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Prevalence of vitamin D3 deficiency in patients with type 2 diabetes and proteinuria

Zastupljenost deficita vitamina D3 kod bolesnika sa dijabetesom melitusom tip 2 i proteinurijom

Tatjana Stojšić Vuksanović*, Violeta Knežević†‡

***General Hospital Subotica, Department of Nephrology, Subotica, Serbia; †University Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, Novi Sad, Serbia; ‡University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia**

Abstract

Background/Aim. Vitamin D3 plays an important role in glucose metabolism, with influence on insulin secretion and sensitivity. Low grade inflammation is present in patients with diabetes type 2 and it is a known fact that vitamin D has an anti-inflammatory effect. Vitamin D deficiency is particularly pronounced in patients with diabetic nephropathy. Vitamin D3 levels during the year are associated with seasonal changes primarily influenced by UV radiation. The aim of the study was to examine the prevalence of vitamin D3 deficiency in patients with diabetic nephropathy. **Methods.** Patients with type 2 diabetes and diabetic nephropathy were included in the study after the vitamin D3 levels were established. The results were classified according to a lower limit level given for each month being reduced or normal values. For the purpose of further research, patients with low vitamin D3 levels were divided into two groups, study and control group, each including 45 patients. The study group received cholecalciferol at the dose necessary to achieve the intended optimal vitamin D3 blood level of 90–100 nmol/L. **Results**. At the beginning of the study, vitamin D3 value for all patients with vitamin D3 deficiency (n = 90) was 45.1 ± 15.6 nmol /L. Vitamin D3 deficiency in the study sample (n = 109) was found in 82.56% of participants, while the normal values of vitamin D3 were found in 17.43% of patients. There is a statistically significant difference in the deviation of vitamin D3 levels from the lower normal values in the whole group of subjects between winter and summer, with the deviation being more pronounced in summer. There is no gender difference in these values, although in both men and women there is a more pronounced deviation in summer. **Conclusion**. Vitamin D3 deficiency is significantly represented in patients with type 2 diabetes and diabetic nephropathy.

Key words:
cholecalciferol; deficiency; diabetes mellitus; prevalence; proteinuria; seasons.

Apstrakt

Uvod/Cilj. Vitamin D3 ima važnu ulogu u metabolizmu glukoze, a ispoljava se kroz uticaj na insulinsku sekreciju i senzitivnost. Upala niskog stepena je prisutna kod bolesnika sa dijabetesom tip 2, a poznato je da vitamin D3 ima značajno antiinflamatorno dejstvo. Nedostatak vitamina D3 je posebno izražen kod bolesnika sa dijabetesnom nefropatijom. Nivo vitamina D3 je tokom godine uslovljen sezonskim promenama, prvenstveno uticajem UV zračenja. Cilj rada je bio da se utvrdi prevalencija deficita vitamina D3 kod bolesnika sa dijabetesnom nefropatijom. **Metode**. Ispitanici sa dijabetesom tipa 2 i dijabetesnom nefropatijom su uključeni u studiju nakon određivanja vrednosti vitamina D3. Rezultati su klasifikovani kao normalni ili sniženi, u odnosu na donju granicu normalnih vrednosti koja je data za svaki mesec u godini. Za potrebe daljeg istraživanja bolesnici sa sniženim nivoom vitamina D3 podeljeni su u dve grupe: studijsku i kontrolnu grupu, svaka sa po 45 bolesnika. Studijska grupa ispitanika je dobijala holekalciferol u dozi potrebnoj za postizanje planiranog optimalnog nivoa vitamina D3 od 90–100 nmol/L u krvi. **Rezultati**. Vrednost vitamina D3 kod svih ispitanika sa deficitom vitamina D3 (n = 90) na početku istraživanja je iznosila 45,1 ± 15,6 nmol/L. Zastupljenost bolesnika sa deficitom vitamina D3 je u ispitivanom uzorku (n = 109) bila 82,56%, dok su normalne vrednosti vitamina D3 imalo 17,43% ispitanika. Utvrđena je statistički značajna razlika u odstupanju nivoa vitamina D3 od donjih normalnih vrednosti između zimskog i letnjeg perioda u celoj grupi ispitanika, pri čemu je odstupanje bilo izraženije u letnjem periodu. Nije utvrđena razlika tih vrednosti između muškaraca i žena, mada je kod

Correspondence to: Tatjana Stojšić Vuksanović, General Hospital Subotica, Department of Nephrology, Subotica, Serbia.
E-mail: belavuk@gmail.com; tatjana.stojsic@bolnicasubotica.com

oba pola odstupanje bilo izraženije u letnjem periodu. **Zaključak**. Deficit vitamina D3 je značajno zastupljen kod bolesnika sa dijabetesnom nefropatijom.

Ključne reči:
vitamin d; nedostatak; dijabetes melitus; prevalenca; proteinurija; godišnja doba.

Introduction

The prevalence of vitamin D3 deficiency is more common in type 2 diabetes mellitus (T2DM) patients compared to the healthy population [1]. Studies have indicated that vitamin D deficiency is a risk for the development of T2DM [2]. Patients with T2DM with vitamin D3 deficiency are at a higher risk of developing nephropathy [3]. Moreover vitamin D3 deficiency increases with the progression of diabetic nephropathy (DN) so that serum 25-hydroxyvitamin D [25(OH)D] appears to be a favourable inverse predictor of DN progression [4]. A significant association between vitamin D deficiency and glycemic control has been also reported [5]. Vitamin D3 is involved in glucose metabolism by improving insulin secretion and sensitivity [6]. Vitamin D3 produces these effects by increasing intracellular free calcium and insulin receptor transcription [7]. These paracrine effects are manifested by its action on vitamin D3 receptors (VDRs), which are widely expressed on various cell types, including pancreatic beta cells [8]. Vitamin D3 also exhibits other characteristics that directly or indirectly affect the expression of the sequelae of diabetes disease. It was found that vitamin D has a beneficial effect on reducing proteinuria, hence it is expected that its action can slow down the progression of DN. The reduction of proteinuria is considered to be an important predictive factor regarding the future outcome of renal function [9]. The indirect mechanism of the beneficial effect of vitamin D3 in diabetic patients is manifested by reducing inflammation. There are indications that diabetes is a condition of low-grade chronic inflammation, the so-called state of "metaflamation". "Metaflamation" is a form of low-grade systemic and chronic inflammation that occurs in metabolic diseases [10]. Its effect on lipid parameters was also investigated. A cross-sectional study of a large number of subjects indicated an association between high values of 25(OH)D and low values of total cholesterol, low density lipoprotein (LDL)-cholesterol, high values of high density lipoprotein (HDL)-cholesterol, and low triglycerides [11]. However, when longitudinal studies were performed, it was not confirmed that the change in the value of 25(OH)D from deficiency to sufficiency has a positive effect on the lipid profile. Namely, an increase in total and HDL-cholesterol, without changes in LDL-cholesterol and triglycerides, was found [12]. In some other studies positive results have been reported. In a study by Ramiro-Lozano and Calvo-Romero [13] a statistically significant reduction in total cholesterol levels and an insignificant trend of decreasing values of LDL-cholesterol, non-HDL cholesterol and triglycerides and no change in the value of HDL-cholesterol were found. In addition, vitamin D deficiency affects bone metabolism. In such conditions, the initial compensatory mechanism is increased secretion of parathyroid hormone (PTH), which stimulates the kidneys to increase phosphate and decrease calcium excretion.

The level of vitamin D3 varies during the year so that serum 25(OH)D concentrations are strongly associated with the exposure to ultraviolet (UV) light [14]. Because of seasonal variations which are very pronounced in temperate climates, 25(OH)D concentrations are highest in late summer and early autumn and lowest in late winter and early spring [15]. Therefore, in order to more accurately assess vitamin D3 deficiency in temperate climates, it is important to define its lower limit values depending on the time of year, which can significantly affect the decision on the need for supplementation and the required dose for its correction.

The aim of the study was to determine the prevalence of vitamin D3 deficiency in patients with T2DM and proteinuria, taking into account its seasonal variations, as well as to examine the influence of vitamin D3 deficiency and its replenishing on some parameters of bone metabolism.

Methods

This non-randomised controlled clinical trial was conducted in the General Hospital in Subotica, Serbia. The 24-week study included patients followed for T2DM with nephropathy defined with proteinuria > 150 mg/24 h, who were treated and followed at the outpatient Clinics for Nephrology and Diabetes. The population included in the study lived in the wider geographic region of Subotica, located at 46 °6 '0"north latitude and 19° 40' 0.01" east longitude, with a pronounced Pannonian-continental climate. The study was conducted and lasted from May 2018 to November 2019. Criteria for inclusion in the study were fulfilled: if patients aged 18 to 75 years with body weight (BW) was > 50 kg and body mass index (BMI) in the range of 18 to 35 kg/m^2 and if patients were treated for T2DM for at least 5 years, were on an antidiabetic diet or oral hypoglycemics, and had satisfactory glycoregulation in the period before enrolment in the study (the target HbA1 values were < 7% based on the criteria for the prevention of microvascular complications); patients with proteinuria > 150 mg/24 h and creatinine clearance > 30 mL/min/1.73 m^2 chronic renal failure (CRF) grade 4 which according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NFK DOQI) is a stage in which a permanent vascular approach is prepared] and in whom another cause of proteinuria was excluded (glomerulonephritis, amyloidosis, malignancies, systemic lupus erythematosus,…); patients who did not use vitamin D supplementation or its analogues for at least 3 months, who did not use dietary calcium, did not use corticosteroid therapy and did not have a history of other kidney diseases and current urinary tract infection; patients who if using antihy-

pertensive therapy, used angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ATB) and who were on this therapy for at least 3 months prior to the enrolment in a study with satisfactorily regulated arterial tension (values around 140/90 mmHg). The exclusion criteria were as follows: lack of cooperation (irregular intake of prescribed therapy, non-attendance at the scheduled outpatient check-ups ...); development of a disease or condition during the examination that could affect the implementation of diagnostic methods; pregnancy; the will of the respondents to no longer participate in this survey.

After the initial screening phase, 90 patients were selected for the study, and were divided into two groups, study (experimental) and control group, each consisting of 45 patients. The study group received cholecalciferol, and the control group received their standard therapy. The lower limit of normal vitamin D values for each patient was determined on the basis of seasonally defined limits for the required level of vitamin D3 given by months of the year, and according to their gender. For the assessment of vitamin D status and for the purpose of this study, values of seasonally defined limits for the required level of vitamin D3 were adapted to our climate conditions (Table 1).

For optimal values of vitamin 25(OH)D3, the level of 90–100 nmol/L was determined. Vigantol® (MERCK KGaA) 20,000 IU/mL in the form of oral drops (500 IU of vitamin D in one drop) was used. The number of cholecalciferol drops was determined on the basis of the difference between the level of vitamin D in the patient's serum and the set optimal levels. The number of drops was increased/decreased in men by changing the winter-summer period by 2 drops, and in women by 1 drop. The dose of cholecalciferol was reduced if the concentration of calcium in the urine in two consecutive controls exceeded the value of 7.5 mmoL/24 hours and in the serum a value greater than 2.6 nmol/L. Subjects in both groups were monitored for 6 months for sedimentation rate, complete blood count, serum and 24-hour urine calcium, phosphorus, alkaline phosphatase, C-reactive protein (CRP), fibrinogen, albumin, proteinuria in 24-hour urine and glomerular filtration rate (GFR) – at the beginning of the study (control examination I), after two (control examination II), four (control examination III) and six months (control examination IV), and lipid status (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, atherosclerosis risk factor – FAKRIZ and atherosclerosis index – INDART), HbA1c values in both groups at the beginning (control examination I) and at the end of the study (control examination IV) and $25(OH)D_3$ level only in the study group at the end of the study (control examination IV). The $25(OH)D_3$ was determined by the chemiluminescence method with acridinium ester-CMIA on the Abbott Architect I 1000 Immunochemical Analyser of MEDLAB laboratory (accreditation number: 03–008, with the accepted requirements prescribed by SRPS ISO 15189: 2014). Abbot tests were used. For blood sampling vacutainers and vacutainer needles Becton Dickinson, ref 367955 were used. The samples were sent on the same day to a central laboratory and were analysed within 6 hours.

Ethical aspects

The study design was approved by the local Ethics Committee. Each participant in the study signed a consent form.

Statistical analysis

Using the International Business Machines Corporation (IBM) Statistical Package for Social Sciences (IBM SPSS) version 20 and STATISTICA version 11 data were analysed. Qualitative data were presented in the form of numbers and percentages while quantitative data with parametric distribution were presented in the form of means, standard deviations (SD) and ranges. The whole tests were two sided. When the *p* value was less than 0.05, it was considered statistically significant; when it was less than 0.001 it was highly statistically significant, and greater than or equal to 0.05 it was statistically insignificant. The relationship between the two continuous variables was determined by quantitative correlation measures (Pearson's correlation coefficient). The results were interpreted and explained. The difference between the mean values of all observed variables at monitored time intervals of the applied therapy (I–IV) at the beginning and end of the study was analysed using the *t*-test and the *Z* test.

Table 1

Seasonally defined limits for the required level of vitamin D3

Month	Minimum level of 25-(OH)D3 (nmol/L)			
	> 50		> 80 female	
	male	female	male	female
July	81	65	131	105
August	87	69	137	109
September	87	71	137	111
October	79	67	129	107
November	69	62	119	102
December	59	57	109	97
January	52	52	102	92
February	50	50	100	90
March	50	50	100	90
April	53	51	103	91
May	61	54	111	94
June	71	60	121	100

Results

From 109 patients who were screened for the study, 19 of them had normal levels of vitamin D3. Thus, the prevalence of patients with vitamin D3 deficiency in the study sample was 82.56%, while the normal values of vitamin D3 were found in 17.43% of the subjects, out of which 10 (52.63%) were men and 9 (47.36%) women.

The clinical and biochemical characteristics of the patients in the experimental and control group are given in Table 2.

The distribution of vitamin D3 values in all study patients with deficient and normal vitamin D3 values, in total and by gender, is shown in Tables 3 and 4.

The average normal levels of vitamin D3 during follow-up by months and by gender in the examined patients are shown in Figure 1.

The normal levels of vitamin D3 by months of follow-up with respect to the lower limit values for women and men are shown in Figure 2.

The average vitamin D3 levels by month, in total and by gender, for patients enrolled in the study and control groups are shown in Figure 3.

The average values of vitamin D3 level per month for all patients in the study and control groups with respect to gender are given in Figures 4 and 5.

Vitamin D3 values measured in patients in both groups (n = 90) at the beginning were 45.1 ± 15.6 nmol/L, in the study group, the mean value was 42.8 ± 15.22 nmol/L and in the control group it was 47.02 ± 16.06 nmol/L. There was a statistically significant difference between deviations in vitamin D3 levels to the lower limit of normal, between summer and winter in the study and control groups together (p = 0.038), as well as in the study group (p = 0.03), where the difference to normal values of this vitamin was higher in summer. The summer period is defined as the period from

Table 2

Clinical and biochemical characteristics of patients in the study and control group

Variables	Study group (n = 45)	Control group (n = 45)	*p*
Mean age (years), M/F	63/65	66.5/63	0.512
BMI (kg/m^2), mean ± SD	29.936 ± 4.392	29.192 ± 4.278	0.418
Duration of diabetes (years), mean ± SD	8.46 ± 4.679	8.52 ± 4.095	0.94
Systolic pressure (mmHG), mean ± SD	130.9 ± 11.324	128.51 ± 10.224	0.296
Diastolic pressure (mmHg), mean ± SD	79.77 ± 5.999	79.88 ± 5.486	0.921
Antihypertensive therapy (n), yes / no	43/2	40/5	
monotherapy	9	8	
dual therapy	20	18	
triple therapy	14	14	
ACEI or ATB (n), yes/no	40/5	37/8	
Oral antidiabetics (n), yes/no	42/3	43/2	
monotherapy	27	20	
dual therapy	15	25	
Hypolipemics (n), yes/no	18/27	23/22	
statins	15/30	18/27	
fibrates	3/42	5/40	
Sedimentation rate (mm/h), mean ± SD	18 ± 14.69	12.9 ± 10.376	0.043
CRP (mg/L), mean ± SD	6.19 ± 7.856	4.11 ± 5.099	0.668
Fibrinogen (g/L), mean ± SD	4.048 ± .979	3.71 ± 0.755	0.88
Albumin (g/L), mean ± SD	43.8 ± 4.145	44.84 ± 5.143	0.306
Calcium (s) (mmol/L), mean ± SD	2.42 ± 0.135	2.426 ± 0.109	0.837
Phosphorus (s) (mmol/L), mean ± SD	1.097 ± 0.164	1.041 ± 0.216	0.132
Alkaline phosphatase (U/L), mean ± SD	76.59 ± 72.09	72.09 ± 21.01	0.733
HbA1c (mmol/moL), mean ± SD	47.8.57 ± 5.076	47.592 ± 5.486	0.331
Cholesterol (mmol/L), mean ± SD	5.352 ± 1.164	5.48 ± 1.119	0.565
Triglycerides (mmol/L), mean ± SD	2.091 ± 1.401	1.830 ± 0.825	0.296
HDL (mmol/L), mean ± SD	1.171 ± 0.297	1.23 ± 0.267	0.201
LDL (mmol/L), mean ± SD	3.42 ± 0.905	3.443 ± 0.960	0.806
FACRIZ, mean ± SD	4.74 ± 1.125	4.547 ± 1.032	0.186
INDART	2.962 ± 0.811	2.834 ± 0.852	0.190
GFR (mL/min), mean ± SD	100.782 ± 33.76	105.31 ± 46.452	0.500
24 h proteinuria (g), mean ± SD	0.683 ± 1.446	0.680 ± 1.161	0.335
Calcium(u) (mmoL/24 h), mean ± SD	3.069 ± 1.496	5.342 ± 3.151	0.00
Vitamin D3 (nmol/L), mean ± SD	43.2 ± 15.082	47.02 ± 16.069	0.198
No. of drops of cholecalciferol (mean ± SD)/ (IU)	4.72 ± 1.448/237	–	

M – males; F – females; BMI – body mass index; ACEI – angiotensin converting enzyme inhibitors; ATB – angiotensin receptor blockers; CRP – C-reactive protein; HDL – high density lipoprotein; LDL – low density lipoprotein; FACRIZ – risk factor for atherosclerosis; INDART – index of atherosclerosis; GFR – glomerular filtration rate [GFR (mL/min) = Cu*Vu(mL)/Cp*1,440 min (C – concentration, V – Volume, u – urine, p – plasma)]; (s) – serum; (u) – urine; IU – international unit.

Table 3

Vitamin D3 levels (nmol/L) in subjects with low values of vitamin D3

Month	All subjects (SG + CG)		Males			Females		
	vitamin D3	(n)	vitamin D3	lower limit value/month	(n)	vitamin D3	lower limit value/month	(n)
January	32.88 ± 10.82	7	36.87 ± 11.2	52	2	27.56 ± 9.4	52	2
February	31.18 ± 6.17	8	31.92 ± 6.56	50	3	29.96 ± 6.6	50	1
March	31.53 ± 12.6	6	31.53 ± 18.9	50	1	34.36 ± 4.1		2
April	38.95 ± 6.75	6	41.13 ± 8	53	1	36.76 ± 5.9	51	2
May	46.28 ± 13.81	6	53.77 ± 4.52	61	1	37.9 ± 15.5	54	2
June	38.05 ± 11.65	6	52.1	71	1	35.24 ± 10.5	60	2
July	53.7 ± 15.1	5	53.42 ± 17.5	81	2	55.1	65	1
August	44.97 ± 19.14	9	53.83 ± 19.35	87	3	33.9 ± 13.5	69	2
September	57.35 ± 16.14	10	53.82 ± 20.24	87	2	60.88 ± 12.5	71	3
October	61.29 ± 13.94	10	57.7 ± 17.54	79	1	66.67 ± 2.4	67	3
November	46.128 ± 8.9	8	52.46 ± 6	69	1	43.85 ± 9.13	62	2
December	46.94 ± 9.31	9	51.27 ± 7.6	59	2	43.48 ± 10.95	57	3
Total	45.1 ± 15.6	90	47.75 ± 15.89		20	42.57 ± 14.98		25

SG – study group; CG – control group.

Table 4

Vitamin D3 levels (nmol/L) in subjects with normal reference values of vitamin D3

Month	All subjects (SG + CG)		Males		Females	
	vitamin D3	(n)	vitamin D3	(n)	vitamin D3	(n)
January	67.2	1			67.2	1
February	57.6	1			57.6	1
March						
April	82.2	1			82.2	1
May	60	1			60	1
June						
July	72.1	1			72.1	1
August	88.25 ± 1.2	2	88.25 ± 1.2	2		
September	93.43 ± 20.11	3	113.1	2	83.6 ± 15.13	1
October	85.2 ± 6.7	5	89.53 ± 3.17	2	78.75 ± 4.88	3
November	81.0 ± 3.2	3	81.0 ± 3.2	3		
December	64	1	64	1		
Total	80.47 ± 13.36	19	86.52 ± 12.38	10	73.75 ± 11.53	9

SG – study group; CG – control group.

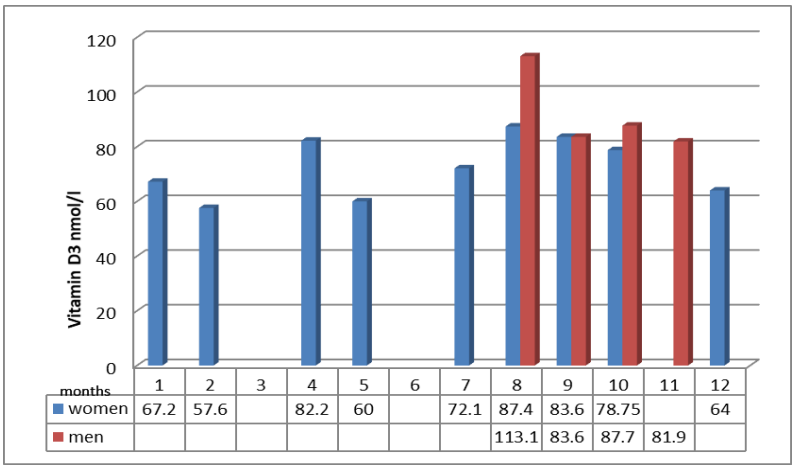


Fig. 1 – The average normal vitamin D3 levels in patients with diabetes mellitus type 2 and diabetic nephropathy during the year from January to December.

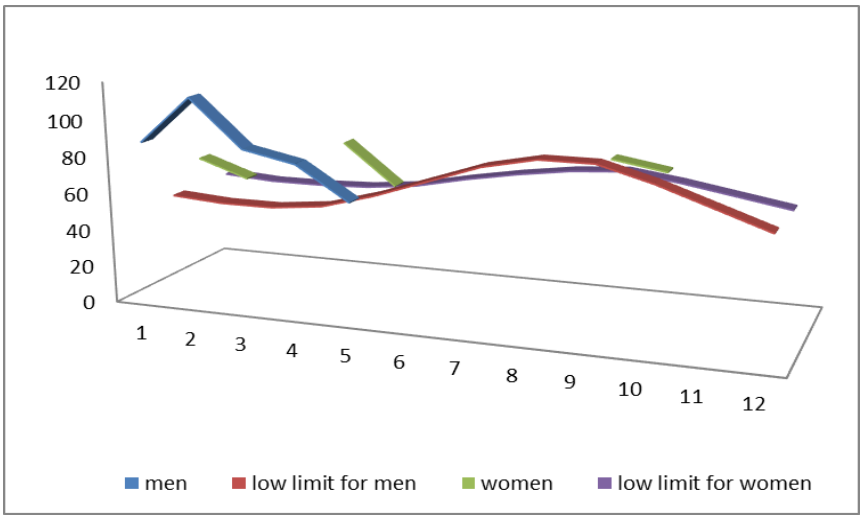
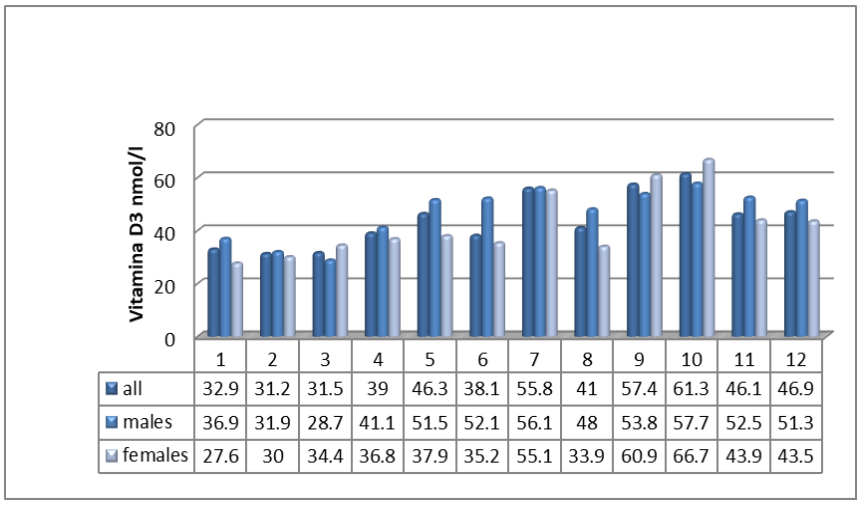
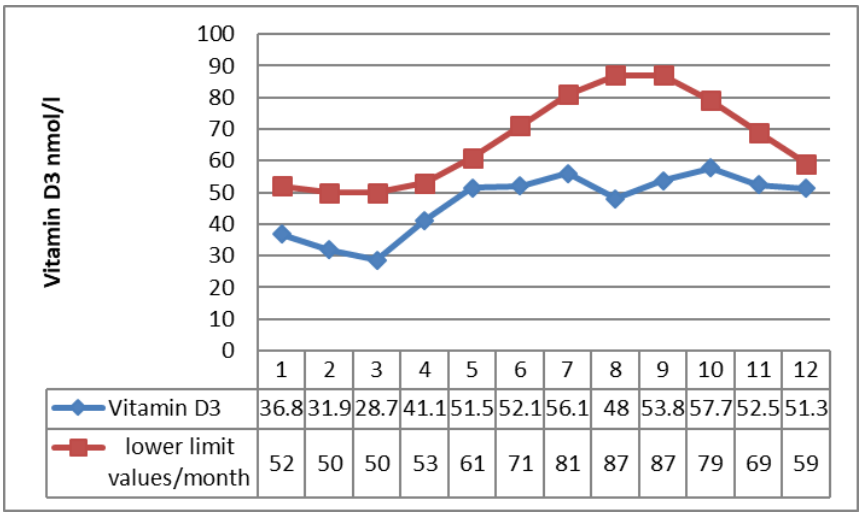


Fig. 2 – The average normal levels of vitamin D3 (nmol/L) in relation to the lower limit (ordinate) in men and women during the year from January to December (abscissa).



	1	2	3	4	5	6	7	8	9	10	11	12
all	32.9	31.2	31.5	39	46.3	38.1	55.8	41	57.4	61.3	46.1	46.9
males	36.9	31.9	28.7	41.1	51.5	52.1	56.1	48	53.8	57.7	52.5	51.3
females	27.6	30	34.4	36.8	37.9	35.2	55.1	33.9	60.9	66.7	43.9	43.5

Fig. 3 – The average vitamin D3 deficiency levels per month during the year, from January to December, in both the study and control groups.



	1	2	3	4	5	6	7	8	9	10	11	12
Vitamin D3	36.8	31.9	28.7	41.1	51.5	52.1	56.1	48	53.8	57.7	52.5	51.3
lower limit values/month	52	50	50	53	61	71	81	87	87	79	69	59

Fig. 4 – The average values of vitamin D3 deficiency during the year in men from month 1 (January) to 12 (December)

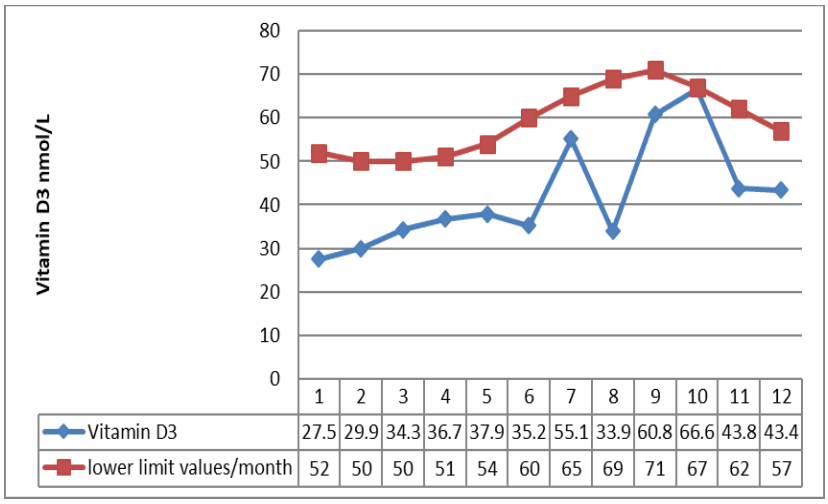


Fig. 5 – The average values of vitamin D3 deficiency during the year in women from month 1 (January) to 12 (December).

April to the end of September and the winter period from November to March. In the control group, there was also a difference between the values of vitamin D3 and the lower limit values for this vitamin, which was more pronounced in summer but this difference was not statistically significant ($p = 0.449$). There was also a difference in vitamin D3 values by gender, which was not statistically significant ($p = 0.21$) and was more pronounced during the summer period in men.

In relation to some parameters of bone metabolism, the following results were obtained: a significant positive correlation in the study group existed between serum vitamin D3 levels and serum calcium levels at the beginning ($r = 0.303$; $p = 0.043$) and at the end of the study ($r = 0.312$; $p = 0.49$), alkaline phosphatase activity at the beginning ($r = 0.298$; $p = 0.047$) and serum phosphorus levels ($r = 0.343$; $p = 0.030$) at the end of the study. There was a variable relationship between the values of vitamin D3 and calcium in urine ($r = 0.109$; $p = 0.491$ and $r = -0.033$; $p = 0.836$, respectively), with the fact that in the initial phase of the increase in the value of vitamin D3 there was a positive correlation and in the later phase it was negative one. The analysis of the relationship between glomerular filtration rate (GFR) and monitored parameters showed a statistically significant correlation only with urinary calcium ($r = 0.46$; $p < 0.01$). Alkaline phosphatase activities in the study group showed a statistically significant difference between control examination I (at the beginning of the study) and III (after four months) ($t = 2.091$; $p = 0.043$) and between control examination I (at the beginning of the study) and IV (after six months) ($t = 2.389$; $p = 0.022$), while in the control group there was no statistically significant difference (Figure 6).

There was a statistically significant difference in the values of serum calcium in the study group between control examination I (at the beginning of the study) and II (after two months) ($t = -3.894$; $p = 0.00$) as well as between control examination I (at the beginning of the study) and III (after four months) ($t = -2.027$; $p = 0.049$) and control examination I (at the beginning of the study) and control examination IV (after six months) ($t = -2.624$; $p = 0.012$), while between control examination II (after two months) and III (after four months), the value of the difference was at the limit of statistical significance ($t = 1.964$; $p = 0.056$). In the control group,

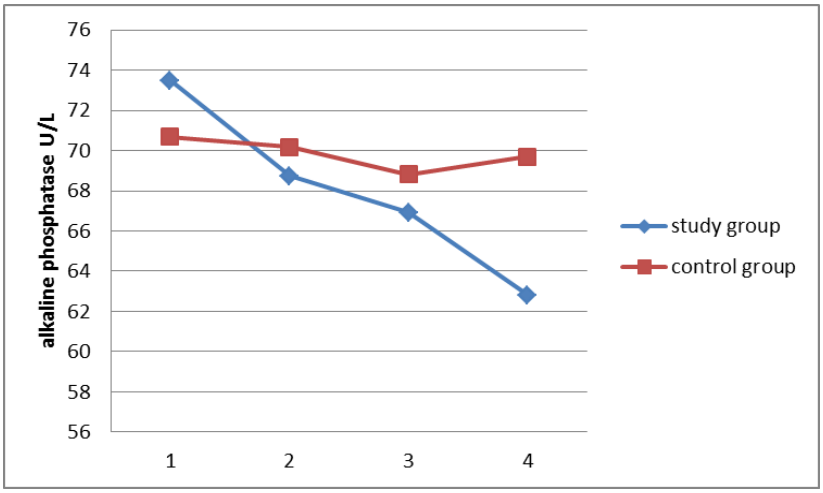


Fig. 6 – Average values of alkaline phosphatase in the both groups. 1 –at the beginning of the study; 2 – after two months; 3 – after four months; 4 – after six months.

there were no statistically significant differences in serum calcium levels among between control examnations (Figure 7).

The value of calcium in urine in the initial phase of the study in the study group increased and a statistically significant difference was found between control examination I (at the beginning of the study) and II (after two months) (t = 3.387; p = 0.002), as well as between control examination I (at the beginning of the study) and III (after four months) when the increase in calcium levels was less pronounced (t = 2.664; p = 0.011). Furthermore, the follow-up calcium values were slightly increased but without a statistically significant difference. The average values of calcium in urine remained in the reference range. The average value of calcium in urine in the control group was higher than the value in the study group, without significant changes among control examinations during the follow-up (Figure 8).

Discussion

It is known that, there is an increased frequency of vitamin D3 deficiency in the patients with T2DM [16]. Lower levels of vitamin D3 have also been found to be strongly correlated with a higher prevalence of microvascular complications such as nephropathy and retinopathy [17]. This study is, to our knowledge, the first one which took into account seasonally defined limit values for this vitamin, by months of the year and by gender of patients, when determining vitamin D levels.

The vitamin D3 value at the beginning of the study in all included patients in the study and control groups (n = 90) was 45.1 ± 15.6 nmol/L. In a study by Majiti and Lochan [18], the value of vitamin D3 level was 49.6 ± 8.9 nmol/L in the group of subjects older than 45 years. The difference in the

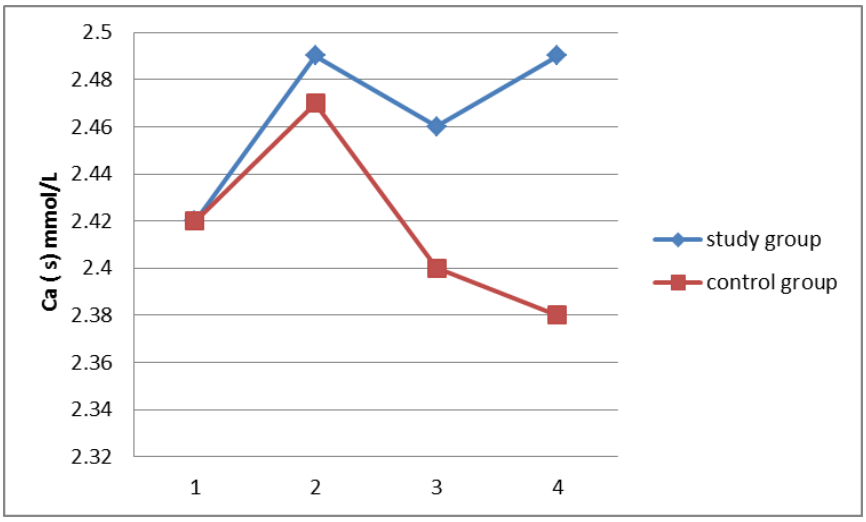


Fig. 7 – Average serum calcium values during the follow-up in both groups. 1 –at the beginning of the study; 2 – after two months; 3 – after four months; 4 – after six months.

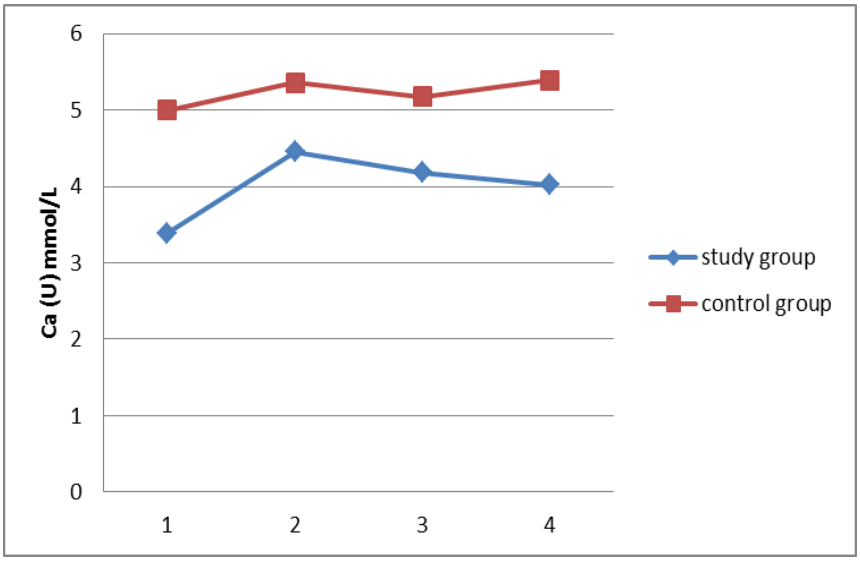


Fig. 8 – Average levels of calcium in the urine during the follow-up in both groups. 1 –at the beginning of the study; 2 – after two months; 3 – after four months; 4 – after six months.

obtained results in these studies can be explained by the different age structure of the study participants.

The prevalence of vitamin D3 deficiency in the study sample of 109 patients was 82.56%, while the normal vitamin D3 values were found in 17.43% of the subjects, out of which 10 (52.63%) were men and 9 (47.36 %) women. These results were obtained using the classification of vitamin D3 levels as reduced and normal values, which was performed according to the data of Bolland et al. [19] and modified according to our geographical conditions (Table 1). This data shows the lower normal limits for vitamin D3 for each month of the year, for men and women separately. This seasonally adapted classification for vitamin D3 deficiency is not commonly used in the patient's follow-up. Better insight into the comparison with the results of other authors can be obtained if the results of this study are expressed through classification according to which the normal values of vitamin D3 are determined by values of > 75 nmol/L, insufficiency with values between 45 and 75 nmol/L and vitamin D3 deficiency as < 45 nmol/L. Based on these criteria, we found vitamin D3 levels to be within the normal range in 18 (16.51%) patients in our sample, while in 48 patients (44.03%) we found insufficiency and in 43 (39.44%) patients, deficiency of vitamin D3.

Vitamin D3 deficiency is known to be more pronounced in diabetic patients than in the healthy population. In a study by Bayani et al. [20], among diabetic patients with a mean age of 51.2 ± 7.98 years, the mean value of vitamin D3 levels was 46.75 ± 25.5 nmol/L, while in the group of healthy subjects with a mean age of 50.6 ± 7.73 years, it was 61.5 ± 33.75 nmol/L. Among diabetic patients, vitamin D3 level was found to be within the reference range in 10.3%, deficiency in 64.2%, and insufficiency in 25% of the patients. The difference in the proportion of patients with vitamin D3 deficiency and insufficiency between our patient group and the cited author group can be partly explained by the difference in the age of diabetic patients, taking into account that expressiveness of vitamin D3 deficiency increases with age [21], also with different climatic conditions (the Pannonian-continental climate in Serbia and the mountainous-continental in northern Iran) as well as their diet. Due to the proximity of the Caspian Lake, it is possible that the fish diet in this population is higher, while the intake of fatty meat, as a nutritional source of vitamin D, is limited in our population.

Some other studies show results that are comparable to our results. In a study of Aljabri [22], patients with DN were found to have the following prevalence of vitamin D status: normal value was found in 17%, deficiency in 55.6% and insufficiency in 27.3% of patients. The mean age of patients was 54.4 ± 16.5 years. In our group of patients who were older than the patients in the mentioned study, the insufficiency (39.44%) was more represented and the deficiency (44.03%) of vitamin D3 was less present.

Because patients were enrolled in the study during the twelve months on a monthly basis, the difference between the deviations in vitamin D levels to the lower limit of normal for each month of the year was analysed on admission to the study. Patients were divided into two periods according to the time of enrolment into the study: summer and winter. The summer period is defined as the period from April to the end of September and the winter period from November to the end of March. A statistically significant difference was found between winter and summer values in the whole sample of participants with vitamin D3 deficiency, as well as in the study group of patients, with the deviation towards the normal values of this vitamin being greater in summer. In the control group, a difference between the measured values of vitamin D3 and the lower limit of normal values was also found, which was more pronounced in summer, and compared to gender, more pronounced in men, but none of the obtained differences was statistically significant. Our results do not correspond to the results of Carnevale et al. [23] who found the presence of vitamin D3 deficiency in 17.8% of subjects in the whole sample in winter and 2.2% of participants in summer, while in women, the deficiency was registered in 27.8% in winter and 3.4% in summer. In men, no difference was found in vitamin D deficiency in summer and winter. In this study, a single cut-off value for vitamin D3 deficiency was set at 75 nmol/L, and in these subjects vitamin D3 was determined twice a year, in February and in August. The study included healthy young adults (men average age 39.4 ± 7 years and women average age 36.9 ± 6.4 years) who are likely to have different dietary habits and more common outdoor activities resulting from good health. It is known that vitamin D synthesis in the skin under the influence of UV rays contributes significantly to its concentration during the summer. It was also found that the skin ability to synthesise vitamin D decreases with age [24].

Recent studies have suggested that there is a difference in vitamin D3 levels in women and men. Seasonally adjusted lower limit values for vitamin D3 have been defined and are different in men and women [15]. This difference is explained by the different BMI between genders [25], differences in lifestyle, cultural and religious factors that are associated with exposure to sunlight, physical activity, and the use of skin protection against UV damage [26]. Gender-related differences in vitamin D metabolism are also of importance [27].

In our patients, no difference was found in the level of vitamin D3 in relation to gender: the mean value at the study enrolment was 42.78 ± 15.37 nmol/L for women and 43.14 ± 15.1 nmol/L for men (p = 0.413). The patients were older middle-aged (men = 64.98 ± 7.58 years and women = 64.19 ± 7.05 years), and no difference was found between the mean BMI in men (29.09 ± 4.0 kg/m^2) and in women (30.07 ± 4.7 kg/m^2) (p = 0.345). The exposure to sunlight through outdoor activities was reduced due to age and associated diseases, and dietary habits in the winter allow more nutritional intake of vitamin D, resulting in a lower vitamin D3 deficit in winter than in summer.

The impact of vitamin D3 deficiency and its supplementation on some parameters of bone metabolism is of importance for monitoring and analysis. It is known that the vitamin D3 deficiency reduces the intestinal absorption of calcium by 15%–30%, which increases the level of serum parathyroid hormone (PTH) [28]. Alkaline phosphatase, which is important for bone formation and mineralisation, is usually

elevated in response to the action of PTH leading to stimulation of osteoblastic activity.

Vitamin D deficiency, however, is most commonly associated with normal serum calcium and phosphate values, high normal or elevated PTH values, normal or elevated alkaline phosphatase activity values, and low 24-hour urinary calcium excretion. Hypocalcemia and hypophosphatemia are rarely seen in patients with severe and long-term vitamin D deficiency [29]. In the study group of patients who used cholecalciferol, serum calcium and phosphate increased as a sign of an increase in vitamin D values, while a decrease in the value of alkaline phosphatase was registered as an expression of suppression, i.e. normalisation of previously elevated PTH values. Heaney et al. [30] found that maximal renal calcium absorption in men occurs when 25(OH)D levels are 70 nmol/L to 90 nmol/L, which otherwise represent the cut-off value for the onset of PTH suppression.

The mechanism by which vitamin D supplements potentially increase the risk of hypercalciuria has not been fully elucidated. Although data suggest that vitamin D supplements increase the risk of hypercalcemia by increasing intestinal calcium absorption, episodes of hypercalciuria are not correlated with hypercalcemia [31]. In a study by Tacheri et al. [32], an increase in calcium in urine from 3.74 mmoL/24 h to 5.7 mmoL/24 h was registered [32], while in our study after the initial increase in calcium urinary values from 3.34 mmoL/24 h to 4.5 mmoL/24 h, a stagnation of its values was registered with a slight decrease by the end of monitoring. In this research, it was concluded that despite the increase in calciuria during supplementation with vitamin D, it was not correlated with the increase in vitamin D values or changes in PTH values [32]. It has been suggested that other, predominantly dietary factors may be associated with hypercalciuria. Thus, in 24 h urine, a significant increase in the value of excreted urea was registered due to increased protein intake, and natrium and sucrose were also found [31]. In our study, a statistically significant correlation was found between vitamin D3 and calcium levels, only in serum, not in urine, at the beginning and end of the follow-up.

PTH levels are increased in patients with vitamin D deficiency, and it is expected that its values decrease during supplementation. A decrease in serum PTH could lead to an increase in urinary calcium due to a decrease in calcium resorption in the renal tubules [32]. The absence of this phenomenon can be explained in two ways. First, the tightly regulated conversion of 25(OH)D to $1.25(OH)_2D_3$ under the action of 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) limits the synthesis of the active form of vitamin D3 and thus prevents excessive intestinal calcium resorption. Second, the increased reabsorption of calcium in the intestines will not result in an increase in calciuria due to additional calcium deposition in the bones in order to restore their mineral content [33].

The limitation of this study is in the relatively small number of monitored patients as well as the lack of PTH values, which was attempted to be compensated by the use for the first time, to our knowledge, a seasonally defined limit values for vitamin D3, by months of the year and by gender of patients, and a detailed analysis of other bone parameters.

Conclusion

Based on our results and in consideration of the natural seasonal variation in vitamin D3 levels, it may be concluded that there is a significant deficiency in vitamin D3 during the whole year in patients with T2DM and nephropathy. Since patients with T2DM lasting more than 5 years are most often elderly, a deficiency of this vitamin can be expected due to its reduced synthesis in the skin which becomes thinner, due to obesity in which vitamin D is deposited in adipose tissue, due to reduced outdoor activity as a consequence of associated comorbidities, as well as due to the altered diet recommended for these patients - which does not contain a sufficient nutritional source of vitamin D. Significant majority of these patients have vitamin D deficiency and therefore its supplementation is recommended due to its multiple beneficial effects. The safety profile of serum and urine calcium values during long-term use is good, a slight reduction in calciuria was found during therapy. Deviations from normal values are more pronounced in the summer. Individual treatment with vitamin D3 supplements is required at intermittent intervals, which should be adjusted according to the diet and exposure to sunlight.

Conflict of interest

The authors have no conflict of interest to declare. There was no outside funding for the study.

REFERENCES

1. *Shaheen S, Chauhan H , Mishra N.* Association between type 2 diabetes mellitus and hypovitaminosis D. Int J Clin Biochem Res 2017; 4(4): 413–5.
2. *Xuan Y, Zhao HY, Liu JM.* Vitamin D and type 2 diabetes mellitus (D2). J Diabetes 2013; 5(3): 261–7.
3. *Aljack HA, Abdalla MK, Idris OF, Ismail AM.* Vitamin D deficiency increases risk of nephropathy and cardiovascular diseases in Type 2 diabetes mellitus patients. J Res Med Sci 2019; 24: 47.
4. *Xiao X, Wang Y, Hou Y, Han F, Ren J, Hu Z.* Vitamin D deficiency and related risk factors in patients with diabetic nephropathy. J Int Med Res 2016; 44(3): 673–84.
5. *Al-Timimi DJ, Ali AF.* Serum 25(OH) D in Diabetes Mellitus Type 2: Relation to Glycaemic Control. J Clin Diagn Res 2013; 7(12): 2686–8.
6. *Alvarez JA, Ashraf A.* Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010; 2010: 351385.
7. *de Boer IH.* Vitamin D and glucose metabolism in chronic kidney disease. Curr Opin Nephrol Hypertens 2008; 17(6): 566–72.
8. *Wang Y, Zhu J, DeLuca HF.* Where is the vitamin D receptor? Arch Biochem Biophys 2012; 523(1): 123–33.

9. *Buhary BM, Almohareb O, Aljohani N, Alrajhi S, Elkaissi S, Sherbeeni S,* et al. Association of Glycosylated Hemoglobin Levels With Vitamin D Status. J Clin Med Res 2017; 9(12): 1013–8.
10. *Zhong J, Gong Q, Mima A.* Inflammatory regulation in diabetes and metabolic dysfunction. J. Diabetes Res 2017; 2017: 5165268.
11. *Ponda MP, Huang X, Odeh MA, Breslow JL, Kaufman HW*. Vitamin d may not improve lipid levels: a serial clinical laboratory data study. Circulation 2012; 126(3): 270–7.
12. *Ponda MP, Dowd K, Finkielstein D, Holt PR, Breslow JL.* The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. Arterioscler Thromb Vasc Biol 2012; 32(10): 2510–5.
13. *Ramiro-Lozano JM, Calvo-Romero JM.* Effects on lipid profile of supplementation with vitamin D in type 2 diabetic patients with vitamin D deficiency. Ther Adv Endocrinol Metab 2015; 6(6): 245–8.
14. *Klingberg E, Oleröd G, Konar J, Petzold M, Hammarsten O.* Seasonal variations in serum 25-hydroxy vitamin D levels in a Swedish cohort. Endocrine 2015; 49(3): 800–8.
15. *Isaia G, Giorgino R, Adami S*. High prevalence of hypovitaminosis D in female type 2 diabetic population. Diabetes Care 2001; 24(8): 1496.
16. *Zoppini G, Galletti A, Targher G, Brangani C, Pichiri I, Trombetta M,* et al. Lower levels of 25-hydroxyvitamin D3 are associated with a higher prevalence of microvascular complications in patients with type 2 diabetes. BMJ Open Diabetes Res Care 2015; 3(1): e000058.
17. *Maiti S, Lochan Das K.* The assessment of vitamin D status in the patient of diabetic nephropathy: a case controletertary institutional based study. Asian J Sci Technol 2015; 6(9): 1794–8.
18. *Holick MF*. Vitamin D deficiency. N Engl J Med 2007; 357(3): 266–81.
19. *Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD*, et al. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency. Am J Clin Nutr 2007; 86(4): 959–64.
20. *Bayani MA, Akbari R, Banasaz B, Saeedi F.* Status of Vitamin-D in diabetic patients. Caspian J Intern Med 2014; 5(1): 40–2.
21. *Maggio D, Cherubini A, Lauretani F, Russo RC, Bartali B, Pierandrei M*, et al. 25(OH)D Serum levels decline with age earlier in women than in men and less efficiently prevent compensatory hyperparathyroidism in older adults.J Gerontol A Biol Sci Med Sci 2005; 60(11): 1414–9.
22. *Aljabri KS*. Vitamin D Deficiency in Saudi Men with Type 2 Diabetes Mellitus. Ann Short Reports 2019; 2: 1037
23. *Carnevale V, Modoni S, Pileri M, Di Giorgio A, Chiodini I, Minisola S,* et al. Longitudinal evaluation of vitamin D status in healthy subjects from southern Italy: seasonal and gender differences. Osteoporos Int 2001; 12(12): 1026–30.
24. *Lucas JA, Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM*, et al. Determinants of vitamin D status in older women livingin a subtropical climate. Osteoporos Int 2005; 16: 1641–8.
25. *Bredella MA.* Sex Differences in Body Composition. Adv Exp Med Biol 2017; 1043: 9–27.
26. *Bruce AF, Theeke L, Mallow J.* A state of the science on influential factors related to sun protective behaviors to prevent skin cancer in adults. Int J Nurs Stud 2017; 4(3): 225–35.
27. *Sanghera DK, Sapkota BR, Aston CE, Blackett PR.* Vitamin D Status, Gender Differences, and Cardiometabolic Health Disparities. Ann Nutr Metab 2017; 70(2): 79–87.
28. *Hashemipour S, Larijani B, Adibi H, Sedaghat M, Pajouhi M, Bastan-Hagh MH,* et al. The Status of Biochemical Parameters in Varying Degrees of Vitamin D Deficiency J Bone Miner Metab 2006; 24(3): 213–8.
29. *Kennel KA, Drake MT, Hurley DL.* Vitamin D deficiency in adults: when to test and how to treat? Mayo Clin Proc 2010; 85(8): 752–7.
30. *Heaney RP, Dowell MS, Bierman J, Hale CA, Bendich A.* Absorbability and cost effectiveness in calcium supplementation. J Am Coll Nutr 2001; 20(3): 239–46.
31. *Malihi Z, Wu Z, Stewart AW, Lawes CM, Scragg R.* Hypercalcemia, hypercalciuria, and kidney stones in long-term studies of vitamin D supplementation: a systematic review and meta-analysis. Am J Clin Nutr 2016; 104(4): 1039–51.
32. *Taheri M, Tavasoli S, Shokrzadeh F, Amiri FB, Basiri A.* Effect of vitamin D supplementation on 24-hour urine calcium in patients with calcium Urolithiasis and vitamin D deficiency. Int Braz J Urol 2019; 45(2): 340–6.
33. *Letavernier E, Daudon M.* Vitamin D, Hypercalciuria and Kidney Stones. Nutrients 2018; 10(3): 366.
34. *Leaf DE, Korets R, Taylor EN, Tang J, Asplin JR, Goldfarb DS,* et al. Effect of Vitamin D Repletion on Urinary Calcium Excretion among Kidney Stone Formers. CJASN 2012; 7(5): 829–34.

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