scientific reports

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25(OH)D3 and F-25(OH)D as indicators of chronic kidney disease progression in patients with rheumatoid arthritis

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Vitamin D (VitD) deficiency has been associated with the development of rheumatoid arthritis (RA) and chronic kidney disease (CKD), but its exact role in patients with RA and CKD remains unclear. This cross-sectional study explored the relationship of 25(OH)D2, 25(OH)D3, and Free 25(OH)D [F-25(OH)D] with CKD progression in patients with RA. Patients with RA ($n = 1514$) were enrolled and divided into the mild, moderate, and severe CKD groups. Total 25(OH)D, 25(OH)D3, and F-25(OH)D in the moderate and severe CKD groups were lower than in the mild CKD group (all $P < 0.05$), while there were no differences in 25(OH)D2 levels ($P = 0.095$). As the severity of CKD progressed, total 25(OH)D, 25(OH)D3, and F-25(OH)D decreased (all *Padj*.<0.05). When progressing from moderate to severe CKD, only 25(OH)D3 decreased significantly (*Padj*.=0.014). Partial correlation and multiple logistic regression analyses revealed a significant association between 25(OH)D3 and the progression of CKD deterioration, as did F-25(OH)D (all $P < 0.05$). Further seasonal stratified analysis showed that this correlation existed only in spring, summer, and autumn for 25(OH)D3 and only in spring and summer for F-25(OH)D ($P < 0.05$). In conclusion, the serum 25(OH)D3 and F-25(OH)D levels may be indicators of CKD progression in patients with RA to plan for timely intervention and management.

Keywords Rheumatoid arthritis, Chronic kidney disease, 25(OH)D metabolites, F-25(OH)D

Rheumatoid arthritis (RA) is a chronic systemic connective tissue inflammatory disease. According to the WHO statistics, 18 million people worldwide were living with RA in 2019, among which 70% were women and 55% were older than 55 years[1]. RA is often characterized by primary inflammatory arthritis (joint inflammation and damage) and subsequent extra-articular connective tissue involvement. With the disease progression, extra-articular organs such as the lungs, cardiovascular system, and kidneys may be affected, resulting in a high risk of disability and even death[2]. At present, there is no effective cure for RA. Management of RA often involves a rehabilitative strategy and medications. Yet, patients with RA often present with renal disease due to systemic inflammation from RA itself or medications[3]. Previous studies reported a considerable incidence of renal dysfunction in RA patients, mainly manifested as hematuria, proteinuria, hypoproteinemia, and so on[3,4]. Moreover, an autopsy of patients with inflammatory RA showed that renal failure was the leading cause of death in 20% of cases[5]. In addition, studies have reported that the incidence of chronic kidney disease (CKD) in patients with RA was >30% higher than in other patients and was an independent risk factor for death in patients with RA[6,7]. Therefore, the clinical recognition and management of renal disease associated with RA lies in adjusting patient management strategy.

Vitamin D (VitD) has been reported associated with the occurrence and progression of RA[8] and CKD[9]. VitD is a secosteroid hormone essential for calcium absorption and bone mineralization, is positively associated with bone mineral density[10], and participates in immunoregulation[11]. Charoenngam et al. found a higher prevalence of RA and relatively low VitD levels in women in the northeastern United States of America, suggesting a

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potential association between the two[12]. Another study from India reported that VitD supplementation could quickly attenuate symptoms in patients with RA and VitD deficiency during the active phase of the disease[13]. Two clinical trials reported similar findings and concluded that VitD supplementation positively affected RA control in patients with RA[14,15]. However, there are also reports of patients with recurrent RA who failed to benefit from VitD supplementation[16]. Thus, the relationship between VitD and the complications of RA progression requires real-world clinical studies to provide support. In addition, the exact role of VitD and its metabolites in patients with RA and CKD remains poorly investigated. The incidence of VitD deficiency is high in patients with CKD, and it plays roles as a cell differentiation and antiproliferation factor by regulating the renin-angiotensin system and the nuclear factor (NF)-κB pathway, which are both involved in CKD pathogenesis and progression[17–19]. Previous studies have shown that patients with CKD and inflammation were at high risk of developing cardiovascular problems compared with those with CKD or inflammation alone[10]. Other studies have shown that patients with RA and CKD had a higher incidence of cardiovascular events[20], diabetes[21], and hypertension[21].

VitD is mainly synthesized in human skin by ultraviolet radiation stimulation. It generally consists of fat-soluble cholesterol metabolites, mainly including 25(OH)D, 1,25(OH)2D, and other metabolites, which have an essential role in the calcium-phosphorus metabolism regulation, maintenance of normal functions of muscle, nerve, and cells, and an essential nutrient for life[22]. Two forms of 25(OH)D are found in circulation: one is the binding type, of which about 85-90% is bound to VitD binding protein (DBP), while the rest (10-15%) is non-specifically bound to albumin (ALB); another is the free type, namely F-25(OH)D, which accounts for about 0.03%. According to the "free hormone theory," only free and non-specifically bound hormone fractions (which are very easy to dissociate) can act on the target cells to exert their biological effects[23]. A 25(OH)D bound to ALB and F-25(OH)D forms bioavailable VitD[24], which can pass through the cell membrane in a free or mediated manner and are hydroxylated into active VitD under the action of liver and kidney 1α hydroxylase, then used by the human body[25].

Although 25(OH)D is an internationally recognized indicator for evaluating VitD reserve, studies have found that such reserves may not be accurately identified under multiple conditions, such as pregnancy[26], nephrotic syndrome[27], end-stage kidney disease (ESKD)[28,29], coronary artery disease[30], and metabolic syndrome[31], which may relate to the content difference of different 25(OH)D metabolites[30,31]. Therefore, it has been suggested that the detection of 25(OH)D should consider the content of different metabolites or forms, such as 25(OH)D2, 25(OH)D3, and F-25(OH)D, rather than only total amounts.

The role of VitD deficiency/insufficiency as a significant risk factor for RA progression is still an area of controversy and has been rarely reported in patients with RA and CKD. Thus, in this study, we explored the association of 25(OH)D metabolites (including 25(OH)D, 25(OH)D2, 25(OH)D3, and F-25(OH)D) with CKD progression in patients with RA to provide clinical evidence for the prevention and control of CKD in patients with RA.

Materials and methods

Patients

All adult patients with RA visiting the Department of Rheumatology of Mianyang Central Hospital between January 2019 and February 2022 were enrolled consecutively in this cross-sectional study. The inclusion criteria were (1) patients above 18 years old and with a 5-year history of RA diagnosed with RA by rheumatologists[32] and (2) diagnosed with CKD after RA was confirmed. The exclusion criteria were (1) patients with thyroid dysfunction or hyperparathyroidism, (2) patients with malignant tumors, (3) co-infection patients during specimen collection, (4) other immune system diseases, (5) patients with hypertension and/or diabetes, (6) patient with CKD history, (7) patients with severe liver disease or malabsorption syndrome, (8) those who received VitD supplement, calcium, hormonal therapy within 1 month of enrollment, or (9) pregnant patients.

All RA patients were assigned to three different CKD severity groups based on their estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR) measured every 1 to 2 months over the last 3 to 6 months, which was recorded in the medical records. (1) Mild CKD group: eGFR ≥ 60 ml/min/1.73 m^2 and ACR < 30 mg/g on at least two of three measurements. (2) Moderate CKD group: eGFR ≥ 60 ml/min/1.73 m^2 and 30 mg/g < ACR ≤ 300 mg/g, or 45 ml/min/1.73 m^2 ≤ eGFR < 60 ml/min/1.73 m^2 and ACR < 30 mg/g, on at least two of three measurements. (3) Severe CKD group: eGFR ≥ 60 ml/min/1.73 m^2 and ACR ≥ 300 mg/g, or 45 ml/min/1.73 m^2 ≤ eGFR < 60 ml/min/1.73 m^2 and ACR ≥ 30 mg/g, or eGFR < 45 ml/min/1.73 m^2, on at least two of three measurements. The criteria were determined according to the CKD classification criteria recommended by KDIGO in 2012[33], as shown in Table 1.

Groups	eGFR (ml/min/1.73m^2)	ACR (mg/g)
Mild CKD	≥60	<30
Moderate CKD	≥60	30–300
	45–59	<30
Severe CKD	≥60	>300
	45–59	>30
	<45	No limit

Table 1. CKD severity groups.

Collection and sample processing

Blood samples were collected after obtaining informed consent from the patients. Briefly, 3 ml of fasting blood was collected in a separation gel-vacuumed blood collection tube (BD, USA) in the morning and then centrifuged at 4000 r/min for 10 min to separate the serum, which was used to measure serum creatinine (Cr), cystatin C (CysC), high-sensitivity C-reactive protein (hsCRP), rheumatoid factor (RF), 25(OH)D2 and 25(OH) D3 levels. Within an hour of blood collection, 5 ml midstream urine samples were collected from all subjects and centrifuged at 3000 r/min for 10 min. The supernatant was used to measure urine albumin (UAlb) and urine creatinine (UCr).

Sample assay

API 4500 high-performance liquid chromatography (AB, USA) and a fat-soluble vitamin detection reagent kit (Fandi Biotechnology Co. Ltd, Chengdu) were used to detect 25(OH)D2 and 25(OH)D3 levels, and the sum of the two was the value of 25(OH)D.

An RT-6100 ELISA analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen) and a commercial ELISA kit (DIA Source Future Diagnostics, Belgium) were used to detect the F-25(OH)D levels. A Cobas c701 automatic biochemical analyzer (Roche, USA) and the original reagents were used to detect Cr, CysC, and hsCRP levels.

A BN II automatic specific protein analyzer (Siemens, DE) and the original reagent were used to detect RF. The A25 automatic biochemical analyzer (BIO Systems, ES) and original reagents were used to detect the UAlb and UCr, and the UAlb/UCr ratio (ACR) was calculated.

Measurement was performed only after calibration and quality control. The eGFR was calculated using the 2012 CKD-EPI Cr-CysC formula[34]:

$$eGFR\ \left(ml/\min/1.73m^2\right) = 135 \times \min\ (Cr/\kappa, 1)^{\alpha} \times \max\ (Cr/\kappa, 1)^{-0.601} \times \min\ (CysC/0.8, 1)^{-0.375} \times \max\ (CysC/0.8, 1)^{-0.711} \times 0.995^{age}\ [\times 0.969\ female]\ \ (male\ \kappa = 0.9,\ female\ \kappa = 0.7;\ male\ \alpha = -0.207,\ female\ \alpha = -0.248)$$

where min represents the minimum value between the calculated value in parentheses and 1, and max represents the maximum value between the calculated value in parentheses and 1.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 (IBM, Armonk, NY, USA). The one-sample Kolmogorov-Smirnov method was used to test the normality of the continuous data. Non-normally distributed continuous data were presented as *M (p25, p75)*. The differences among multiple groups were analyzed using the covariance analysis with adjustment for gender, age, and season. The comparison between the two groups was performed using the Bonferroni test, and the adjusted *P* value (*Padj.*) was used to determine the significant differences between the two groups. The trend analysis of the 25(OH)D metabolite and CKD severity was conducted by the independent-samples Jonckheere-Terpstra test; the significance of the change in 25(OH)D metabolite level for the two groups was analyzed using the mean rank test of Jonckheere-Terpstra and the *Padj.* was used to determine the significance of the changing trend between the two groups. The partial correlation of serum 25(OH)D metabolites with the CKD severity in RA patients was analyzed using the enter method multiple line regression and verified by stepwise method multiple line regression. The association between the serum 25(OH) D metabolites and CKD severity in patients with RA was analyzed by multiple logistic regression. *P* or *Padj.* < 0.05 was considered statistically significant.

Results

General characteristics

A total of 1514 patients with RA, including 341 males and 1173 females, were enrolled in this study. All patients were divided into 1237 patients with mild CKD (271 males vs. 966 females; average age 51), 182 patients with moderate CKD (44 males and 138 females; average age 52), and 95 patients with severe CKD (26 males and 69 females; average age of 56).

25(OH)D metabolites according to gender, age, and seasons

The covariance analysis was used to explore the gender, age, and seasonal differences of 25(OH)D metabolites. The total 25(OH)D levels were different according to gender, age, and season ($F = 8.218$, 4.058, and 37.231, respectively; all $P < 0.05$). 25(OH)D2 was different among different gender and age ($F = 12.872$ and 15.145, both $P < 0.001$), but was not affected by seasons ($F = 1.078$, $P = 0.357$). 25(OH)D3 was different among gender and seasons ($F = 26.520$ and 39.140, both $P < 0.001$) but was not affected by age ($F = 0.001$, $P = 0.989$). F-25(OH)D was not different according to gender, age, or season ($F = 0.178$, 0.041, and 0.802, $P = 0.673$, 0.839, and 0.492, respectively). These results suggest that the relationship between 25(OH)D metabolites and disease is influenced by gender, age, and seasonal factors, except for the F-25(OH)D levels.

Comparison indexes in RA patients according to CKD severity

After adjusting for gender, age, and season, no statistically significant differences in age and gender were observed among mild, moderate, and severe CKD (all $P > 0.05$). Except for 25(OH)D2 and RF, the values of other indexes were statistically different among the three CKD groups (all $P < 0.001$). Compared with the mild CKD group, the levels of serum 25(OH)D, 25(OH)D3, F-25(OH)D, and eGFR were lower in the severe and moderate CKD groups (all *Padj.*<0.05), while the levels of serum Cr, CysC, and hsCRP were higher (all *Padj.*< 0.001). However, when comparing the severe CKD group with the moderate CKD group, there were no statistically significant

Item	Mild CKD group ($n=1237$)	Moderate CKD group ($n=182$)	Severe CKD group ($n=95$)	χ^2, P
Gender (Male/Female)*	271/966	44/138	26/69	1.831, 0.400
Age (y)	51(46, 59)	52(44, 59)	56(47, 61)	4.687, 0.096
25(OH)D (ng/ml)☺	23.80(18.69, 28.92)	20.93(16.83, 25.56)△	19.12(13.83, 24.89)△	49.447, < 0.001
25(OH)D2 (ng/ml)☺	1.77(0.87, 5.38)	2.80(0.94, 6.11)	2.66(0.69, 7.26)	4.713, 0.095
25(OH)D3 (ng/ml)☺	18.50(14.43, 23.73)	16.09(12.93, 21.95)△	13.00(10.60, 20.25)△	51.874, < 0.001
F-25(OH)D (pg/ml)☺	6.03(4.77, 7.81)	5.89(4.60, 6.90)▲	4.87(3.79, 7.05)△	23.512, < 0.001
Cr (μmol/L)	51.1(44.3, 59.7)	57.1(43.0, 75.8)△	105.9(70.7, 133.8)△♀	138.418, < 0.001
CysC (mg/L)	0.93(0.82, 1.07)	1.22(0.95, 1.52)△	1.96(1.63, 2.34)△♀	259.684, < 0.001
eGFR (ml/min/1.73m^2)	95.0(83.1, 108.4)	77.1(62.8, 97.5)△	42.3(33.3, 57.3)△	217.965, < 0.001
ACR (mg/g)	6.48(4.07, 11.22)	42.86(30.32, 70.10)△	102.43(30.81, 305.48)△	437.484, < 0.001
hsCRP (mg/L)	12.79(3.27, 35.99)	23.84(8.00, 55.14)	28.13(10.40, 57.30)	45.472, < 0.001
RF (IU/ml)	85.8(14.08, 306.9)	76.5(11.8, 311.0)	142.0(12.2, 347.5)	1.087, 0.581

Table 2. Comparison of indicators among different CKD groups. *The 2×3 table χ^2 test was used, ☺ the covariance analysis was used, and the others used the Kruskal-Wallis test. After multiple comparisons by Kruskal-Wallis, ▲ represents compared with the Mild CKD group, *Padj.*<0.05; △ represents compared with the Mild CKD group, *Padj.*<0.001; ♀ represents compared with the Moderate CKD group, *Padj.*<0.001.

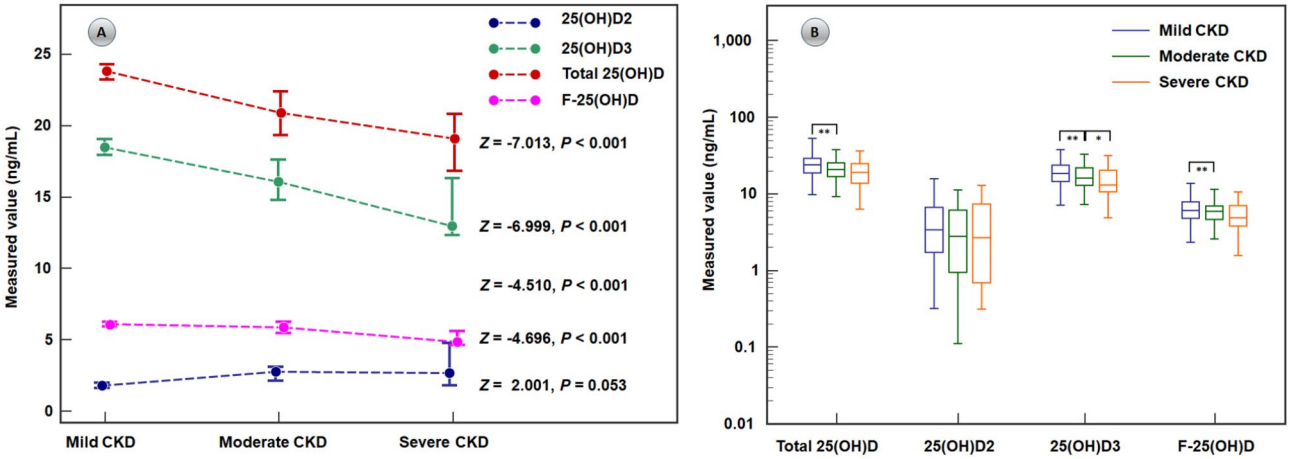

Fig. 1. Changing 25(OH)D metabolites trends with the increased CKD severity in RA patients (unit: ng/ml). (**A**) By the Jonckheere-Terpstra test, a significant decreasing trend was observed in the total 25(OH)D, 25(OH)D3, and F-25(OH)D levels as CKD severity gradually increased, but not in 25(OH)D2 level. (**B**) By post-hoc multiple comparisons of the Jonckheere-Terpstra test, only the change in the level of 25(OH)D3 showed a statistically significant difference in the contiguous CKD severity groups, while changes in 25(OH)D and F-25(OH)D levels only showed statistically significant differences from mild to moderate CKD groups.

differences in the other indexes, except for eGFR, Cr, and CysC (all *Padj.*<0.001). The results are shown in Table 2.

Changing trend of 25(OH)D metabolite according to CKD severity

CKD severity was inversely correlated with serum total 25(OH)D, 25(OH)D3, and F-25(OH)D levels ($P<0.001$), but there were no statistically significant changes in 25(OH)D2 ($P=0.053$) (Fig. 1A). From mild to moderate CKD, there was a statistical difference in the decrease degree for the total 25(OH)D, 25(OH)D3, and F-25(OH)D (all *Padj.*<0.05), while from moderate to severe CKD, only the decrease degree of total 25(OH)D3 showed a statistical significance (*Padj.*=0.014). No changes in the other two metabolites were found among groups (all *Padj.*>0.05) (Fig. 1B).

Partial correlation analysis of 25(OH)D metabolites and CKD severity in patients with RA after adjusting for confounders

The multivariable partial correlation analysis showed that, when using enter method, only F-25(OH)D ($r_{partial}$=-0.139) showed a correlation with CKD severity before adjustment; yet, after adjusting for age, gender, season, hsCRP, and RF, F-25(OH)D ($r_{partial}$=-0.148, $P<0.001$) and 25(OH)D3 ($r_{partial}$=-0.055, P=-0.032) showed correlations with CKD severity. Using the stepwise method, before and after adjustment, F-25(OH)D ($r_{partial}$=-0.136 and −0.145, both $P<0.001$) and 25(OH)D3 ($r_{partial}$=-0.171 and −0.160, both $P<0.001$)

Observational index	Before adjustment			After adjustment *		
	$r_{partial}$	t	*P*	$r_{partial}$	t	*P*
Enter method						
Total 25(OH)D	−0.043	−1.679	0.093	0.035	1.345	0.179
25(OH)D2	0.031	1.206	0.228	0.043	1.674	0.094
25(OH)D3	0.022	0.846	0.398	**−0.055**	**−2.143**	**0.032**
F-25(OH)D	**−0.139**	**−5.438**	**< 0.001**	**−0.148**	**−5.818**	**< 0.001**
Stepwise method**						
25(OH)D3	**−0.171**	**−6.735**	**< 0.001**	**−0.160**	**−6.306**	**< 0.001**
F-25(OH)D	**−0.136**	**−5.344**	**< 0.001**	**−0.145**	**−5.681**	**< 0.001**

Table 3. The partial correlation coefficient of serum 25(OH)D levels with the severity of CKD in RA patients. Significant values are given in bold. *Adjusted factors included age, gender, season, hsCRP, and RF. These results showed that F-25(OH)D and 25(OH)D3 are partially correlated with CKD severity in RA patients after adjusting for age, gender, season, hsCRP, and RF. ** Stepwise method excluded 25(OH)D2 and Total 25(OH)D with $P > 0.10$ or multicollinearity.

Observational index	Before adjustment			After adjustment **		
	$r_{partial}$	t	*P*	$r_{partial}$	t	*P*
Spring						
25(OH)D3	**−0.115**	**−2.724**	**0.007**	**−0.119**	**−2.817**	**0.005**
F-25(OH)D	**−0.172**	**−4.110**	**< 0.001**	**−0.182**	**−4.351**	**< 0.001**
Summer						
25(OH)D3	**−0.198**	**−4.436**	**< 0.001**	**−0.187**	**−4.174**	**< 0.001**
F-25(OH)D	**−0.143**	**−3.170**	**0.002**	**−0.143**	**−3.179**	**0.002**
Autumn						
25(OH)D2	**−0.155**	**−2.408**	**0.017**	**-0.141**	**−2.207**	**0.026**
25(OH)D3	**−0.313**	**−5.064**	**< 0.001**	**−0.305**	**−4.875**	**< 0.001**
F-25(OH)D	**−0.133**	**−2.068**	**0.040**	**−0.130**	**−2.024**	**0.047**
Winter						
25(OH)D3	−0.029	−0.431	0.667	−0.009	−0.128	0.898
F-25(OH)D	−0.012	−0.180	0.857	−0.028	−0.411	0.682

Table 4. Partial correlation coefficient between 25(OH)D levels and CKD severity; stratified by season. Significant values are given in bold. ** The stepwise method was used, and adjusted factors included age, gender, hsCRP, and RF. These results showed that F-25(OH)D and 25(OH)D3 showed a partial correlation with CKD severity during spring, summer, and autumn; meanwhile, 25(OH)D2 showed a partial correlation with CKD severity only during autumn.

consistently showed correlations with CKD severity. The results are shown in Table 3. These data suggest that confounders may primarily interfere with correctly assessing the relationship between 25(OH)D3 and CKD severity but not for F-25(OH)D.

The influence of seasonal factors was further analyzed. The results showed that, in spring, summer, and autumn, but not winter, both F-25(OH)D and 25(OH)D3 showed a partial correlation with CKD severity regardless of adjustment for age, gender, hsCRP, and RF or not (before adjustment: $r_{partial}$=-0.115~-0.313, all $P < 0.05$; after adjustment $r_{partial}$=-0.119~-0.305, all $P < 0.05$). Meanwhile, in autumn, 25(OH)D2 showed a partial correlation with CKD severity ($r_{partial}$=-0.155 and − 0.141, $P = 0.017$ and 0.026) (Table 4). These results suggested that the season should be considered an important factor when analyzing the relationship between 25(OH)D metabolites and CKD severity. F-25(OH)D and 25(OH)D3 correlated better with CKD severity except in winter.

Logistic regression analysis of 25(OH)D metabolites and CKD severity in patients with RA

After adjusting for age, gender, hsCRP, and RF, and taking the mild CKD group as the control group, multiple logistic regression analysis of each 25(OH)D metabolites and the progression of CKD showed that 25(OH)D3 and F-25(OH)D were correlated to CKD severity (for the moderate group: $OR = 0.956$ and 0.847; for the severe group: $OR = 0.895$ and 0.766, all $P < 0.001$) without considering the influence of season. Further analysis after consideration of seasonal factors showed that, in spring and summer, 25(OH)D3 and F-25(OH)D presented a significant correlation with CKD severity (for the moderate group: 25(OH)D3 $OR = 0.960$ and 0.943, F-25(OH)D $OR = 0.824$ and 0.809; for the severe group: 25(OH)D3 $OR = 0.932$ and 0.901, F-25(OH)D $OR = 0.727$ and 0.786, all $P < 0.05$). In autumn, only 25(OH)D3 presented a significant correlation with CKD severity (for the moderate group: $OR = 0.919$; for the serious group: $OR = 0.735$, all $P < 0.05$) (Table 5). In winter, no 25(OH)D metabolites

Season	Moderate CKD*			Severe CKD*		
	OR (95%CI)	Wald χ2	*P*	OR (95%CI)	Wald χ2	*P*
All seasons (*n* = 1514)						
25(OH)D3	**0.956(0.931, 0.981)**	11.255	**0.001**	**0.895(0.861, 0.931)**	30.942	**< 0.001**
F-25(OH)D	**0.847(0.779, 0.922)**	14.762	**< 0.001**	**0.766(0.680, 0.864)**	18.867	**< 0.001**
Spring (*n* = 558)						
25(OH)D3	**0.960(0.923, 0.999)**	3.998	**0.046**	**0.932(0.875, 0.991)**	4.9778	**0.026**
F-25(OH)D	**0.824(0.732, 0.928)**	10.176	**0.001**	**0.727(0.604, 0.875)**	11.334	**0.001**
Summer (*n* = 486)						
25(OH)D3	**0.943(0.895, 0.994)**	4.688	**0.030**	**0.901(0.850,0.956)**	12.117	**< 0.001**
F-25(OH)D	**0.809(0.666, 0.983)**	4.570	**0.033**	**0.786(0.646,0.956)**	5.777	**0.016**
Autumn (*n* = 240)						
25(OH)D3	**0.919(0.860,0.981)**	6.399	**0.011**	**0.735(0.640,0.845)**	18.916	**< 0.001**
F-25(OH)D	NA	NA	NA	NA	NA	NA
Winter (*n* = 230)						
25(OH)D3	NA	NA	NA	NA	NA	NA
F-25(OH)D	NA	NA	NA	NA	NA	NA

Table 5. Logistic regression analysis of 25(OH)D metabolites with CKD severity in RA patients. Significant values are given in bold. *Relative to the mild CKD group. Stepwise logistic regression was used, and adjusted factors included age, gender, season, hsCRP, and RF. "NA" means the variable is excluded from regression analysis because $P > 0.1$.

showed a correlation with CKD severity. The results further suggested the potential influence of seasonal factors on the association between 25(OH)D metabolites and CKD severity. In addition, besides 25(OH)D3, F-25(OH)D is also an important indicator of CKD severity, even when seasonal factors are considered.

Discussion

Measuring 25(OH)D remains the most widely used method to assess VitD deficiency. Based on the 2011 Endocrine Society Clinical Practice Guidelines[35], VitD status is defined as optimal (i.e., serum 25(OH)D concentration: 30–150 ng/mL) and suboptimal (i.e., serum 25(OH)D concentration < 30 ng/mL, hypovitaminosis D). A serum 25(OH)D level < 10 ng/mL (25 nmol/L) represents VitD deficiency and leads to a reduction in serum 1,25(OH)2D. Yet, studies have suggested that confiding factors such as age, gender, race, and physical activity, among others, are important for correctly assessing 25(OH)D[36]. The main effects of age on VitD include intestinal resistance of calcium absorption to circulating 1,25(OH)2D, decreased VDR, decreased renal production of 1,25(OH)2D by the aging kidney, decreased skin production of VitD, and substrate deficiency of VitD[37]. Werdoia et al. assessed the impact of gender difference on VitD status and its relationship with the extent of coronary artery disease (CAD) and discovered that lower 25(OH)D levels observed in females had a more relevant role in conditioning the severity of CAD[38]. Another study found that vitD insufficiency/deficiency was highly prevalent in patients with stage 5 CKD on HD, and lower values seem to be related to female gender[39].

This study explored the relationship between 25(OH)D metabolites and the progression of RA complicated with CKD. After performing multivariable partial correlation analysis and after adjusting for age, gender, season, hsCRP, and RF, F-25(OH)D and 25(OH)D3 showed a correlation with CKD severity, but it was not observed for total 25(OH)D and 25(OH)D2. As the severity of CKD increased from mild to moderate, total 25(OH)D, 25(OH)D3, and F-25(OH)D decreased, while from the moderate to severe CKD status, only 25(OH)D3 showed a significant difference. These results indicated that gender, age, hsCRP, RF, and season were all influencing factors in correctly assessing the relationship between 25(OH)D3 and F-25(OH)D and CKD severity. 25(OH)D3 has a potential role in the pathogenesis of RA by inhibiting the expression of IL-6, which may be the principle of its anti-inflammatory effect[31]. F-25(OH)D demonstrates enhanced absorption and conversion into biologically active 1,25-(OH)D within the renal system, thereby exerting profound physiological effects[40]. Hence, it is imperative to consider evaluating the levels of 25(OH)D3 and F-25(OH)D in the comprehensive management of patients with RA to prevent the onset and progression of CKD.

Next, the duration and intensity of sunshine exposure were considered since VitD is produced by the skin exposed to sunlight. During this process, ultraviolet-B (UVB) light stimulates the conversion of 7-dehydrocholesterol (provitamin D) in the skin to pre-VitD, which isomerizes into VitD. Hence, season acts as a contextual factor potentially confounding associations between vitD and disease course, including RA[15]. In this study, patients with RA were enrolled in the Department of Rheumatology of Mianyang Central Hospital, China. As a second-tier city in western China, Mianyang is located in the northern temperate hilly region north of the Tropic of Cancer, and it has uneven light throughout the four seasons. Therefore, the influence of seasons was analyzed. It was found that regardless of whether adjusting for potential impact factors, F-25(OH)D and 25(OH)D3 showed a partial correlation with CKD severity in spring, summer, and autumn but not in winter. After adjusting the above-influencing factors and taking the mild CKD group as the control group, 25(OH)D3 and F-25(OH)D presented an independent correlation with CKD severity in spring and summer.

For treating VitD deficiency in adults, oral ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) is preferred. Nevertheless, although there is a clinical consensus to administer supplemental therapy to VitD-deficient patients with RA, the choice of D2 or D3 often baffles clinicians. The present study tends to support supplementation with D3. Meanwhile, other studies have shown that selecting D2 preparations may lead to competition with D3 hydroxylation, affecting the serum 25(OH)D level, but more research data are needed to support it in different diseases[41,42]. The results also showed that although there were no statistically significant differences, 25(OH)D2 presented a superficial upward trend with CKD progression in patients with RA, and the distribution of 25(OH)D2 value was extremely heterogeneous (0.5-20.27 ng/ml). Therefore, the relation between 25(OH)D2 and disease progression needs further study.

This study has a few limitations. First, the study had a relatively small sample size, especially those with moderate and severe CKD. In addition, there was a lack of ethnic diversity and a lack of further follow-up. Drugs like anticonvulsants, cholestyramine, and orlistat can influence VitD metabolism, but the data were not collected.

In conclusion, this study indicates that 25(OH)D insufficiency or deficiency is common in patients with RA and CKD. It was also revealed for the first time that the 25(OH)D metabolites, 25(OH)D3 and F-25(OH)D, are closely related to the progression of CKD in patients with RA. Therefore, monitoring the serum 25(OH)D3 and F-25(OH)D levels in patients with RA may be valuable for risk prediction of CKD progression in patients with RA.

Data availability

All data generated or analysed during this study are included in this published article.

Received: 25 March 2024; Accepted: 10 April 2025
Published online: 25 April 2025

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Acknowledgements

The author would like to thank Prof. Jiafu Feng for providing technical guidance and a testing platform (including reagents, materials, and analysis tools) for this study.

Author contributions

Conceived and designed the experiments: Jie Tang, Yuwei Yang, and Wenbing Deng. Obtained the funding: Jie Tang and Yuwei Yang. Patient management: Jinmei Zou and Yan Wu. Performed the experiments: Wenqiang Jiang, Bei Xu. Analyzed the data: Jie Tang and Yuwei Yang. Drafted the original manuscript: Jie Tang, Yuwei Yang, and Lijuan Wu. All authors critically reviewed and approved the final manuscript.

Funding

This study was financially supported by the Basic Application Project of the Science & Technology Department of Sichuan Province [2019YJ0701] and the Incubation Project of Mianyang Central Hospital, School of Medicine, University of Electronic Science and Technology of China [2022FH005]. The authors have no other relevant affiliations or financial involvement with any organization apart from those disclosed.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All procedures were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This study was approved by the Medical Ethics Committee of Mianyang Central Hospital, School of Medicine, University of Electronic Science and Technology of China (approval No.: S-2018-085, approval date: Oct. 09, 2018). All patients provided written informed consent before treatment. All methods were carried out in accordance with relevant guidelines and regulations.

Additional information

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