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Effect of Vitamin D on proteinuria, lipid status, glycoregulation and C-reactive protein in patients with type-2 diabetes mellitus

Efekat vitamina D na proteinuriju, lipidni status, glikoregulaciju i C-reaktivni protein kod bolesnika sa dijabetes melitusom tip 2

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Abstract

Background/Aim. Vitamin D insufficiency/deficiency is often present in patients with type-2 diabetes mellitus (DM) and could present a risk factor for rapid progression of diabetic nephropathy and for higher incidence of cardiovascular events. The aim of this study was to examine the influence of vitamin D supplementation on proteinuria, cholesterol, triglycerides, C-reactive protein (CRP) and hemoglobin A1c in patients with type-2 DM and vitamin D insufficiency/deficiency. **Methods.** This prospective, cohort study included 90 patients with type-2 DM and vitamin D insufficiency/deficiency divided into 3 equal groups: with normal proteinura, with microproteinuria and with macroproteinuria. Therapy included six months of supplementation with cholecalciferol drops: first two months with 20,000 IU twice weekly, than if level of vitamin D was below normal the same dose was given next four months. If the level of vitamin D was normal 5,000 IU was given twice weekly. At the begining and at the end of the study the levels of urea, creatinine, fasting blood glucose, calcium, phosphorus, cholesterol, triglycerides, CRP, hemoglobin A1c, intact parathyroid hormone, 24-hour urine protein and creatinine clearance were determined. Levels of calcium, phosphorus and vitamin D were also checked 2 months after beginning of therapy due to possible correction of cholecalciferol dose. **Results.** The lowest level of vitamin D before therapy was found in patients with macroproteinuria, while at the end of the study the significantly higher level of vitamin D was found in all three groups. After 6 months of therapy a significant decrease of 24-hour urine protein, cholesterol, triglycerides, hemoglobin A1c in all three groups, and CRP in patients with normal proteinuria and microproteinuria were found. Significantly negative correlation between vitamin D and 24-hour urine protein, cholesterol and CRP was found in patients with macroproteinuria. Also, significantly negative correlation was found between vitamin D and hemoglobin A1c, in patients with normal proteinuria, vitamin D and CRP in patients with microproteinuria. **Conclusion.** A preventive use of high-dose cholecalciferol supplementation in patients with type-2 DM (with or without proteinuria) decreases cholesterol, triglycerides, proteinuria, CRP and hemoglobin A1c.

Key words:
c-reactive protein; cholesterol; diabetes mellitus, type 2; diabetic nephropathies; glycated hemoglobin a; vitamin d; treatment outcome; proteinuria; triglycerides.

Apstrakt

Uvod/Cilj. Nedostatak vitamina D je često prisutan kod bolesnika sa dijabetes melitusom (DM) tip 2 i može biti faktor rizika od brže progresije dijabetesne nefropatije i veće incidencije kardiovaskularnih događaja. Cilj studije bio je da se ispita uticaj supstitucije vitamina D na proteinuriju, holesterol, trigliceride, C-reaktivni protein (CRP) i hemoglobin A1c kod bolesnika sa DM tip 2 i nedostatkom vitamina D. **Metode.** Prospektivnom, kohortnom studijom obuhvaćeno je 90 bolesnika sa DM tip 2 i nedostatkom (insuficijencija/deficijencija) vitamina D svrstanih u tri grupe po 30 bole-

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snika: I – sa normalnom proteinurijom, II – sa mikroproteinurijom i III – sa makroproteinurijom. Sprovedena je šestomesečna nadoknada vitamina D holekalciferol kapima: tokom prva dva meseca sa 20 000 i.j. dva puta nedeljno, a zatim, je kod bolesnika kod kojih je nivo vitamina D ostao snižen nastavljeno sa istom dozom još četiri meseca. Kod bolesnika kod kojih se nivo vitamina D normalizovao, nastavljeno je sa 5 000 i.j. dva puta nedeljno. Na početku i na kraju ispitivanja meren je nivo uree, kreatinina, jutarnje glikemije, kalcijuma, fosfora, holesterola, triglicerida, CRP, hemoglobina A1c, intaktnog paratireoidnog hormona, 24-časovne proteinurije i klirensa kreatinina. Zbog eventualne korekcije doze holekalciferola vrednosti kalcijuma, fosfora i vitamina D proverene su i dva meseca posle započinjanja supstitucije. **Rezultati.** Najniži nivo vitamina D pre terapije imali su bolesnici u grupi sa makroproteinurijom, dok je na kraju ispitivanja utvrđen statistički značajno povišen nivo vitamina D, u sve tri grupe. Nakon šestomesečne primene vitamina D, postignuto je statistički značajno sniženje nivoa 24-časovne proteinurije, holesterola, triglicerida i hemoglobin A1c u sve tri ispitivane grupe, a CRP u grupi sa normalnom proteinurijom i mikroproteinurijom. Statistički značajna negativna korelacija između vitamina D i 24-časovne proteinurije, holesterola i CRP dokazana je u grupi sa makroproteinurijom. Statistički značajna negativna korelacija dokazana je između vitamina D i HBA1c u grupi sa normalnom proteinurijom i vitamina D i CRP u grupi sa miroproteinurijom. **Zaključak.** Supstitucija vitamina D visokim dozama holekalciferola i njegova preventivna primena kod bolesnika sa DM tip 2 (sa ili bez proteinurije) snižava holesterol, trigliceride, proteinuriju, CRP i hemoglobin A1c.

Ključne reči:
c-reaktivni protein; holesterol; dijabetes melitus, tip-2; dijabetičke nefropatije; hemoglobin a, glikozilovan; vitamin d; lečenje, ishod; proteinurija; trigliceridi.

Introduction

There were 382 million people with diabetes mellitus (DM) worldwide in 2013, and it is estimated that number will rise to 585 million in 2035 [1]. It was estimated that more than 700 million people in 2015 had DM or glucose intolerance, but half of it was unrecognized [2]. Approximately one third of the patients with DM will develop diabetes nephropathy (DN) and chronic kidney disease (CKD) [1]. The DM and DN are the main causes of end stage renal disease (ESRD) in the USA, with prevalence of 800 million people [3]. The DN is chronic microvascular complication of DM, presenting as clinical syndrome, manifesting with persistent albuminuria [urine albumin/creatinine ratio (UACR) > 300 mg/g], arterial hypertension, decreasing of glomerular filtration rate and increased cardiovascular events [4]. Considering high direct and indirect costs for treating ESRD patients with renal transplantation or hemodialysis, continuous research are conducting to prevent or slow progression of DN.

It is already proved that changed life habits like regular physical exercise, reduction of body weight in obese patients, reduced intake of salt, proteins and alcohol, smoking cessation, tight control of blood pressure and glucose level, slow down DN progression [4]. Certain drugs can have renoprotective effect: angiotensin converting enzyme inhibitors (ACEI) [5], angiotensin II receptor blockers (ARBs) [5], alsikiren [6], sodium-glucose cotransporter 2 (SGLT2) inhibitors [7], pentoxifylline [8], nonsteroidal mineralocorticoid receptor antagonist- finerenone [9], fenofibrate [10], allopurinol [11], spirinolactone [12] and hydrochlorothiazide [12].

Vitamin D is essential hormone obtained from food (10%–20%) and skin synthesis. Apart from its primary role on calcium and phosphorus homeostasis, it is believed that vitamin D has certain renoprotective effect, antifibrotic, anti-inflammatory effect, inhibits renin angiotensin aldosterone system (RAAS), role in maintaining cardiomyocyte health and insulin sensitivity and role in reducing albuminuria in patients with CKD and DM [13–17].

Studies have confirmed low serum 25-hydroxivitamin D [25(OH)D] levels in patient with CKD [13, 14]. Low vitamin D level seems to be associated with impaired glucose metabolism including DM [18]. Risk factors for vitamin D deficiency in patient with DN remain unclear [18]. The prevalence of vitamin D deficiency is 25% in lean patients and 35% in obese patients [19].

Proteinuria seems to be the most important target to treat in order to prevent cardiovascular events in patients with DN and CKD [13, 14, 17, 20, 21]. Paricalcitol and cholecalciferol can decrease proteinuria in patients with CKD or DM [22–25]. It is assumed that high dose of cholecalciferol like 40,000 international units (IU) weekly could decrease albuminuria and urine transforming growth factor beta-1 (TGF-β1) in patients with DM and Vitamin D insufficiency/deficiency [23].

If we take all in consideration, we can conclude that vitamin D supplementation in patients with vitamin D insufficiency/deficiency could have renoprotective effect and potentially slow progression of DN.

Considering that measuring vitamin D level in blood is easy feasible and its supplementation is not expensive, we decided to conduct the study in patients with type-2 DM and vitamin D insufficiency/deficiency. The aim of this study was to assess the effect of vitamin D on 24-hour urine protein, cholesterol, triglycerides, C-reactive protein (CRP), fasting blood glucose (FBG) and hemoglobin A1c (HBA1c) in patients with type-2 DM and vitamin D insufficiency/deficiency. Those parameters were chosen as possible predictors of progression of DN.

Methods

This 6-month prospective, cohort study included 90 patients patients with type-2 DM and vitamin D insufficiency/deficiency.

Inclusion criteria were: males and females between 18 and 75 years diagnosed with type-2 diabetes mellitus and vitamin D insufficiency (50–75 nmol/L) or deficiency (< 50 nmol/L), medical therapy for type-2 DM during three months

before screening in stable dose (also during study), creatinine clearance > 60 mL/min/1.73 m^2, therapy with ACEI or ARBs at least three months before screening in stable dose during that period (also during study).

Exclusion criteria: glomerulonephritis, connective tissue diseases, serum calcium (corrected for albumin) > 2.45 mmol/L, serum phosphorus > 1.65 mmol/L, congestive heart failure, previous myocardial infarction, poorly regulated arterial hypertension, malignant disease, liver cirrhosis, hepatitis B or hepatitis C infection, HIV, currently enrolled in another trial, women who are pregnant/nursing and previous treatment with vitamin D during six months before screening.

Patients were divided into three equal groups according to initial 24-hour urine protein: I group – patients with normal proteinuria (< 150 mg/24 h); II group – patients with microproteinuria (150–500 mg/24 h); III group – patients with macroproteinuria (> 500 mg/24 h).

Treatment consisted of cholecalciferol drops (Vigantol® 20,000 IU/mL oral drops, solution – Cholecalciferol; Merck KgaA, Germany) in period of six months. During first two months patients received cholecalciferol 20,000 IU twice weekly. After two months patients with normal level of vitamin D received cholecalciferol 5,000 IU twice weekly, and patients with low level of vitamin D received cholecalciferol 20,000 IU twice weekly next four months.

The following variables were analyzed in patients: gender, age, body mass, height, body mass index, urea, creatinine, FBG, calcium, phosphorus, total cholesterol, triglycerides, CRP, HBA1c, intact parathyroid hormone (iPTH) and 24-hour urine protein and creatinine clearance [using CKD-Epidemiology Collaboration (EPI) formula [26]].

All parameters are measured at screening and after six months of vitamin D therapy, except calcium, phosphorus and vitamin D, which are measured also after two months of therapy.

The research was approved by Ethics Committee of Military Medical Academy, Belgrade, Serbia (date of approval 1/21/2016). All patients signed the inform consent.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 19.0 computer program (IBM, USA, 2011). All continuous variables were described in the form of the median [interquartile range (IQR): range between 25th and 75th percentile], because the data distribution was not normal (Shapiro-Wilk test; $p < 0.05$). The categorical variables were expressed as percentages and examined using the χ^2 test. Relationship between variables was tested by Spearmen's coefficient correlation. Cohen's criteria for correlation are follows: $r < 0.29$ is small correlation, $r = 0.30–0.49$ is moderate correlation and $r > 0.50$ strong correlation. Intra-group comparisons of continuous variables were performed by the non-parametric Wilcoxon signed rank test (two groups – before and after vitamin D therapy). Inter-group comparisons of continuous variables were performed by the non-parametric Kruskal Wallis test (three groups according to proteinuria). Comparisons of nonparametric variables between 2 groups were performed by the Mann-Whitney *U* test. The normality distribution of data was tested with the Shapiro-Wilk test (subject number in the group less than 50). All the analyses were evaluated at the level of statistical significance of $p < 0.05$.

Results

A total of 90 patients with type-2 DM and vitamin D insufficiency/deficiency group were divided in three equal groups according to the basal proteinuria levels: the first group with normal proteinuria, second with microproteinuria, and third with macroproteinuria. All patients were treated with cholecalciferol drops during patients 6 months. Out of 48 (53.33%) male and 42 (46.67%) female patients; 22 (24.44%) patients had vitamin D insufficiency, and 68 (75.55%) patients had vitamin D deficiency. Basic sociodemographic and anthropometric patient characteristics are presented in Table 1.

Average duration of type-2 DM in group with normal proteinuria was 10.00 years (5.75–14.25), in group with microproteinuria also 10.00 years (5.75–15.25) and in group with macroproteinuria 15.00 years (10.00–20.00).

Type-2 DM was treated with oral hypoglycemic drugs in 16 (53.33%) patients in the first group, 13 (43.33%) patients in the second group and 11 (36.67%) patients in the third group (χ^2; $p = 0.425$), 13 (43.33%) patients received insulin therapy in the first group, 15 patients (50.00%) in the second group and 17 patients (56.66%) in the third group (χ^2; $p = 0.587$), while 1 (3.33%) patient received combination therapy in the first group, 2 (6.66%) patients in the second group and also 2 (6.66%) patients in the third group (χ^2; $p = 0.809$).

Table 1

Demographic characteristics of type-2 diabetes mellitus patients according to 24-hour urine protein

Parameter	24-hour urine protein (mg/24 h)			*p*
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Gender, n (%)				
male	16 (53.3)	17 (56.7)	15 (50.0)	0.875*
female	14 (46.7)	13 (43.3)	15 (50.0)	
Age (years), median (IQR)	62.00 (58.00–69.00)	62.00 (52.50–69.00)	66.00 (62.25–69.50)	0.281**
Heigh (m), median (IQR)	1.70 (1.05–1.77)	1.73 (1.70–1.83)	1.71 (1.68–1.80)	0.035**
Weight (kg), median (IQR)	80.00 (74.25–85.00)	89.00 (79.88–91.25)	87.50 (79.00–91.00)	0.010**
BMI (kg/m^2), median (IQR)	27.50 (25.62–28.70)	27.45 (26.52–30.12)	28.40 (26.31–91.62)	0.120**

BMI – body mass index; *χ^2-test; **Kruskal Wallis test; IQR – interquartile range: 27–75 percentiles.

Table 2

Vitamin D level in the patients with type-2 diabetes mellitus, before and six months after vitamin D supplementation (S)

24-hourour urine protein	Vitamin D (nmol/L)		*p*
	before S	after S	
Normal proteinuria, median (IQR)	42.53 (30.40–46.25)	79.65 (69.09–92.12)	< 0.001**
Microproteinuria, median (IQR)	47.03 (33.18–53.88)	86.65 (70.50–92.85)	< 0.001**
Macroproteinuria, median (IQR)	28.49 (22.40–47.67)	69.22 (54.74–78.04)	< 0.001**
p	0.006*	0.009*	

***– Kruskal Wallis test; **– Wilcoxon Signed Ranks test; IQR- interquartile range: 25–75 percentiles.**

Table 3

Parameters of inflammation,glycoregulation and renal function in relation to 24-hour urine protein before and six months after vitamin D supplementation (S)

Parameter	24-hour urine protein (mg/24 h)			*p*
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
CRP (mg/L), median (IQR)				
before S	1.48 (0.99–3.08)	1.60 (0.95–2.22)	1.52 (1.01–2.25)	0.786*
after S	1.42 (0.87–2.96)	1.16 (0.80–2.15)	1.22 (0.95–2.16)	0.809*
p	< 0.001**	0.001**	0.943**	
FBG (mmol/L), median (IQR)				
before S	8.60 (6.85–10.02)	7.50 (6.67–9.35)	9.40 (7.90–10.60)	0.036*
after S	7.90 (6.68–8.93)	7.95 (5.70–8.23)	8.05 (7.13–8.95)	0.366*
p	0.001**	0.020**	0.001**	
Hemoglobin A_{1C} (%), median (IQR)				
before S	6.97 (6.60–7.80)	7.15 (6.57–8.42)	7.80 (6.72–8.70)	0.192*
after S	6.80 (6.20–7.70)	6.95 (6.40–7.60)	7.10 (6.80–8.62)	0.069*
p	0.001**	0.001**	0.016**	
Creatinine (μmol/L), median (IQR)				
before S	76.50 (66.75–90.25)	82.00 (71.00–96.15)	86.00 (74.75–106.00)	0.149*
after S	73.00 (64.00–82.50)	83.50 (70.50–94.25)	82.00 (71.50–104.75)	0.136*
p-value	0.273**	0.090**	0.022**	
Creatinine clearance (mL/min), median (IQR)				
before S	84.30 (64.05–91.55)	68.60 (61.00–94.25)	63.20 (60.57–89.97)	0.101*
after S	86.30 (71.15–95.45)	74.15 (63.82–90.70)	68.80 (62.00–90.07)	0.143*
p	0.286**	0.049**	0.040**	
Urea (mmol/L), median (IQR)				
before S	5.95 (5.10–7.13)	6.90 (5.47–8.20)	8.40 (5.37–9.72)	0.004*
after S	5.85 (5.15–6.57)	6.20 (5.20–7.95)	8.15 (5.47–9.22)	0.003*
p	0.032**	0.147**	0.484**	

***Kruskal Wallis test; **Wilcoxou Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**
CRP – C-reactive protein; FBG – fasting blood glucose.

The lowest level of vitamin D, before therapy, was found in the patients with macroproteinuria, on average 28.49 nmol/L (22.40–47.67). After six months of supplementation a significantly increased vitamin D level was found in patients in all three groups (Table 2).

After six months of vitamin D supplementation, significantly decreased CRP level was found in the group with normal proteinuria ($p < 0.001$) and the group with microproteinuria ($p = 0.001$). All three groups had significantly decreased levels of HBA1c and FBG (Table 3).

We performed a correlation between vitamin D level and levels of CRP, FBG and HBA1c after six months of cholecalciferol therapy and we found a moderately negative correlation between increased vitamin D level and decreased CRP level in the group with microproteinuria ($r = -0.368$; $p = 0.046$), and in the group with macroproteinuria ($r = -0.375$; $p = 0.041$). We found a moderately negative correlation ($r = -0.342$; $p = 0.064$) between increased vitamin D level and decreased HBA1c only in group with normal proteinuria.

We found the significantly decreased serum creatinine level, after therapy with cholecalciferol, only in the group with macroproteinuria ($p = 0.022$). Increased creatinine clearance level was significant in the group with microproteinuria, 68.60 mL/min (61.00–94.25) at the beginning of the study, after therapy 74.15 mL/min (63.82–90.70) ($p = 0.049$), and in the group with macroproteinuria where we have increase from 63.20 mL/min (60.57–89.97) to 68.80 mL/min (62.00–90.07) ($p = 0.040$) (Table 3).

We did not find calcium level over 2.45 mmol/L and *p* level over 1.65 mmol/L. The iPTH was significantly decreased after therapy only in the group with normal proteinuria ($p = 0.003$), while in the group with microproteinuria level of iPTH was significantly increased from 5.05 pmol/L (4.00–6.15) to 5.10 pmol/L (3.35–6.22) ($p = 0.021$) (Table 4).

Table 4

Parameters of calcium and phosphate homeostasis, and lipid profile in relation to 24 h-proteinuria before and six months after vitamin D supplementation (S)

Parameter median	24-hour urine protein (mg/24 h)			*p*-values
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Calcium (mmol/L), median (IQR)				
before S	2.39 (2.31–2.42)	2.38 (2.29–2.40)	2.3 (2.26–2.37)	0.002*
after S	2.38 (2.32–2.42)	2.34 (2.30–2.40)	2.35 (2.28–2.40)	0.401*
p	0.628**	0.354**	0.002**	
Phosphorus (mmol/L), median (IQR)				
before S	1.10 (1.01–1.19)	1.10 (0.99–1.17)	.16 (1.02–1.30)	0.123*
after S	1.06 (0.91–1.19)	1.07 (0.98–1.19)	1.10 (1.00–1.29)	0.149*
p	0.165**	0.684**	0.348**	
IPTH (pmol/L), median (IQR)				
before S	3.40 (2.18–5.05)	5.05 (4.00–6.15)	7.10 (6.48–9.20)	< 0.001*
after S	3.25 (2.10–4.50)	5.10 (3.35–6.22)	7.02 (5.05–9.70)	< 0.001*
p	0.003**	0.021**	0.363**	

***Kruskal Wallis test; **Wilcoxon Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**
iPTH – intact parathyroid hormone.

Table 5

Parameters of proteinuria and lipid profile in relation to 24-hour urine protein before and six months after vitamin D supplementation (S)

Parameter median	24-hour urine protein (mg/24 h)			*p*-values
	normal (< 150)	microproteinuria (150–500)	macroproteinuria (> 500)	
Total cholesterol (mmol/L), median (IQR)				
before S	5.01 (4.36–5.32)	5.52 (4.84–6.32)	5.66 (4.73–6.36)	0.032*
after S	4.45 (3.90–5.10)	4.66 (84.14–5.30)	4.79 (4.39–5.77)	0.333*
p	< 0.001**	< 0.001**	< 0.001**	
Triglyceride (mmol/L), median (IQR)				
before S	1.81 (1.31–2.41)	2.16 (1.61–2.80)	1.81 (1.58–2.90)	0.194*
after S	1.44 (1.10–1.77)	1.43 (1.16–1.71)	1.49 (1.40–2.22)	0.116*
p	< 0.001**	< 0.001**	< 0.001**	
24-hour urine protein (mg/24 h), median (IQR)				
before S	0.071 (0.046–0.101)	0.221 (0.182–0.273)	0.967 (0.707–4.993)	< 0.001*
after S	0.060 (0.026–0.085)	0.136 (0.100–0.200)	0.664 (0.319–5.140)	< 0.01*
p	0.003**	0.001**	0.015**	

***Kruskal Wallis test; **Wilcoxon Signed Ranks test; IQR – interquartile range: 27–75 percentiles.**

Significantly decreased levels of total cholesterol and triglycerides, after six months of vitamin D supplementation, were achieved in all three groups ($p < 0.001$) (Table 5).

We found significantly decreased 24-hour urine protein in all three groups, after six months of vitamin D supplementation, in the group with normal proteinuria ($p = 0.003$), in the group with microproteinuria ($p < 0.001$) and in the group with macroproteinuria ($p = 0.015$) (Table 5).

We performed correlation between vitamin D blood level and levels of total cholesterol, triglycerides and 24-hour urine protein, after six months therapy of cholecalciferol, we found strong negative correlation ($r = - 0.570$; $p < 0.001$) only in the group with macroproteinuria, between increased vitamin D level and decreased total cholesterol level, and between increased vitamin D level and decreased 24-hour urine protein ($r = -0.685$; $p < 0.001$) at the end of the study (Figures 1 and 2).

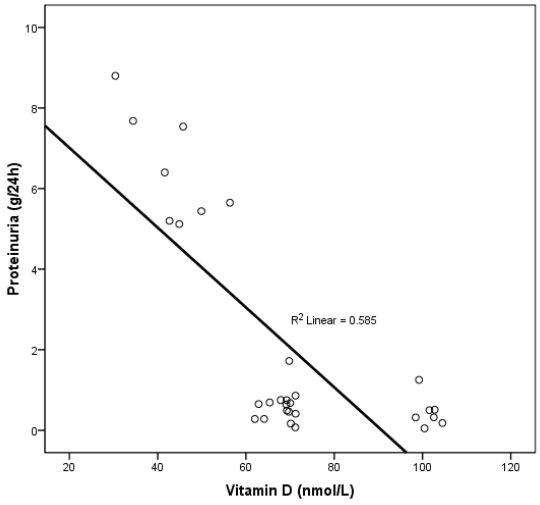

Fig. 1 – Correlation between vitamin D level and 24-hour urine protein in the group with macroproteinuria six months after vitamin D supplementation (Sperman's rho -0.685; $p < 0.001$).

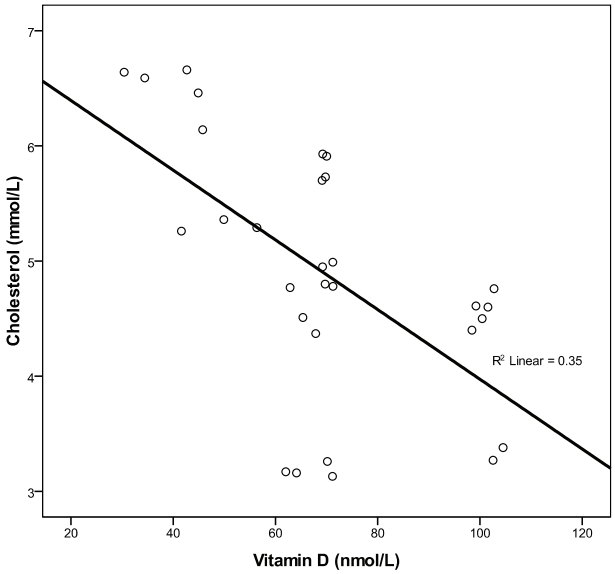

Fig. 2 – Correlation between vitamin D level and total cholesterol level in the group with macroproteinuria six months after vitamin D supplementation (Spearman's rho -0.570; $p < 0.001$).

Discussion

We conducted prospective, cohort study about effect of the six months therapy with cholecalciferol on 24-hour urine protein, lipid status, glicoregulation and parameters of inflammation in the patients with type-2 DM and vitamin D insufficiency/deficiency. We chose cholecalciferol because it is cheap comparing to others vitamin D drugs.

Based on other experience on vitamin D supplementation, we treated patients with higher dose of cholecalciferol, 20,000 IU twice weekly, until normalization of vitamin D level [23, 27–29]. There were no adverse events particularly meaning hypercalcemia or hyperphosphatemia.

After six-month therapy, level of vitamin D was significantly higher in all three groups. The lowest level of vitamin D, before therapy, was found in the patients with macroproteinuria. These patients had highest level of serum creatinine and lowest level of creatinine clearance at the beginning of the study, which is in correlation with data from other authors that vitamin D deficiency is more often in the patients with type-2 DM with macroalbuminuria and in CKD [13, 18, 24, 30].

In the patients with normal 24-hour urine protein, significantly decreased iPTH level was found. Since all patients had normal iPTH level, it is not clear if this decrease is of clinical importance. Although if we consider positive effect of vitamin D on bone turnover by increased osteoclast activation, which leads to increased bone volume, trabecular thickness and osteoid surface [13, 24], probably this iPTH decrease that we found may be clinically important [31].

We measured HBA1c and FBG as parameters for glycoregulation. All patients had a significantly decreased levels of HBA1c and FBG after six months of the therapy with cholecalciferol. We found moderately negative correlation of HBA1c ($r = -0.342$; $p = 0.064$) between increased vitamin D level and decreased HBA1c level in the patients with normal proteinuria. Better glycoregulation after vitamin D supplementation was confirmed by other authors [27, 31, 32]. That effect is achieved by increased insulin sensitivity through stimulation of expression of insulin receptors in skeletal muscles and through activation of peroxisome proliferator activator receptor δ (PPAR δ), RAAS (known as inhibitor of insulin action on peripheral tissues) inhibition [27, 31, 32]. Vitamin D also increases insulin releasing by stimulating intracellular level of calcium in pancreatic beta cells [13, 31]. Vitamin D probably has direct effect on beta cell function by binding to vitamin D receptor (VDR) expressed on beta cells. It is proved that mice without functional VDR have damaged glucose stimulated insulin secretion [13–15, 33].

Chronic inflammation plays crucial role in development of DM and DN [4, 13, 34, 35] and vitamin D can directly or indirectly diminish that effect [14, 31, 36]. Anti-inflammatory effect is achieved by reduced releasing of proinflammatory cytokines (TNF-α, IL-6, IL-12, IL-8, IL- 1β, IFN-γ), blocking of dendritic cell differentiation, inhibition of lymphocytes proliferation, inhibition of foam cells generation, decreased macrophages cholesterol intake and improved development of regulatory T lymphocytes or increased releasing of anti-inflammatory cytokines, like IL-10 [4, 13, 31, 33, 35, 36]. We chose CRP as biomarker of inflammation. After six months of supplementation with vitamin D we found significantly decreased CRP level in the patients with normal proteinuria and microproteinuria with moderately negative correlation between increased vitamin D level and decreased CRP level in the patients with microproteinuria ($r = -0.368$; $p = 0.046$) and macroproteinuria ($r = -0.375$; $p = 0.041$).

Proteinuria is the main target to treat in order to prevent and slow down DN and decrease incidence of cardiovascular events [20, 21, 24]. Vitamin D decreases proteinuria by RAAS inhibition and reduces renal fibrosis by reduction of TGF-β/SMAD pathway [15–17]. Vitamin D, apart of renal RASS inhibition, reduces renin expression in heart thereby decreasing arterial blood pressure [20, 21]. Vitamin D, by slowing down the fibrosis, slows down progression of left ventricle hypertrophy and development of heart failure, lowers brain natriuretic peptide level, reduces gene expression important for atherosclerosis/vascular growth factors [20, 21]. All our patients had significantly decreased proteinuria after six-month therapy. Patients with macroproteinuria had strong negative correlation ($r = -0.685$; $p < 0.001$) between increased vitamin D level and decreased 24-hour urine protein at the end of the study.

We found different data about influence of vitamin D on lipid status in literature [13, 29, 37]. All our patients had significantly decreased levels of total cholesterol and triglycerides after therapy. Patients with macroproteinuria have strong negative correlation ($r = -0.570$; $p < 0.001$) between increased vitamin D and decreased total cholesterol.

We can conclude that after six months of supplementation with vitamin D and correction of vitamin D insufficiency/deficiency all our patients had significantly decreased levels of proteinuria, HBA1c, CRP, total cholesterol and triglycerides. Significantly negative correlation was found between increased vitamin D level and decreased 24-hour urine protein, total cholesterol and CRP in the patients with ma-

croproteinuria. These patients had lowest level of creatinine clearance at the beginning, but at the end of the study we found significantly increased creatine clearance. Based on that we can confirm that vitamin D had renoprotective effect especially in the patients with macroproteinuria and initial CKD. According to our finding of significantly decreased 24-hour urine protein, levels of total cholesterol, triglycerides, HBA1c and CRP in other two groups, but without significant correlation, except in HBA1c in the patients with normal proteinuria and in CRP in the patients with microproteinuria, we can conclude that vitamin D has renoprotective role in type-2 DM in all patients. Based on our results we can assume that vitamin D, apart of renoprotective effect in type-2 DM, could prevent cardiovascular events, which was not studied during our research but could be the goal for future studies.

Limitation of the study and future tasks

Considering limited number of patients in all groups, it would be possible to draw conclusion that a higher number of patients and longer follow up would provide us more specific results. We could investigate effect of vitamin D on proinflammatory cytokines which we have not done in our study. Future research could include new urinary and serum biomarkers marked by other authors as early biomarkers of development and progression of DN.

Conclusion

Vitamin D supplementation in higher dose than conventional and its prolonged preventive use in patients with type-2 DM, has renoprotective effect resulting in decreased 24-hour urine protein, total cholesterol, triglycerides, HBA1c and CRP, especially in the patients with macroproteinuria. If we consider that positive effect is achieved in the patients with normal proteinuria and microproteinuria it is possible that we should treat all patients with type-2 DM with vitamin D supplementation, but we need further trial to confirm that.

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