analysis was used to demonstrate the relationship between vitD levels and extent of LA fibrosis (%). Receiver operating characteristic (ROC) analysis was performed to determine the sensitivity and specificity of vitD cut-off levels for predicting the severity of LA fibrosis (<20 vs. ≥20% LA fibrosis). To adjust for multiple testing, a Bonferroni correction was applied (0.05/7 = 0.007) and multivariable Cox regression models were fitted using covariates with $p < 0.007$ in the univariable analysis. A two-tailed $p < 0.05$ was considered statistically significant for all other analyses.

Results

The comparison of baseline characteristics of the healthy control group and AF group are represented in Table 1. Serum 25(OH)D levels were significantly lower in AF group (25.8 ± 7.6 ng/ml vs. 31.0 ± 9.5 ng/ml, $p = 0.004$). Additionally, LA diameter and LA volume index (LAVI) were higher in the AF group compared to the control group ($p < 0.05$).

A total of 32 (66.7%) AF patients revealed any degree of LA fibrosis with a median LA enhancement at DE-MRI of 5 (range 0–50)%. Patients were assigned to one of the four groups (Utah I–IV) based on the percentage of LA wall enhancement as defined in DECAAF trial (Fig. 1). AF patients were classified as follows, 30 (62.5%) in Utah I, 5 (10.4%) in Utah II, 6 (12.5%) in Utah III, and 7 (14.6%) in Utah IV. There was no statistically significant intergroup difference between Utah I (median 31 ng/ml; mean 30 ± 4.6 ng/ml) vs. Utah II (median 38.5 ng/ml; mean 28.5 ± 6.0 ng/ml) and Utah III (median 18 ng/ml; mean 20.4 ± 8.4 ng/ml) vs. Utah IV (median 15 ng/ml; mean 16.4 ± 3.9 ng/ml) (Fig. 2A). Thus, we preferred to categorize AF patients into 2 as extent of LA fibrosis

Table 1
Baseline clinical, laboratory, and follow-up characteristics of the study groups ($n=96$).

	Control group ($n=48$)	AF group ($n=48$)	p
Clinical parameters			
Age (years)	48.9 ± 7.3	48.5 ± 8.4	0.806
Female gender	24 (50.0%)	20 (41.7%)	0.539
BMI (kg/m^2)	28.9 ± 4.2	27.6 ± 5.2	0.176
Current smokers	15 (31.2%)	17 (35.4%)	0.829
Alcohol[a]	10 (20.8%)	7 (14.6%)	0.594
Physical activity			
<15 min/day	13 (27.1%)	14 (29.2%)	0.919
15–30 min/day	22 (45.8%)	20 (41.7%)	
>30 min/day	13 (27.1%)	14 (29.2%)	
Sun exposure (h/week)	1.44 ± 0.86	1.43 ± 0.87	1.00
Wearing clothes which restrict exposure to sunlight	4 (8.3%)	2 (4.2%)	0.677
Heart rate (bpm)	76.2 ± 7.7	74.2 ± 8.6	0.218
Systolic blood pressure (mmHg)	125.1 ± 6.9	124.5 ± 9.8	0.729
Diastolic blood pressure (mmHg)	80.6 ± 5.9	78.9 ± 6.9	0.145
Echocardiographic parameters			
LVEF (%)	67.0 ± 4.6	68.0 ± 4.3	0.256
LA diameter (mm)	32.6 ± 3.6	37.4 ± 3.4	<0.001*
LAVI (ml/m^2)	28.5 ± 1.8	31.8 ± 4.2	<0.001*
Laboratory parameters			
Fasting plasma glucose (mg/dl)	86.0 ± 8.1	87.7 ± 8.8	0.331
Serum creatinine (mg/dl)	0.91 ± 0.15	0.90 ± 0.21	0.959
Total cholesterol (mg/dl)	200.0 ± 43.0	196.0 ± 27.6	0.593
Triglyceride (mg/dl)	135.9 ± 50.2	135.5 ± 43.4	0.971
LDL-cholesterol (mg/dl)	125.0 ± 29.5	121.6 ± 29.6	0.578
HDL-cholesterol (mg/dl)	44.3 ± 6.5	46.8 ± 9.0	0.122
25(OH)D (ng/ml)	31.0 ± 9.5	25.8 ± 7.6	0.004*
iPTH (pg/ml)	37.2 ± 10.2	39.2 ± 11.4	0.352
Calcium (mg/dl)	9.46 ± 0.16	9.45 ± 0.17	0.993
Phosphorus (mg/dl)	3.48 ± 0.27	3.50 ± 0.29	0.697
C-reactive protein, mg/dL	0.24 (0.03–0.50)	0.43 (0.10–1.51)	<0.001

Numerical variables were presented as the mean ± standard deviation or median (range), and categorical variables were defined as number (percentage).
AF, atrial fibrillation; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; iPTH, intact-parathyroid hormone; LAVI, left atrial volume index; LDL, low-density lipoprotein; LVEF, left ventricle ejection fraction; SBP, systolic blood pressure.
[a] Alcohol intake is defined as having up to 1 drink per day for women and up to 2 drinks per day for men in which heavy drinkers and abusers were excluded.
* $p<0.05$.

<20% (including Utah I and II) vs. ≥20% (including Utah III and IV) according to DECAAF trial.

Baseline clinical, laboratory, and follow-up characteristics of the AF group according to the severity of LA fibrosis (<20% in 35 patients vs. ≥20% in 13 patients) are demonstrated in Table 2. Age, duration of AF history, LAVI, serum iPTH levels, and the rate of early and late AF recurrence after cryoablation were significantly higher in patients with LA fibrosis of ≥20%. However, serum 25(OH)D levels were significantly lower in patients with LA fibrosis of ≥20% as compared to LA fibrosis of <20% (17.9 ± 6.0 vs. 28.7 ± 5.8, $p<0.001$) (Fig. 2B). In multivariable logistic regression analysis model including age, preablation serum 25(OH)D level, duration of AF history, and LAVI, only preablation serum 25(OH)D level was associated with moderate-extensive LA fibrosis independent of other measures (OR: 0.72, 95% CI: 0.54–0.97, $p=0.028$) (Table 3). Furthermore, correlation analysis revealed a strongly negative relationship between serum 25(OH)D level and the extent of LA fibrosis ($r=-0.487$, $p<0.001$) (Fig. 3A). Receiver operating characteristic curve was used to explore the relation between the preablation 25(OH)D level and moderate–severe LA fibrosis. The area under the curve was 0.882 (95% CI: 0.772–0.992, $p<0.001$). Using a cut-off level of <23.5 ng/ml, the preablation 25(OH)D level was associated with moderate–severe LA fibrosis with a sensitivity of 88.6% and specificity of 84.6% (Fig. 3B).

A total of 208 PVs (100%) were successfully isolated with mean freezing cycles of 2.32 ± 0.4 per PV. At a mean 16.5 ± 2.6 months follow-up, AF recurrence after blanking period was observed in 10 (20.8%) patients. Clinical, laboratory and follow-up characteristics of the AF group in regard to occurrence of late AF recurrence are shown in Table 4. Age, LA diameter, LAVI, iPTH, median extent of LA fibrosis, and the rate of patients with early recurrence and moderate–severe LA fibrosis were significantly higher in patients with late recurrence ($p<0.05$). But, serum 25(OH)D levels were significantly lower in patients with AF recurrence (16.1 ± 4.8 vs. 28.4 ± 5.9, $p<0.001$). In multivariable

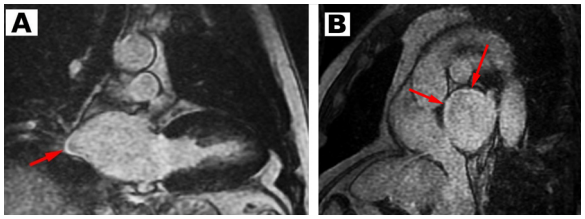

Fig. 1. (A) Cardiac DE-MRI showing LA posterior wall late gadolinium enhancement (LA fibrosis extent: 8%). (B) Cardiac DE-MRI showing LA superior and supero-medial wall late gadolinium enhancement (stage III; LA fibrosis extent: 25%) in a 56-year-old male patient with lone atrial fibrillation for 25 years. DE-MRI, delayed-enhanced magnetic resonance imaging; LA, left atrial.

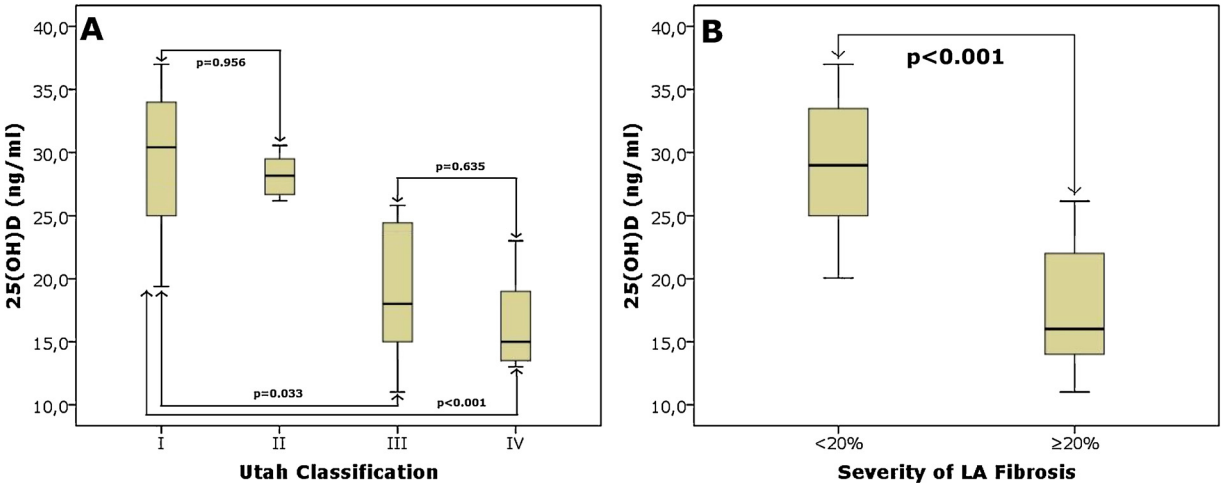

Fig. 2. Box-plot graph showing the comparison of serum 25(OH)D levels according to the extent of left atrial (LA) fibrosis using Utah classification (class I–IV) (A) and LA fibrosis <20% vs. ≥20% (B).

Table 2
Baseline clinical, laboratory, and follow-up characteristics of the AF group according to the severity of LA fibrosis ($n = 48$).

	LA fibrosis <20% ($n = 35$)	LA fibrosis ≥20% ($n = 13$)	p
Clinical parameters			
Age (years)	46.2 ± 8.4	54.8 ± 3.4	0.001
Female gender	14 (40.0%)	6 (46.2%)	0.750
BMI (kg/m^2)	27.6 ± 5.2	27.8 ± 5.7	0.904
Current smokers	11 (31.4%)	6 (46.2%)	0.498
Alcohol	4 (11.4%)	3 (23.1%)	0.370
Physical activity			
<15 min/day	10 (28.6%)	4 (30.8%)	0.196
15–30 min/day	17 (48.6%)	3 (23.1%)	
>30 min/day	8 (22.9%)	6 (46.2%)	
Sun exposure (h/week)	1.45 ± 0.88	1.38 ± 0.84	0.800
Wearing clothes which restrict exposure to sunlight	2 (5.7%)	0 (0.0%)	0.527
Heart rate (bpm)	74.5 ± 8.1	73.0 ± 10.1	0.581
Systolic blood pressure (mmHg)	125.1 ± 9.7	124.0 ± 10.2	0.696
Diastolic blood pressure (mmHg)	77.9 ± 6.6	76.3 ± 7.1	0.196
EHRA score	2.97 ± 0.61	2.92 ± 0.76	0.822
Duration of AF history (months)	40 (12–216)	96 (24–312)	0.010
Echocardiographic parameters			
LVEF (%)	68.3 ± 3.7	67.2 ± 5.7	0.457
LA diameter (mm)	36.9 ± 3.0	38.8 ± 4.1	0.100
LAVI (ml/m^2)	30.3 ± 3.6	35.8 ± 3.2	<0.001*
Laboratory parameters			
Fasting plasma glucose (mg/dl)	87.3 ± 8.5	88.7 ± 9.7	0.642
Serum creatinine (mg/dl)	0.87 ± 0.14	0.91 ± 0.19	0.322
Total cholesterol (mg/dl)	202.0 ± 27.2	195.0 ± 22.4	0.193
Triglyceride (mg/dl)	136.3 ± 42.4	133.4 ± 47.9	0.838
LDL-cholesterol (mg/dl)	119.0 ± 28.9	129.0 ± 31.2	0.278
HDL-cholesterol (mg/dl)	45.8 ± 7.7	49.5 ± 11.9	0.217
25(OH)D (ng/ml)	28.7 ± 5.8	17.9 ± 6.0	<0.001*
iPTH (pg/ml)	35.7 ± 9.0	48.7 ± 12.1	<0.001*
Calcium (mg/dl)	9.44 ± 0.18	9.48 ± 0.14	0.506
Phosphorus (mg/dl)	3.50 ± 0.28	3.49 ± 0.32	0.870
C-reactive protein, mg/dL	0.32 (0.10–1.54)	0.65 (0.13–0.92)	<0.001
Follow-up parameters			
Follow-up (months)	16.3 ± 2.6	17.0 ± 2.5	0.388
Early recurrence	5 (14.3%)	7 (53.8%)	0.009
Late recurrence	0 (0.0%)	10 (76.9%)	<0.001

Numerical variables were presented as the mean ± standard deviation or median (range), and categorical variables were defined as number (percentage).
AF, atrial fibrillation; BMI, body mass index; DBP, diastolic blood pressure; EHRA, European Heart Rhythm Association; HDL, high-density lipoprotein; iPTH, intact-parathyroid hormone; LAVI, left atrial volume index; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure.
[a]Alcohol intake is defined as having up to 1 drink per day for women and up to 2 drinks per day for men in which heavy drinkers and abusers were excluded.
* $p < 0.05$.

Table 3
Univariate and multivariate binominal logistic regression analyses demonstrating independent predictors of LA fibrosis of ≥20%.

Parameters	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age (years)	1.42 (1.12–1.80)	0.004	1.47 (0.86–2.52)	0.162
Duration of AF history (months)	1.01 (1.001–1.02)	0.026	1.04 (0.99–1.08)	0.081
LAVI (ml/m^2)	1.54 (1.20–1.99)	0.001	1.42 (0.88–2.30)	0.147
25(OH)D (ng/ml)	0.77 (0.67–0.89)	<0.001	0.72 (0.54–0.97)	0.028*
iPTH (pg/ml)[a]	1.12 (1.04–1.20)	0.002	–	–
C-reactive protein, mg/dL	3.40 (0.52–22.1)	0.201		

AF, atrial fibrillation; CI, confidence interval; iPTH, intact-parathyroid hormone; LA, left atrium; LAVI, left atrial volume index; OR, odds ratio.
[a] Just 25(OH)D is included in the multivariate analysis because of a strong negative correlation between 25(OH)D and iPTH.
* $p < 0.05$. $R^2 = 0.837$.

Cox regression analysis, LAVI (HR: 1.42, 95% CI: 1.01–2.01, $p = 0.045$) and the extent of LA fibrosis (HR: 1.14, 95% CI: 1.01–1.28, $p = 0.034$) were independently associated with late AF recurrence during follow-up (Table 5).

Discussion

Major findings of our study included the following: (a) serum 25(OH)D levels were significantly lower in patients with lone paroxysmal AF compared to healthy control subjects; (b) serum 25(OH)D level was significantly lower in patients with moderate–severe LA fibrosis compared to patients with minimal-mild LA fibrosis; (c) in multivariable logistic regression analysis, only serum 25(OH)D level was associated with moderate-extensive LA fibrosis; and (d) the extent of LA fibrosis and LAVI were independently associated with AF recurrence during follow-up. To the best of our knowledge, this is the first study in the literature evaluating the association of serum vitD status with the extent of LA fibrosis at DE-MRI in patients with paroxysmal AF undergoing cryoablation.

Besides its crucial role in mineral homeostasis and skeletal health, vitD metabolites also act on extraskeletal tissues including cardiovascular system [9]. VitD metabolites such as 25(OH)D and 1,25(OH)D act via binding their intracellular receptors. It was shown that vitD receptors were also found in cardiomyocytes, vascular smooth muscle cells, and endothelial cells [23–25]. VitD

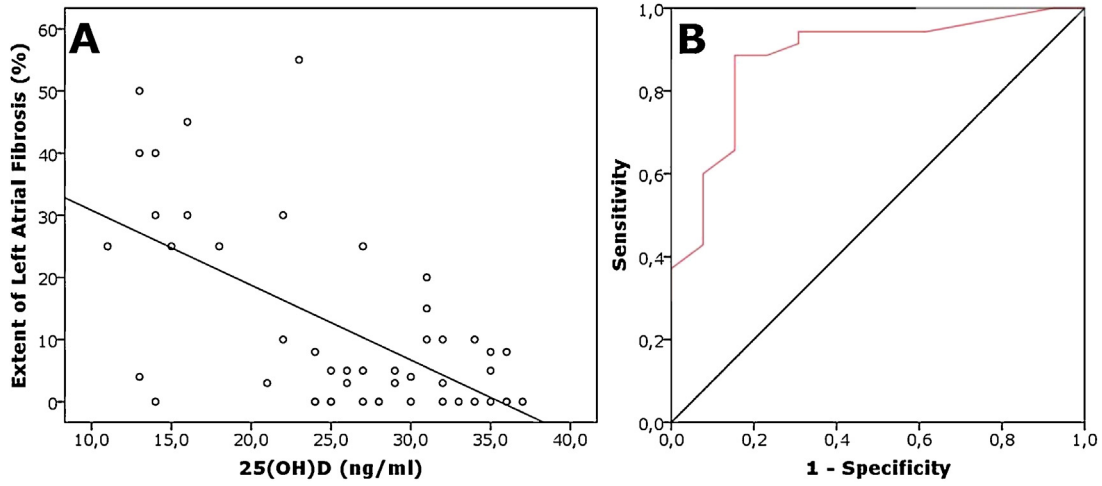

Fig. 3. Correlation analysis showing a significantly negative correlation between serum 25(OH)D levels and extent of left atrial (LA) fibrosis (A). Receiver operating characteristic curve representing the cut-off point of serum 25(OH)D level in prediction of moderate–severe LA fibrosis in delayed-enhanced magnetic resonance imaging (B).

Table 4
Clinical, laboratory, and follow-up characteristics of the patient group in regard to occurrence of late AF recurrence ($n = 48$).

	Recurrence (−) ($n = 38$)	Recurrence (+) ($n = 10$)	p
Clinical parameters			
Age (years)	46.7 ± 8.3	55.3 ± 3.6	0.003*
Female gender	16 (42.1%)	4 (40.0%)	0.599
BMI (kg/m^2)	27.8 ± 5.1	26.9 ± 6.1	0.617
Current smokers	11 (28.9%)	6 (60.0%)	0.134
Alcohol	4 (10.5%)	3 (30.0%)	0.147
Physical activity			
<15 min/day	10 (26.3%)	4 (40.0%)	0.630
15–30 min/day	17 (44.7%)	3 (30.0%)	
>30 min/day	11 (28.9%)	3 (30.0%)	
Sun exposure (h/week)	1.47 ± 0.89	1.30 ± 0.78	0.578
Wearing clothes which restrict exposure to sunlight	2 (5.3%)	0 (0.0%)	0.623
Heart rate (bpm)	74.4 ± 8.0	73.3 ± 11.2	0.732
Systolic blood pressure (mmHg)	124.4 ± 9.7	124.8 ± 10.7	0.915
Diastolic blood pressure (mmHg)	78.6 ± 6.7	75.3 ± 7.1	0.174
EHRA score	2.95 ± 0.65	3.00 ± 0.66	0.823
Duration of AF history (months)	48 (12–312)	90 (24–300)	0.148
Echocardiographic parameters			
LVEF (%)	68.1 ± 3.8	67.6 ± 6.1	0.746
LA diameter (mm)	36.9 ± 3.0	39.4 ± 4.2	0.040*
LAVI (ml/m^2)	30.4 ± 3.5	37.1 ± 2.1	<0.001*
Laboratory parameters			
Fasting plasma glucose (mg/dl)	87.1 ± 8.6	90.0 ± 9.7	0.360
Serum creatinine (mg/dl)	0.89 ± 0.16	0.90 ± 0.21	0.122
Total cholesterol (mg/dl)	200.0 ± 27.3	190.0 ± 23.9	0.141
Triglyceride (mg/dl)	135.7 ± 41.2	134.7 ± 53.7	0.947
LDL-cholesterol (mg/dl)	120.0 ± 28.3	127.5 ± 35.2	0.487
HDL-cholesterol (mg/dl)	45.5 ± 7.9	48.9 ± 11.4	0.244
25(OH)D (ng/ml)	28.4 ± 5.9	16.1 ± 4.8	<0.001*
iPTH (pg/ml)	35.8 ± 8.8	52.0 ± 11.7	<0.001*
Calcium (mg/dl)	9.44 ± 0.17	9.50 ± 0.14	0.289
Phosphorus (mg/dl)	3.49 ± 0.27	3.52 ± 0.36	0.823
C-reactive protein, mg/dL	0.29 (0.1–1.54)	0.77 (0.54–1.92)	<0.001
Cardiac MRI parameters			
Extent of LA fibrosis (%)	3.0 (0–25)	30 (25–50)	<0.001*
LA fibrosis <20%	35 (92.1%)	0 (0.0%)	<0.001*
LA fibrosis ≥20	3 (7.9%)	10 (100%)	
Follow-up parameters			
Follow-up (months)	16.4 ± 2.6	16.8 ± 2.7	0.648
Early recurrence	5 (13.2%)	7 (70.0%)	0.001*

Numerical variables were presented as the mean ± standard deviation or median (range), and categorical variables were defined as number (percentage).
AF, atrial fibrillation; BMI, body mass index; DBP, diastolic blood pressure; EHRA, European Heart Rhythm Association; HDL, high-density lipoprotein; iPTH, intact-parathyroid hormone; LA, left atrium; LAVI, left atrial volume index; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; SBP, systolic blood pressure.
[a]Alcohol intake is defined as having up to 1 drink per day for women and up to 2 drinks per day for men in which heavy drinkers and abusers were excluded.
* $p < 0.05$.

Table 5
Univariate and multivariate Cox regression analyses representing the predictors of AF recurrence after cryoballoon-based catheter ablation.

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (years)	1.31 (1.10–1.55)	0.002	1.15 (0.80–1.66)	0.448
LA diameter (mm)[b]	4.07 (0.81–20.5)	0.089	–	–
LAVI (ml/m^2)	1.61 (1.25–2.07)	<0.001	1.42 (1.01–2.01)	0.045*
25(OH)D (ng/ml)	0.79 (0.69–0.89)	<0.001	0.81 (0.65–1.00)	0.053
iPTH (pg/ml)[a]	1.10 (1.05–1.16)	<0.001	–	–
Extent of LA fibrosis (%)	1.08 (1.05–1.11)	<0.001	1.14 (1.01–1.28)	0.034*
Early recurrence	9.73 (2.5–37.80)	<0.001	17.8 (1.72–183.9)	0.016*
C-reactive protein, mg/dL	5.28 (1.42–19.6)	0.013	–	–

AF, atrial fibrillation; CI, confidence interval; iPTH, intact-parathyroid hormone; LA, left atrium; LAVI, left atrial volume index; OR, odds ratio.
[a] Just 25(OH)D is included in the multivariate analysis because of a strong negative correlation between 25(OH)D and iPTH.
[b] LA diameter is not included in multivariate analysis because of a strong association with LAVI.
* $p < 0.007$. $R^2 = 0.837$.

metabolites act via endocrine, autocrine, and paracrine pathways and modulate vascular endothelial cell function, RAS, and arterial/myocardial remodeling. Although much more data concurrent with each other revealed an association between vitD status and several cardiovascular pathologies such as coronary heart disease, hypertension, and left ventricular hypertrophy [9], the relationship between vitD status and cardiac arrhythmias, particularly with AF, was unclear. Also the results of all those studies investigating the association of vitD status with AF were contradictory [14–16,26].

AF develops as a complex interaction between structural, electrical, and contractile remodeling of the LA [17,27]. The RAS components such as angiotensin II, aldosterone, and transforming growth factor-β1 contribute to these remodeling processes through their proliferative, proinflammatory, profibrotic, and prothrombotic actions [28]. Consequently, as a result of all those neurohormonal and cellular interactions, LA fibrosis occurs as a hallmark of arrhythmogenic structural remodeling. LA fibrosis is known as a prerequisite for LA substrate formation, which plays a role in both initiation and maintenance of AF [28]. Furthermore, it has been shown that the main metabolite of vitD, 1,25-(OH)D, is a potent negative modulator of the RAS [11] and inflammation [12]. Furthermore, vitD receptors are also found in myocytes and fibroblasts in the heart [29]. A number of animal studies confirmed that vitD receptors were important in mediating cardiac hypertrophy, whilst the association between vitD receptors and mediation of cardiac fibrosis is less clear [13,30]. Because of the potential link between vitD, the RAS, and inflammation, it has been proposed that vitD status might be associated with the development of LA fibrosis and recurrence of AF after catheter ablation. In a study by Chen et al. [16] consisting of nonvalvular persistent AF patients, 25(OH)D levels were found to be negatively correlated with LA diameter and hsCRP levels. Thus, Chen et al. [16] confirmed that the structural remodeling of LA and inflammation was significantly associated in patients with vitD deficiency. Furthermore, in an animal study, Hanafy et al. [31] demonstrated that administration of 1,25(OH)D to the rabbit LA significantly increased the action potential duration and confirmed its direct electromechanical effects. Thus, these studies suggest further studies to show whether vitamin D supplementation in patients with AF and low-level vitamin D might improve AF burden. In recent years, cardiac DE-MRI has emerged as an effective method to noninvasively assess and quantify the extent of LA fibrosis, which was shown to be associated with AF recurrence after catheter ablation [22]. But, there was no study in the literature evaluating the association of vitD status with extent of LA fibrosis quantified by using DE-MRI. Our study has significant differences from previous studies including that (i) it consisted of patients with lone paroxysmal AF as a unique study population by which other possible risk factors for LA fibrosis have been excluded; (ii) for the first time we assessed the association of vitD level and the extent of LA fibrosis using DE-MRI; (iii) prognostic value of vitD status after catheter ablation was also available in our study population.

The main strength of our study is the demonstration of a close association of lower 25(OH)D level with an increased extent of LA fibrosis quantified by using DE-MRI. Also, as a confirmation of previous studies, the extent of LA fibrosis was found as an independent predictor for AF recurrence during follow-up [7,22]. As vitD status is readily modifiable, the presence of such

a relationship between vitD status and the extent of LA fibrosis using DE-MRI would have potential clinical implication. Preablation serum 25(OH)D levels may identify patients at higher risk for greater extent of LA fibrosis with high relevance for clinical routine practice, and close follow-up in those patients is warranted. Additionally, no clinical study has examined the utility of replacing vitD with medical interventions in such a population. Further large-scale randomized controlled studies are needed to confirm whether vitD replacement would reduce the extent of LA fibrosis.

The results of our study should be interpreted in the light of several limitations. First, this is a single-center study with a small sample size including a unique subgroup of AF patients. Second, MR imaging in this study was performed with 1.5 T scanner and significant improvements in LA wall imaging with greater spatial resolution and improved signal to noise ratio are expected at higher magnetic field (3 T). Third, lack of long-term continuous monitoring methods during follow-up, which was capable of detection of silent AF episodes, is another limitation of our study. Also, this is a clinical study, and a pathophysiological link between serum 25(OH)D level and LA fibrosis cannot be proposed with these data. Our findings did not show a causal relationship, but only demonstrated an association. Also, cardiac MRI has not been performed in our control group, which may also be helpful to support the association of vitamin D status with atrial fibrosis. Furthermore, measurement of serum 25(OH)D levels at a single time point (in summer or winter months) might have led us to underestimate the association between vitamin D status and development of AF recurrence during follow-up. Lastly, our analyses were limited to individuals of Turkish population, and the results may not be generalizable to other racial/ethnic groups because of significant ethnic variability for vitD levels.

In conclusion, lower levels of serum 25(OH)D as an extensively validated surrogate for vitD stores are significantly associated with more extensive LA fibrosis in patients with lone paroxysmal AF, which might be implicated in the pathophysiology of AF recurrence after cryoablation. Our data are hypothesis generating and selection of appropriate patients for the ablation procedure using serum 25(OH)D levels as an indicator of LA fibrosis needs to be investigated in each individual population. Further large-scale studies are needed to elucidate the exact role of vitD deficiency and replacement on LA fibrosis and AF recurrence after catheter ablation.

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Conflict of interest

Authors do not have any conflict of interest.

Author contributions

UC conceptualized and designed the study along with NÖ and AO and together with KA collected the data and drafted the article. The statistical work was carried out by UC along with TH, who also performed the data analysis and interpreted it, in addition to the image processing. NÖ and AO critically revised the work. All the authors have given their approval for the article.

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