

Clinical Observation and Analysis of Patients with Idiopathic Membranous Nephropathy Combined with Low 25(OH) D₃ Levels

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Research article

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Abstract

Background: To investigate the correlations between 25-hydroxyvitamin D₃ [25(OH) D₃] levels and selected clinical indicators and renal pathology at initial onset of idiopathic membranous nephropathy (IMN) and to analyze changes in the main quantifiable indicators in IMN patients with different 25(OH) D₃ levels.

Methods: A total of 109 patients admitted to the Department of Nephrology of the First Affiliated Hospital of Guangxi Medical University between August 2015 and April 2019 and diagnosed with IMN were reviewed. Comparative analysis of the effects of different 25(OH)D₃ levels on clinical and pathological indicators of IMN was performed, and the changes in serum albumin, eGFR, serum creatinine and other clinical indicators in these patients during follow-up were analyzed.

Results: The 109 patients with IMN had 25(OH)D₃ deficiency [25(OH)D₃<70 nmol/L]. The incidence of nephrotic syndrome, serum phosphorus level, hs-CRP level, and ESR were higher in the 25(OH)D₃<25nmol/L group than in the 25(OH)D₃≥25nmol/L group. In contrast, the TP, ALB, serum calcium, and IgG levels were lower in the 25(OH)D₃<25nmol/L group (P<0.05). No pathological parameters differed significantly between the two groups (P>0.05). Bivariate correlation analysis showed that 25(OH)D₃ was positively correlated with nephrotic syndrome, the TP level, the ALB level, the eGFR, the serum calcium level and the IgG level (P<0.05) and negatively correlated with age, blood pressure, the Scr level, 24-hour UP quantitation, the serum phosphorus level, the hs-CRP level and the ESR (P<0.05). In single-factor repeated measures analysis of variance, the ALB levels in the 25(OH)D₃<25nmol/L and 25(OH)D₃≥25nmol/L groups showed an increasing trend but were lower in the 25(OH)D₃<25nmol/L group than in the 25(OH)D₃≥25 nmol/L group at each follow-up time point (P<0.001).

Conclusion: Reduced 25(OH)D₃ levels are prevalent among IMN patients. During follow-up, serum albumin increased significantly in patients administered vitamin D supplementation. 25(OH)D₃ may play an important role in IMN development.

Introduction

Membranous nephropathy (MN) refers to a group of glomerular diseases with epithelial-side immune complex deposition and diffuse thickening of the glomerular basement membrane as a pathological feature. MN of unknown etiology is called idiopathic membranous nephropathy (IMN). IMN is a common glomerular disease that causes nephrotic syndrome in the elderly population, accounting for approximately 25% of cases of nephrotic syndrome, and is more common in men than in women (1.1~2.4:1)^[1]. In most patients, onset is insidious, and approximately 80% of patients have nephrotic syndrome, 20% to 25% have asymptomatic proteinuria, 30% to 50% have microscopic hematuria, and 20% to 40% have varying degrees of hypertension and renal function injury. In recent years, clinical studies have found that the incidence of IMN is increasing annually, that the age of onset is gradually becoming lower, and that the prognosis is vastly different. A 2010 multicenter study from Spain suggested that the spontaneous response rate of IMN patients was 31.7% and that 29.4% developed end-stage renal disease (ESRD) or died^[2].

Vitamin D₃ is a naturally occurring fat-soluble vitamin also called cholecalciferol. It is a ring-opened form of cholesterol and is a steroid hormone. Two hydroxylation reactions are required for its biological activity as a hormone. First, the reaction producing 25(OH)D₃ from vitamin D₃ is catalyzed in the liver by 25-hydroxylase, and 1 α -hydroxylase then catalyzes the production of 1,25-dihydroxyvitamin D₃ from 25(OH)D₃ in the proximal tubular epithelial cells of the kidney. 25(OH)D₃ binds to nuclear receptors in target cells and produces effects by regulating gene expression. Vitamin D receptors are widely present in cells of human tissues, including podocytes in the kidney^[3]. The kidney is not only an important location of active vitamin D₃ synthesis but also an important organ that affects the metabolism of active vitamin D₃. The classic physiological role of vitamin D₃ is to regulate calcium and phosphorus metabolism. In addition, it also has nonclassical physiological effects, such as regulating immune function, cell growth and differentiation.

In clinical work, we found that patients with IMN often have hypocalcemia and low serum 25(OH)D₃ levels. This pattern suggests that the pathophysiology of IMN is related to active vitamin D₃. However, the current research on active vitamin D₃ and kidney diseases focuses mostly on chronic kidney disease and diabetic nephropathy. Few studies have addressed active vitamin D₃ in IMN, and domestic and international studies on the renoprotective effects of active vitamin D₃ are primarily limited to basic experimental research over clinical research. Therefore, we used a cohort of 109 IMN patients as the research object to understand the correlations between 25(OH)D₃ levels and other clinical indicators as well as renal pathological changes. By following up and observing the

changes in clinical indexes of patients with different levels of active vitamin D₃, the clinical significance of 25(OH)D₃ in the course of IMN treatment was evaluated.

Methods

Patients

We performed a retrospective analysis of 109 patients who were hospitalized in the Department of Nephrology of the First Affiliated Hospital of Guangxi Medical University between August 2015 and April 2019. All patients were diagnosed with membranous nephropathy by renal biopsy and pathology, and all had a follow-up time of longer than 6 months. We excluded patients with secondary membranous nephropathy caused by etiologies such as infection (hepatitis virus or HIV, etc.), autoimmune disease (systemic lupus erythematosus, etc.), metabolic disease (diabetes, etc.), malignant tumors, drug or heavy metal exposure, and familial or other factors. In addition, patients with clear or suspected acute kidney injury, vitamin D supplementation before renal biopsy, and failure to follow the 2012 Kidney Disease Improving Global (KDIGO) guidelines were excluded. Of the patients, 67 were male (61.47%) and 42 were female (38.53%). The male:female ratio was 1.60:1, the age range was 15 to 72 (43.61 ± 14.21) years, and 87 patients (79.82%) were over 30 years old. Among the patients, there were 54 cases (49.54%) of nephrotic syndrome.

Data collection

The fasting venous blood 25(OH)D₃ level, routine blood count, serum total protein level, albumin level, renal function, 24-hour urinary protein (UP) quantification, blood calcium and phosphorus levels, high-sensitivity C-reactive protein (hs-CRP) level, erythrocyte sedimentation rate (ESR), and immunoglobulin G (IgG) level at admission were collected. The glomerular filtration rate (GFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula according to the patient's sex, age, and serum creatinine level.

Each patient underwent renal biopsy within 3 days after admission. The renal pathological results of all patients, including the Ehrenreich-Churg stage (divided into stages I, II, III, and IV; if two or more stages existed simultaneously, the highest stage was recorded), glomerular sclerosis ratio, and qualitative tubule lesion assessment results (renal interstitial fibrosis and renal tubular atrophy), were recorded.

According to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines^[4], a 25(OH)D₃ concentration of ≥ 75 nmol/L indicates sufficiency, a concentration of < 75 nmol/L and ≥ 25 nmol/L indicates insufficiency, and a concentration of < 25 nmol/L indicates a lack. At admission, the level of 25(OH)D₃ was < 75 nmol/L in all patients; thus, daily vitamin D supplementation (600 IU) was begun in all patients on the third day after admission, and the patients were divided into two groups: the 25(OH)D₃ ≥ 25 nmol/L group and the 25(OH)D₃ < 25 nmol/L group. The follow-up data were collected from the medical records of the inpatients and outpatients.

Statistical methods

SPSS 17.0 software was used for statistical analysis. The statistical results for normally distributed data are presented as $\bar{x} \pm s$ values, and the t-test was used to compare the means of the two groups. Nonnormally distributed data are presented as M (P25, P75), and nonparametric tests were used for comparisons between the two groups. Repeated measures data were analyzed by repeated measures analysis of variance; count data are expressed as the numbers of cases (percentages), and comparisons between groups were evaluated by χ^2 tests. Correlations between two continuous variables were analyzed by Pearson correlation analysis, and correlations between categorical variables or graded data were analyzed by Spearman correlation analysis. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of IMN patients

All 109 patients had insufficiency or a lack of 25(OH)D₃, although the degree of kidney disease was different. There were 54 cases (49.54%) of nephrotic syndrome but 91 (83.48%) of hypoproteinemia; some patients exhibited a variable decrease in the estimated glomerular filtration rate (eGFR), an increase in serum creatinine, and dysregulation of calcium and phosphorus metabolism at onset.

Clinical characteristics of IMN patients in different pathological stages

The renal pathological stages of the 109 patients ranged from 1-3, and patients were grouped according to the stage of MN. Because there was only 1 case in IMN 3 stage, it was not included in the analysis. Except for the combination of hypertension, the difference in renal pathological stage was not statistically significant (Table 1).

Table 1:
Clinical characteristics of IMN patients in different pathological stages

	Stage 1 n=32)	Stage 2 n=76)	Statistics	P
Age/year	41.41±14.36	44.79±14.05	-1.135	0.259
Gender/male[n%]	17(53.1%)	49(64.5%)	1.220	0.269
Hypertension[n%]	8(25.0%)	36(47.4%)	4.667	0.031
Nephrotic syndrome[n%]	13(40.6%)	41(53.9%)	1.599	0.206
25(OH)D ₃ (nmol/L)	14.60(6.79,26.37)	12.12(6.79,20.81)	-0.674	0.500
TP/g/L	45.21±8.44	45.17±8.49	0.019	0.985
ALB/g/L	24.72±6.68	23.89±5.63	0.660	0.511
TC/mmol/L	8.97±3.80	8.40±3.02	0.819	0.414
TG/mmol/L	1.72(0.89,2.58)	1.98(1.22,3.20)	-1.433	0.152
LDL-C/mmol/L	6.11±3.40	5.55±2.50	0.956	0.341
Scr/μmol/L	76.00(66.50,86.75)	77.00(63.00,96.75)	-0.441	0.659
UA/μmol/L	389.44±106.39	398.46±120.20	-0.368	0.714
eGFR [ml(min·1.73m ²)]	97.86±22.52	93.69±27.66	0.755	0.452
24hUP/g/24h	4.25±3.52	5.31±3.87	-1.331	0.186
blood calcium/mmol/L	1.97±0.13	1.97±0.14	-0.145	0.885
blood phosphorus/mmol/L	1.00(1.00,1.75)	1.00(1.00,1.00)	-0.145	0.884
hs-CRP/mg/L	5.93(3.36,18.34)	8.08(4.39,17.37)	-0.646	0.518
ESR/mm/h	33.94±24.38	37.59±20.86	-0.790	0.431
IgG/g/L	5.49±2.38	5.59±2.81	-0.186	0.853

Clinical characteristics of IMN patients with different 25(OH)D₃ levels

The proportion of patients with nephrotic syndrome, the blood phosphorus level, the hs-CRP level, and the ESR level were higher but the TP level, ALB level, blood calcium level and IgG level were lower in the 25(OH)D₃ <25nmol/L group than in the 25(OH)D₃ ≥25nmol/L group (P <0.05). There was no statistically significant difference in pathological indicators between the two groups (Table 2).

Table 2:
Clinical characteristics of IMN patients with different 25(OH)D₃ levels

	<25nmol/L	≥25nmol/L	Statistics	P
	n=82)	n=27)		
Age/year	42.98±14.94	45.56±11.74	-0.817	0.416
Gender/male[n(%)]	50(61.0%)	17(63.0%)	0.034	0.854
Hypertension[n(%)]	35(42.7%)	10(37.0%)	0.267	0.605
Nephrotic syndrome[n(%)]	46(56.1%)	8(29.6%)	5.692	0.017
TP/g/L	42.27±6.21	53.51±9.06	-7.230	<0.001
ALB/g/L	22.00±4.47	30.22±5.87	-7.642	<0.001
Scr/μmol/L	83.87±31.39	81.59±26.14	0.339	0.735
UA/μmol/L	390.16±112.72	408.26±126.50	-0.702	0.484
eGFR [ml/(min·1.73m ²)]	94.33±27.11	95.60±23.89	-0.218	0.828
24hUP/g/24h	5.24±4.02	4.08±2.91	1.384	0.169
blood calcium/mmole/L	1.94±0.12	2.08±0.14	-5.177	<0.001
blood phosphorus/mmole/L	1.23[1.10,1.39]	1.08[1.01,1.19]	-3.061	0.002
hs-CRP/mg/L	8.62(4.92,19.54)	5.26(3.00,12.65)	-2.180	0.029
ESR/mm/h	41.39±21.38	22.40±16.85	4.203	<0.001
IgG/g/L	5.04±2.74	6.98±1.98	-3.383	0.001
Kidney pathology indicators				
Spherical sclerosis[n(%)]	39[47.6%]	12[44.4%]	0.079	0.778
Tubular atrophy[n(%)]	53(64.6%)	15(55.6%)	0.713	0.398
Renal interstitial fibrosis[n(%)]	46(56.1%)	15(55.6%)	0.002	0.961

Correlation between the 25(OH)D₃ level and clinical indicators in IMN patients

Correlation analysis showed that 25(OH)D₃ was correlated with multiple clinical indicators. Specifically, the 25(OH)D₃ level was positively correlated with nephrotic syndrome, the TP level, the ALB level, the eGFR, the blood calcium level and the IgG level (all P <0.05) and negatively correlated with age, blood pressure, the serum creatinine level, the 24-h UP quantitation, the blood phosphorus level, the hs-CRP level and the ESR (all P <0.05) (Table 3).

Table 3:
Correlation between the 25(OH)D₃ level and
clinical indicators

Clinical indicators	<i>r</i>	<i>P</i>
Age	-0.923	<0.001
Gende	-0.048	0.619
Hypertension	-0.335	<0.001
Nephrotic syndrome	0.513	<0.001
TP	0.963	<0.001
ALB	0.889	<0.001
Scr	-0.701	<0.001
UA	0.016	0.868
eGFR	0.985	<0.001
24hUP	-0.204	0.034
blood calcium	0.984	<0.001
blood phosphorus	-0.938	<0.001
hs-CRP	-0.620	<0.001
ESR	-0.783	<0.001
IgG	0.913	<0.001

Follow-up results

According to the level of 25(OH)D₃, the patients were divided into the 25(OH)D₃≥25nmol/L group and the 25(OH)D₃<25nmol/L group. Serum albumin, eGFR, and serum creatinine were analyzed at 7 time points, and the changes in the above clinical indicators in the two groups were analyzed.

Changes in serum albumin (Table 4, Figure 1)

The difference in the ALB level between the two groups was statistically significant ($F_{\text{group}} = -7.642$, $P_{\text{group}} < 0.001$). The ALB level in the 25(OH)D₃<25nmol/L group was lower than that in the 25(OH)D₃≥25nmol/L group (all $P < 0.05$). The ALB level showed a time-dependent trend ($F_{\text{time}} = 2.593$, $P_{\text{time}} = 0.036$), and the overall ALB level showed an increasing trend in both groups. There was an interaction effect between the grouping and the follow-up time ($F_{\text{interaction}} = 4.756$, $P_{\text{interaction}} = 0.001$).

Changes in eGFR (Table 4, Figure 2)

There was no statistically significant difference in the eGFR between the two groups ($F_{\text{group}} = -0.218$, $P_{\text{group}} < 0.828$), and there was no interaction between the grouping and the follow-up time ($F_{\text{interaction}} = 1.258$, $P_{\text{interaction}} = 0.283$).

Changes in serum creatinine (Table 4, Figure 3)

There was no statistically significant difference in the serum creatinine level between the two groups ($F_{\text{group}} = -0.441$, $P_{\text{group}} = 0.659$), and there was no interaction between the grouping and the follow-up time ($F_{\text{interaction}} = 2.106$, $P_{\text{interaction}} = 0.074$).

Table 4:
Changes in serum albumin, eGFR and serum creatinine

Groups	N	0months	1month	2months	3months	4months	5months	6months
ALB								
25(OH)D ₃ ≥ 25nmol/L	82	21.99±4.47	29.37±5.98	31.02±6.38	32.12±6.58	33.01±6.70	34.27±6.89	35.10±8.02
25(OH)D ₃ < 25nmol/L	27	30.22±5.87*	33.07±5.42*	35.15±6.02*	35.53±5.38*	36.66±5.41*	37.51±4.53*	37.00±5.47*
eGFR								
25(OH)D ₃ ≥ 25nmol/L	82	94.33±27.11	92.14±20.77	89.86±22.16	91.42±22.22	90.25±20.63	88.07±21.46	90.73±23.49
25(OH)D ₃ < 25nmol/L	27	95.60±23.89	94.01±18.18	90.17±90.17	85.35±85.35	86.89±21.84	84.48±19.85	88.40±19.42
Scr								
25(OH)D ₃ ≥ 25nmol/L	82	83.86±31.39	84.41±22.15	86.56±29.53	84.42±25.06	85.25±24.50	88.69±24.18	87.24±27.59
25(OH)D ₃ < 25nmol/L	27	81.59±26.14	80.32±16.8	83.37±16.88	87.74±24.41	89.42±26.79	90.58±23.69	85.43±20.83
Compared with 25(OH)D ₃ ≥ 25nmol/L group *P<0.05								

Discussion

In recent years, the incidence and prevalence of idiopathic membranous nephropathy have shown an increasing trend. ZHU [5] found that the incidence of IMN among patients with primary glomerular diseases increased from 16.8% in 2003-2007 to 29.35% in 2008-2012. In China, Yu Xiaofang [6] also found that the detection rate of IMN in renal biopsy was 11.1%, a significant increase from the previous rate. The pathogenesis of IMN is not yet clear. Newly discovered autoantigens, such as PLA2R [7] and THSD7A [8], and complement system activation have been found to be involved in the pathogenesis of MN [9], providing us with a better understanding of the causes of IMN. However, the effects of IMN treatment differ greatly, and many researchers are constantly looking for biological indicators for the disease status and prognosis of IMN.

Vitamin D is a steroid derivative that first undergoes a reaction catalyzed by 25-hydroxylase in the liver to produce 25(OH)D₃ and then undergoes a reaction catalyzed by 1- α -hydroxylase in the kidney to produce active vitamin D₃, which binds to intracellular vitamin D₃ receptors. Vitamin D exerts biological effects, participating in the regulation of calcium and phosphorus in the body and the differentiation, growth and function of immune cells [10]. Current research has shown that vitamin D₃ receptors are expressed in more than 30 cells, tissues and organs in humans [11]; thus, active vitamin D₃ is associated with diseases of many body systems, such as the circulatory system and endocrine system. 25(OH)D₃ that enters the blood from the liver is a precursor of active vitamin D₃. Because of its high concentration in blood, good stability and long half-life, 25(OH)D₃ can accurately reflect the nutritional vitamin D status in the body and is thus generally used as a clinical indicator for evaluating vitamin D sufficiency [12]. We found in clinical work that not only patients with chronic kidney disease but also patients with nephrotic syndrome often have low 25(OH)D₃ levels. Earlier studies showed that the incidence of low 25(OH)D₃ levels in patients with nephrotic syndrome exceeds 90% [13]. The results of our study showed that IMN patients generally have low 25(OH)D₃ levels (a detection rate of 100% and the <25nmol/L as high as 75.41%), with an average level of 12.27 (range: 6.79-24.91) nmol/L. This study retrospectively analyzed the correlation of 25(OH)D₃ levels with clinical and pathological indicators in 109 patients diagnosed with IMN, and repeated measures analysis of variance was used to analyze changes in selected clinical indicators at 6 months after treatment initiation.

A total of 109 patients with IMN were enrolled in this study, with a male:female ratio of 1.60:1. Of the patients, 79.82% were over 30 years old and approximately 41.28% had hypertension, similar to the percentages in other domestic and international reports; in addition, 49.54% had nephrotic syndrome, less than that reported in related studies. Pathological analysis of the kidneys showed that the glomerular lesions were mainly stage I and II. Grouping the patients by IMN stage and comparing the differences in the related clinical indicators showed that patients with stage II IMN had a higher risk of developing hypertension, consistent with the results of previous studies [2]. Grouping patients by 25(OH)D₃ level revealed significant differences in the levels of serum albumin, blood lipids, CRP and other indicators. Correlation analysis between the 25(OH)D₃ level and clinical indicators showed that 25(OH)D₃ was correlated with age, hypertension, nephrotic syndrome, 24-hour urinary protein, serum albumin levels, blood lipid levels, CRP levels and other indicators.

High levels of proteinuria and hypoproteinemia are necessary for the diagnosis of nephrotic syndrome. A high level of proteinuria is also the main predictor chronic kidney disease progression. Low serum albumin is an independent risk factor for a poor response to immunosuppressive therapy in IMN patients [6]. Many studies have shown that a high level of proteinuria is associated with vitamin D deficiency [14, 15]. Vitamin D deficiency aggravates urinary protein excretion and causes a further decrease in serum albumin. A high level of proteinuria in IMN patients is related to podocyte damage. Active vitamin D₃ can protect podocytes through a variety of mechanisms, as follows. Active vitamin D₃ can inhibit the renin-angiotensin system (RAS), negatively regulate the expression of angiotensin II and renin, and reduce podocyte shedding and damage. Active vitamin D₃ can inhibit TGF-β/Smad pathway signaling, activate the BMP-7/Smad pathway, inhibit the protein expression of desmin, and reduce podocyte damage [16]. Active vitamin D₃ regulates the Wnt/β-catenin pathway in podocytes and inhibits podocyte epithelial-mesenchymal transition (EMT). Active vitamin D₃ regulates TRPC6 expression to reduce podocyte damage, urinary protein excretion, etc. A high level of proteinuria also exacerbates the lack of vitamin D. Mechanistically, a high level of proteinuria leads to loss of the vitamin D binding protein [17] and a decrease in the Megalin receptor content. In this study, the 24-hour urinary protein quantitation was negatively correlated with the 25(OH)D₃ level, and the serum albumin level was positively correlated with the 25(OH)D₃ level. Serum albumin levels were significantly different in patients with different levels of 25(OH)D₃: in patients with 25(OH)D₃ <25 nmol/L, the serum albumin level was significantly lower than that in patients with 25(OH)D₃ ≥25 nmol/L. However, the difference in 24-hour urinary protein quantification was not statistically significant. This discrepancy may have occurred because of an incorrect methodology of retaining the 24-hour urine sample, leading to experimental errors, the large fluctuation in the daily urinary protein quantitation of patients with MN (may differ according to from the patient's protein intake, body position and activity level) and other factors.

C-reactive protein is an acute-phase protein that is synthesized by the liver under stimulation by inflammatory factors such as IL-6. hs-CRP is an extremely sensitive inflammatory marker. When the body undergoes immune injury or infection, level of hs-CRP in the blood increases. Chronic inflammation of the kidney is an important factor in the progression of renal impairment in CKD patients. Recent studies have shown that vitamin D deficiency can also lead to increased CRP levels and that supplementation with paricalcitol can reduce urinary protein excretion and downregulate hs-CRP [18, 19]. The current study showed that among IMN patients, the hs-CRP level in the 25(OH)D₃ <25nmol/L group was significantly higher than that in the 25(OH)D₃ ≥25nmol/L group. The hs-CRP and 25(OH)D₃ levels were negatively correlated, indicating that the hs-CRP level was correlated with the vitamin D₃ level and may aggravate proteinuria through an inflammatory reaction.

In terms of pathological changes, the current study indicates that the stage of MN is not directly related to the prognosis of the disease [20] and that renal interstitial fibrosis and renal tubular atrophy are influencing factors for the prognosis of MN [21, 22]. In this study, there were no statistically significant differences in the clinical indicators (including 24-hour urinary protein, total blood cholesterol, and other indicators that affect the prognosis of MN) according to the stage of MN. As the 25(OH)D₃ level decreased, the proportion of pathological manifestations, such as glomerular sclerosis, renal tubular atrophy, and renal interstitial fibrosis, increased, but the difference was not statistically significant. In recent years, many studies have indicate that 25(OH)D₃ can exert renoprotective effects by antagonizing renal fibrosis. The main mechanism may act as follows: 1 Active vitamin D₃ may delay or prevent TGF-β-mediated tubular epithelial-myofibroblast transdifferentiation (TEMT) by inhibiting TGF-β expression, thereby delaying renal tubular interstitial fibrosis [23]. 2 Active vitamin D achieves anti-inflammatory effects by inhibiting the pathway initiated by the transcription factor NF-κB, thereby preventing renal tubular interstitial fibrosis [24]. 3 Active vitamin D can inhibit an overactivated RAS, delay the progression of chronic kidney disease, and reduce renal tubular interstitial fibrosis [25]. 4 Active vitamin D can reduce urinary protein excretion, reduce renal interstitial inflammation, and thus reduce renal tubular interstitial fibrosis. 5 Active vitamin D can induce the expression

of HGF, and HGF can antagonize the TGF- β /Smad pathway through various mechanisms and protect against interstitial fibrosis [26, 27]. Active vitamin D can also inhibit EMT, and blockade of EMT can reduce renal interstitial fibrosis [28]. In this study, the difference in the proportion of renal interstitial fibrosis in pathological tissues from the patients in the two groups stratified by 25(OH)D₃ level was not statistically significant, possibly because of an insufficient sample size.

Therefore, we speculate that 25(OH)D₃ may be related to the efficacy of IMN treatment. In this study, by comparing the serum albumin levels, serum creatinine levels, and eGFRs between two groups of patients with different baseline levels of 25(OH)D₃ across a 6-months treatment course, we found that during follow-up, the serum albumin level was gradually increased in both groups but was lower in the 25(OH)D₃ <25nmol/L group than in the 25(OH)D₃ \geq 25nmol/L group. This finding indicated that the overall disease severity in patients in the 25(OH)D₃ \geq 25nmol/L group was milder than that in patients in the 25(OH)D₃ <25nmol/L group. However, there was no statistically significant difference in the serum creatinine level or the eGFR between the two groups, and no grouping by time interaction effect was noted, possibly because MN is characterized by a long natural course, but the follow-up time of this study was short—thus, the serum creatinine levels and eGFR in the follow-up data are insufficient to reflect the progress of renal function. If we extend the follow-up time to obtain more data, the results may change significantly.

In summary, vitamin D deficiency is common in IMN patients, and approximately 75.41% of patients have a 25(OH)D₃ level of <25 nmol/L. The level of 25(OH)D₃ is correlated with the serum albumin level, 24-hour urinary protein quantitation, blood lipid level, hs-CRP level and other clinical indicators that affect the severity of IMN. 25(OH)D₃ may be involved in the pathogenesis and progression of IMN. During follow-up, the serum albumin levels of all patients increased significantly, but the overall serum albumin levels of patients in the 25(OH)D₃ <25nmol/L group were still lower than those of patients in the 25(OH)D₃ \geq 25nmol/L group. However, whether prospective supplementation with active vitamin D₃ can improve the prognosis of IMN requires further prospective cohort studies.

The limitations of this study include the following: 1. The 24-hour urinary protein excretion was not regularly measured in all patients during follow-up, and because this study was retrospective, we were unable to further analyze the correlation between 25(OH)D₃ and proteinuria remission in IMN. 2. IMN progresses slowly, but the follow-up time in this study was short. The follow-up time needs to be extended to monitor the renal function of patients in order to further analyze the impact of 25(OH)D₃ on the prognosis of IMN.

Conclusion

Reduced 25(OH)D₃ levels are prevalent among IMN patients. During follow-up, serum albumin increased significantly in patients administered vitamin D supplementation. 25(OH)D₃ may play an important role in IMN development.

List Of Abbreviations

IMN: idiopathic membranous nephropathy; 25(OH) D₃: 25-hydroxyvitamin D₃; ALB: albumin; ESR: Erythrocyte sedimentation rate; TP: Total protein; ESRD: End-stage renal disease

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University Approval Number: 2019KY-E-067. The data are anonymous, and the requirement for informed consent was therefore waived.

Consent for publication

Consent for publication was not required.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None of the authors have any conflicts of interests to declare.

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Not applicable

Authors' contributions

FZ conceived the study. FZ and YH carried out the research. FZ, YH and ZHY analyzed the data. FZ and ZHY wrote the paper. All authors read and approved the final manuscript.

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Figures

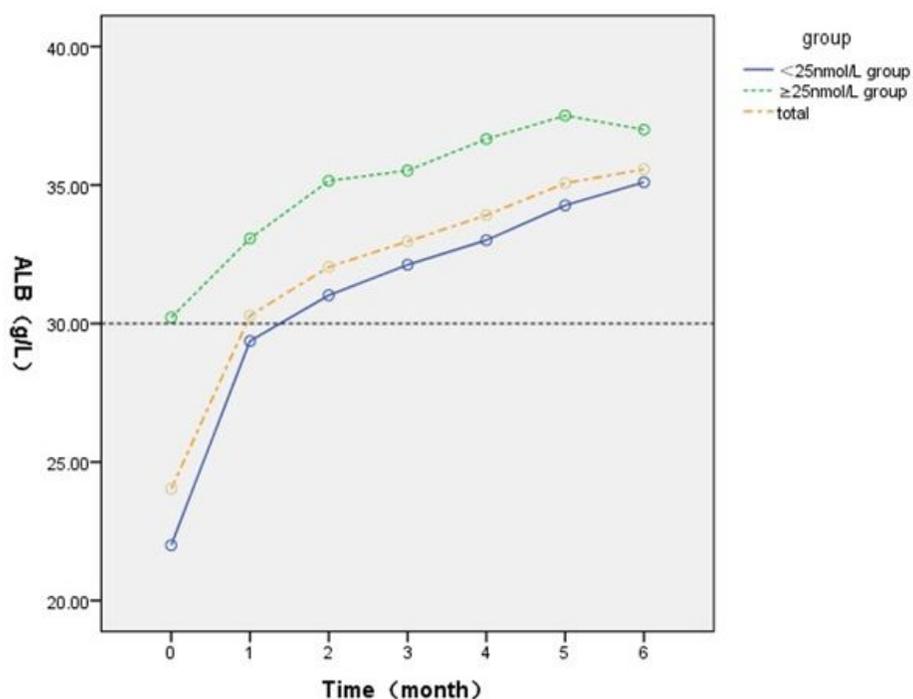


Figure 1

Changes in serum albumin during follow-up.

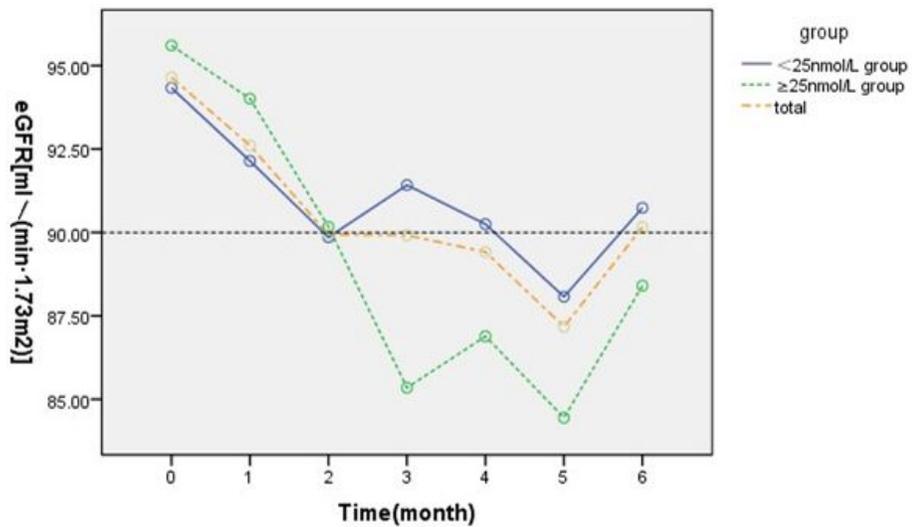


Figure 2

Changes in eGFR during follow-up.

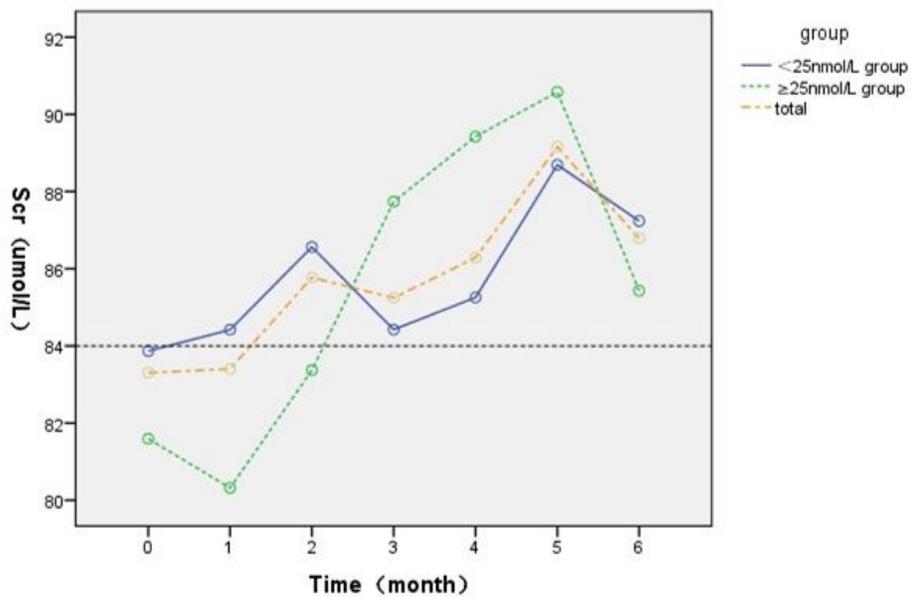


Figure 3

Changes in serum creatinine during follow-up.