Clinical observation and analysis of patients with idiopathic membranous nephropathy combined with low 25(OH)D$_3$ levels

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Research article

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Abstract

Background: To investigate correlations between 25-hydroxyvitamin D_3 [25(OH)D_3] levels and selected clinical indicators and renal pathology at initial onset of idiopathic membranous nephropathy (IMN) and to analyse changes in the main quantifiable indicators in IMN patients with different 25(OH)D_3 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)D_3 levels on clinical and pathological indicators of IMN was performed, and changes in serum albumin, the estimated glomerular filtration rate (eGFR), serum creatinine and other clinical indicators in these patients during the follow-up were analysed.

Results: The 109 patients with IMN had 25(OH)D_3 deficiency [25(OH)D_3<70 nmol/L]. The incidence of nephrotic syndrome, serum phosphorus levels, high-sensitivity C-reactive protein (hs-CRP) levels, and erythrocyte sedimentation rates (ESRs) were higher in the 25(OH)D_3<25 nmol/L group than in the 25(OH)$D_3 \geq$25 nmol/L group. In contrast, total protein (TP), albumin (ALB), serum calcium, and IgG levels were lower in the 25(OH)D_3<25 nmol/L group (P<0.05). No pathological parameters differed significantly between the two groups (P>0.05). Bivariate correlation analysis showed that 25(OH)D_3 was positively correlated with nephrotic syndrome, TP levels, ALB levels, the eGFR, serum calcium levels and IgG levels (P<0.05) and negatively correlated with age, blood pressure, Scr levels, 24-hour urinary protein (UP) quantitation, serum phosphorus levels, hs-CRP levels and the ESR (P<0.05). In single-factor repeated measures analysis of variance, the ALB levels in the 25(OH)D_3<25 nmol/L and 25(OH)$D_3 \geq$25 nmol/L groups showed an increasing trend but were lower in the 25(OH)D_3<25 nmol/L group than in the 25(OH)$D_3 \geq$25 nmol/L group at each follow-up time point (P<0.001).

Conclusion: Reduced 25(OH)D_3 levels are prevalent among IMN patients. During the follow-up, serum albumin increased significantly in patients receiving vitamin D supplementation. 25(OH)D_3 may play an important role in IMN development.

Background

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 pathological features. MN of unknown aetiology 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 all cases of nephrotic syndrome, and is more common in men than in women (1.1~2.4:1)[1]. In most patients, the onset is insidious, and approximately 80% of patients have nephrotic syndrome, 20% to 25% have asymptomatic proteinuria, 30% to 50% have microscopic haematuria, 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 vastly differs. A 2010 multicentre study from Spain suggested that the spontaneous response rate of IMN patients was 31.7%, and that 29.4% of patients developed end-stage renal disease (ESRD) or died [2].

Vitamin D_3 is a naturally occurring fat-soluble vitamin also called cholecalciferol. It is a ring-opened form of cholesterol and a steroid hormone. Two hydroxylation reactions are required for its biological activity as a hormone. First, the reaction producing 25-hydroxyvitamin D_3 [25(OH)D_3] from vitamin D_3 is catalysed in the liver by 25-hydroxylase, and 1α-hydroxylase then catalyses the production of 1,25-dihydroxyvitamin D_3 from 25(OH)D_3 in the proximal tubular epithelial cells of the kidney. 25(OH)D_3 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 D_3 synthesis but also an important organ affecting the metabolism of active vitamin D_3. The classic physiological role of vitamin D_3 is regulation of calcium and phosphorus metabolism. In addition, vitamin D_3 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 hypocalcaemia and low serum 25(OH)D_3 levels. This pattern suggests that the pathophysiology of IMN is related to active vitamin D_3. However, current research on active vitamin D_3 and kidney diseases focuses mostly on chronic kidney disease and diabetic nephropathy. Few studies have addressed active vitamin D_3 in IMN, and domestic and international studies on the renoprotective effects of active vitamin D_3 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)D_3 levels and other clinical indicators as well as renal pathological changes. By following up and observing changes in the clinical indexes of patients with different levels of active vitamin D_3, the clinical significance of 25(OH)D_3 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 MN by renal biopsy and pathology, and all had a follow-up time of longer than 6 months. We excluded patients with secondary MN caused by aetiologies such as infection (hepatitis virus, HIV, etc.), autoimmune disease (systemic lupus erythematosus, etc.), metabolic disease (diabetes, etc.), malignant tumours, 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 older than 30 years. Among the patients, 54 cases (49.54%) of nephrotic syndrome were documented.

Data collection

The fasting venous blood 25(OH)D_3 level, routine blood count, serum total protein level, albumin level, renal function, 24-hour urinary protein (UP) quantification, blood calcium and phosphorus levels, the high-sensitivity

C-reactive protein (hs-CRP) level, the erythrocyte sedimentation rate (ESR), and the 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 $25(OH)D_3$ concentration ≥75 nmol/L indicates sufficiency, a concentration <75 nmol/L and ≥25 nmol/L indicates insufficiency, and a concentration <25 nmol/L indicates deficiency. At admission, the $25(OH)D_3$ level was <75 meg/L in all patients; thus, daily vitamin D supplementation (600 IU) was initiated in all patients on the third day after admission, and the patients were divided into two groups: the $25(OH)D_3$≥25 nmol/L group and the $25(OH)D_3$<25 nmol/L group. 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 x ± 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 analysed by repeated measures analysis of variance; count data are expressed as the numbers of cases (percentages), and comparisons between groups were evaluated by c^2 tests. Correlations between two continuous variables were analysed by Pearson correlation analysis, and correlations between categorical variables or graded data were analysed by Spearman correlation analysis. P <0.05 was considered statistically significant.

Results

Clinical characteristics of the IMN patients

All 109 patients had insufficiency or a lack of $25(OH)D_3$, although the degree of kidney disease was different. Fifty-four cases (49.54%) of nephrotic syndrome but 91 (83.48%) cases of hypoproteinaemia were identified; some patients exhibited a variable decrease in the estimated GFR (eGFR), an increase in serum creatinine, and dysregulation of calcium and phosphorus metabolism at onset.

Clinical characteristics of the 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 only 1 case had stage 3 IMN, this category 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 the IMN patients in different pathological stages

	Stage 1 (n=32)	Stage 2 (n=76)	Statistics	P
Age（years）	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（umol/L）	76.00(66.50,86.75)	77.00(63.00,96.75)	-0.441	0.659
UA（umol/L）	389.44±106.39	398.46±120.20	-0.368	0.714
eGFR [ml/(min·1.73 m^2)]	97.86±22.52	93.69±27.66	0.755	0.452
24-h UP（g/24 h）	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 $25(OH)D_3$ levels

The proportion of patients with nephrotic syndrome, the blood phosphorus level, the hs-CRP level, and the ESR level were higher, but the total protein (TP) level, albumin (ALB) level, blood calcium level and IgG level were lower in the $25(OH)D_3$ <25 nmol/L group than in the $25(OH)D_3 \geq 25$ nmol/L group (P <0.05). No statistically significant difference in pathological indicators was observed between the two groups (Table 2).

Table 2: Clinical characteristics of the IMN patients with different 25(OH)D$_3$ levels

	⬚25 nmol/L (n=82)	≥25 nmol/L (n=27)	Statistics	P
Age（years）	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（umol/L）	83.87±31.39	81.59±26.14	0.339	0.735
UA（umol/L）	390.16±112.72	408.26±126.50	-0.702	0.484
eGFR [ml/(min·1.73 m^2)]	94.33±27.11	95.60±23.89	-0.218	0.828
24-h UP（g/24 h）	5.24±4.02	4.08±2.91	1.384	0.169
Blood calcium（mmol/L）	1.94±0.12	2.08±0.14	-5.177	<0.001
Blood phosphorus（mmol/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

Correlations between 25(OH)D$_3$ levels and clinical indicators in IMN patients

Correlation analysis showed that 25(OH)D$_3$ was correlated with multiple clinical indicators. Specifically, the 25(OH)D$_3$ 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, 24-h UP quantitation, the blood phosphorus level, the hs-CRP level and the ESR (all P <0.05) (Table 3).

Table 3: Correlations between $25(OH)D_3$ levels and clinical indicators

Clinical indicators	r	P
Age	-0.923	<0.001
Gender	-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
24-h UP	-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 $25(OH)D_3$, the patients were divided into the $25(OH)D_3 \geq 25$ nmol/L group and the $25(OH)D_3 < 25$ nmol/L group. Serum albumin, the eGFR, and serum creatinine were analysed at 7 time points, and changes in the above clinical indicators in the two groups were analysed.

Changes in serum albumin (Table 4, Figure 1)

The difference in the ALB level between the two groups was statistically significant (F_{group} = -7.642, P_{group} <0.001). The ALB level in the $25(OH)D_3 < 25$ nmol/L group was lower than that in the $25(OH)D_3 \geq 25$ nmol/L group (all P <0.05). The ALB level showed a time-dependent trend (F_{time} = 2.593, P_{time} = 0.036), and the overall ALB level showed an increasing trend in both groups. An interaction effect was identified between the grouping and the follow-up time ($F_{interaction}$ = 4.756, $P_{interaction}$ =0.001).

Changes in the eGFR (Table 4, Figure 2)

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

Changes in serum creatinine (Table 4, Figure 3)

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

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

Groups	N	0 months	1 month	2 months	3 months	4 months	5 months	6 months
ALB								
25（OH）D$_3$<25 nmol/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$_3$≥25 nmol/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$_3$<25 nmol/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$_3$≥25 nmol/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$_3$<25 nmol/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$_3$≥25 nmol/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 the 25(OH)D$_3$<25 nmol/L group, *P<0.05

Discussion

In recent years, the incidence and prevalence of IMN have shown increasing trends. 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%, demonstrating 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 substantially, and many researchers are constantly seeking biological indicators for the disease status and prognosis of IMN.

Vitamin D is a steroid derivative that first undergoes a reaction catalysed by 25-hydroxylase in the liver to produce $25(OH)D_3$ and then undergoes a reaction catalysed by 1-α-hydroxylase in the kidney to produce active vitamin D_3, which binds to intracellular vitamin D_3 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 D_3 receptors are expressed in more than 30 cells, tissues and organs in humans [11]; thus, active vitamin D_3 is associated with diseases of many body systems, such as the circulatory system and endocrine system. $25(OH)D_3$ that enters the blood from the liver is a precursor of active vitamin D_3. Because of its high concentration in blood, good stability and long half-life, $25(OH)D_3$ 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]. In clinical work, we found that not only patients with chronic kidney disease but also patients with nephrotic syndrome often have low $25(OH)D_3$ levels. Earlier studies showed that the incidence of low $25(OH)D_3$ levels in patients with nephrotic syndrome exceeds 90% [13]. The results of our study showed that IMN patients generally have low $25(OH)D_3$ levels (a detection rate of 100% and a rate of <25 nmol/L as high as 75.41%), with an average level of 12.27 (range: 6.79-24.91) nmol/L. This study retrospectively analysed the correlations of $25(OH)D_3$ levels with clinical and pathological indicators in 109 patients diagnosed with IMN, and repeated measures analysis of variance was used to analyse 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 these patients, 79.82% were older than 30 years, and approximately 41.28% had hypertension, similar to the percentages in other domestic and international reports. In addition, 49.54% of the patients had nephrotic syndrome, which is lower than the rates reported in related studies. Pathological analysis of the kidneys showed that glomerular lesions were mainly stage I and II. Grouping the patients by IMN stage and comparing differences in related clinical indicators showed that patients with stage II IMN had a higher risk of developing hypertension, which is consistent with the results of previous studies [2]. Grouping patients by $25(OH)D_3$ level revealed significant differences in the levels of serum albumin, blood lipids, CRP and other indicators. Correlation analysis between the $25(OH)D_3$ level and clinical indicators showed that $25(OH)D_3$ 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 hypoproteinaemia 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 D_3 can protect podocytes through a variety of mechanisms as follows. Active vitamin D_3 can inhibit the renin-angiotensin system (RAS), negatively regulate the expression of angiotensin II and renin, and reduce podocyte shedding and damage. Active vitamin D_3 can inhibit TGF-β/Smad pathway signalling, activate the BMP-7/Smad pathway, inhibit the protein expression of desmin, and reduce podocyte damage [16]. Active vitamin D_3 regulates the Wnt/β-catenin pathway in podocytes and inhibits podocyte epithelial-mesenchymal transition (EMT). Active vitamin D_3 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, 24-hour urinary protein quantitation was negatively correlated with the $25(OH)D_3$ level, and the serum albumin level was positively correlated with the $25(OH)D_3$ level. Serum albumin levels were significantly different in patients with different levels of $25(OH)D_3$: in patients with $25(OH)D_3$ <25 nmol/L, the serum albumin level was significantly lower than that in patients with $25(OH)D_3 \geq 25$ 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 for retaining the 24-hour urine sample, leading to experimental errors, a large fluctuation in the daily urinary protein quantitation of patients with MN (possibly differing according to the patient's protein intake, body position and activity level) and other factors.

C-reactive protein is an acute-phase protein synthesized by the liver under stimulation by inflammatory factors such as IL-6. hs-CRP is an extremely sensitive inflammatory marker. When the body experiences immune injury or infection, the hs-CRP level 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 $25(OH)D_3$ <25 nmol/L group was significantly higher than that in the $25(OH)D_3 \geq 25$ nmol/L group. hs-CRP and $25(OH)D_3$ levels were negatively correlated, indicating that the hs-CRP level was correlated with the vitamin D_3 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, no statistically significant differences were observed in the clinical indicators (including 24-hour urinary protein, total blood cholesterol, and other indicators affecting the prognosis of MN) according to the stage of MN. As the $25(OH)D_3$ 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 indicated that $25(OH)D_3$ can exert renoprotective effects by antagonizing renal fibrosis. The main mechanism may act as follows: (1) Active vitamin D_3 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, decrease 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]. (6) Active vitamin D can also inhibit EMT, and EMT blockade 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 $25(OH)D_3$ level was not statistically significant, possibly because of an insufficient sample size.

Therefore, we speculate that $25(OH)D_3$ 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 $25(OH)D_3$ across a 6-month treatment course, we found that during the follow-up, the serum albumin level gradually increased in both groups but was lower in the $25(OH)D_3$ <25 nmol/L group than in the $25(OH)D_3 \geq 25$ nmol/L group, indicating that the overall disease severity in the patients in the $25(OH)D_3 \geq 25$ nmol/L group was milder than that in the patients in the $25(OH)D_3$ <25 nmol/L group. However, no statistically significant difference in the serum creatinine level or the eGFR was observed 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 eGFRs in the follow-up data are insufficient to reflect renal function progress. 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 $25(OH)D_3$ level <25 nmol/L. The level of $25(OH)D_3$ is correlated with the serum albumin level, 24-hour urinary protein quantitation, the blood lipid level, the hs-CRP level and other clinical indicators that affect the severity of IMN. $25(OH)D_3$ may be involved in the pathogenesis and progression of IMN. During the follow-up, the serum albumin levels of all patients increased significantly, but the overall serum albumin levels of the patients in the $25(OH)D_3$ <25 nmol/L group were still lower than those of the patients in the $25(OH)D_3 \geq 25$ nmol/L group. However, whether prospective supplementation with active vitamin D_3 can improve the prognosis of IMN requires further prospective cohort studies.

The limitations of this study include the following: 1. 24-hour urinary protein excretion was not regularly measured in all patients during the follow-up, and because this study was retrospective, we were unable to further analyse the correlation between $25(OH)D_3$ and proteinuria remission in IMN. 2. IMN progresses slowly, but the follow-up time in this study was short. The follow-up time must be extended to monitor the renal function of patients to further analyse the impact of $25(OH)D_3$ on the prognosis of IMN.

Conclusion

Reduced $25(OH)D_3$ levels are prevalent among IMN patients. During the follow-up, serum albumin increased significantly in patients receiving vitamin D supplementation. $25(OH)D_3$ may play an important role in IMN development.

List Of Abbreviations

IMN: idiopathic membranous nephropathy; $25(OH)D_3$: 25-hydroxyvitamin D_3; 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 analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

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

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Authors' contributions

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

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Figures
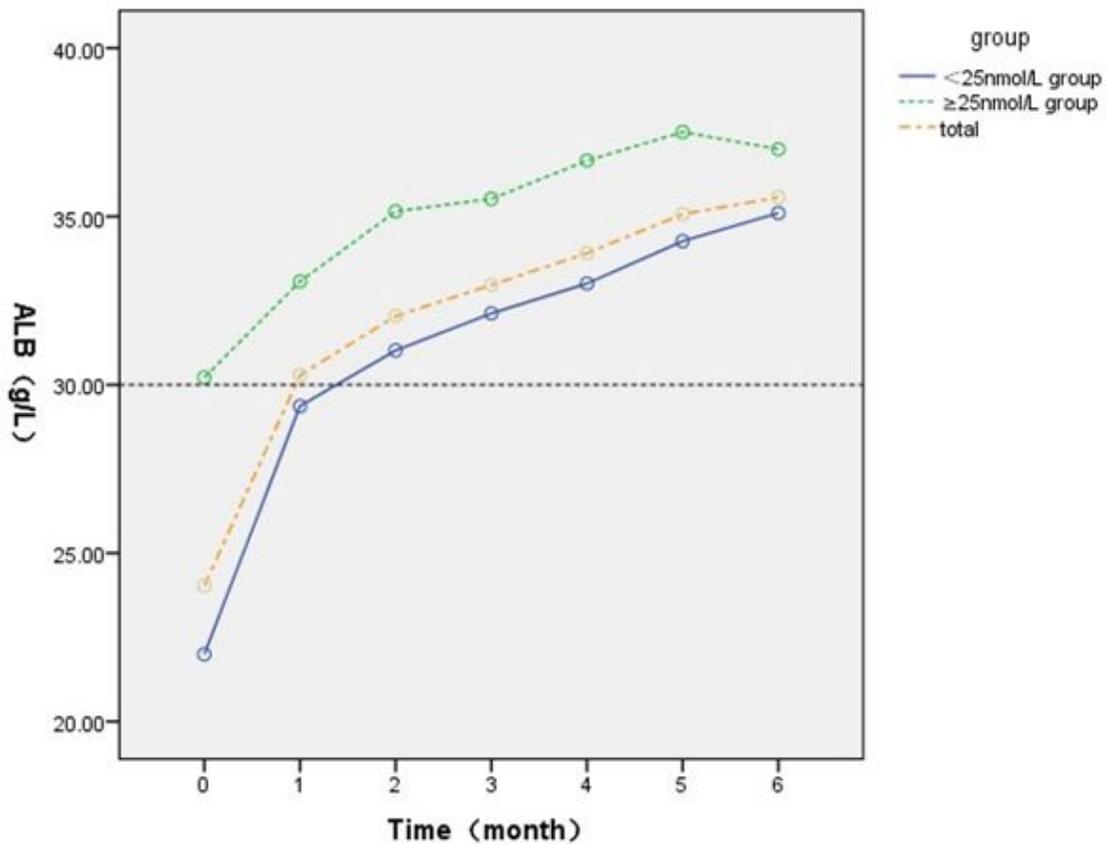
Figure 1

Changes in serum albumin during follow-up.
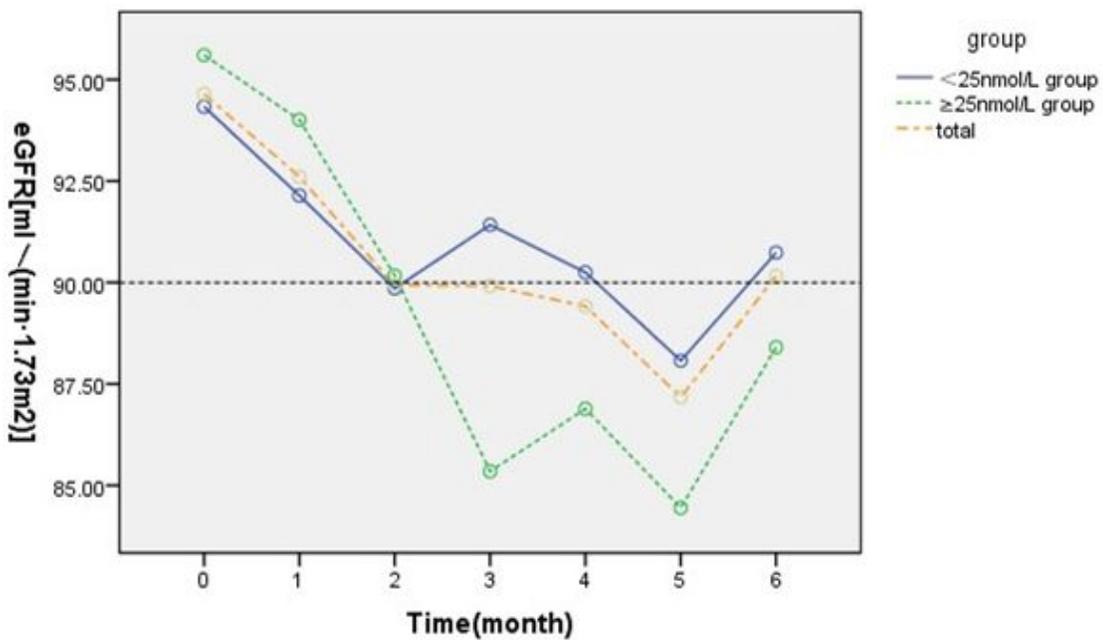
Figure 2

Changes in eGFR during follow-up.
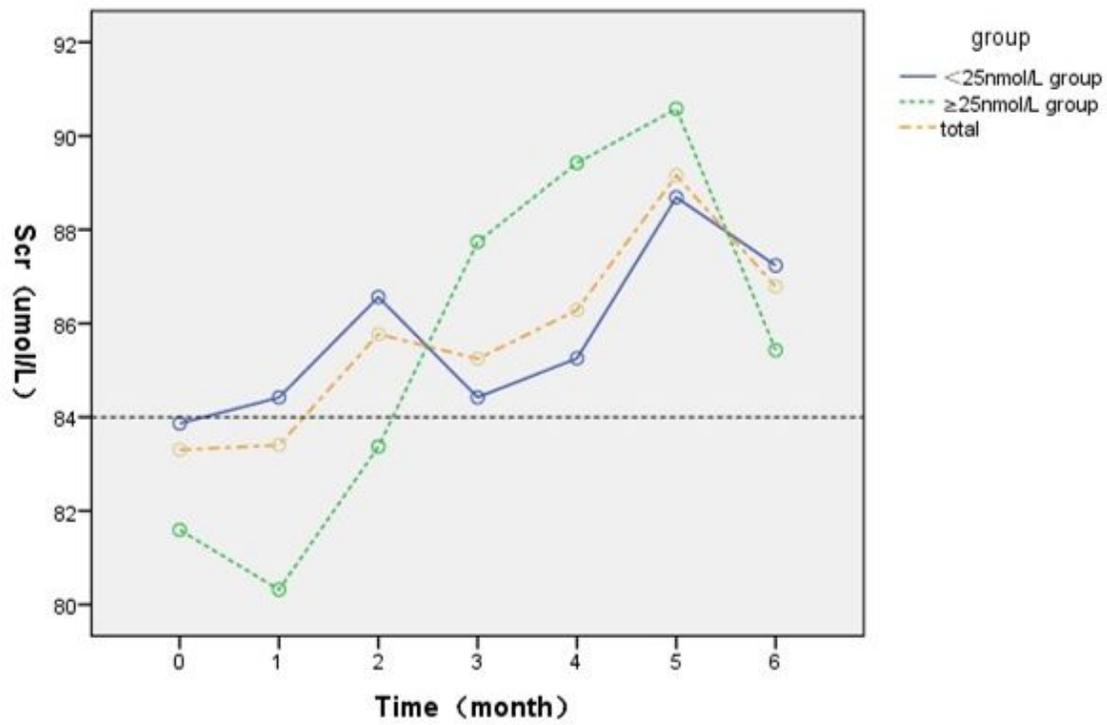
Figure 3

Changes in serum creatinine during follow-up.