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Original Scientific Paper

ERECTILE DYSFUNCTION AND VITAMIN D SERUM CONCENTRATION ASSOCIATED ONLY IN DIABETICS

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SUMMARY – Recently, erectile dysfunction (ED) has become more prevalent, with many risk factors being linked to this condition. We measured vitamin D (VD) serum concentration in 32 ED patients and 28 controls to test the hypothesis that ED patients have lower VD, which was based upon previous links between ED risk factors and VD deficiency. Exclusion criteria were postoperative, anatomical or neurogenic ED, prostate cancer and lower urinary tract symptoms. Erectile function was assessed with the International Index of Erectile Function-5 questionnaire. VD sampling was performed during winter months, thus avoiding seasonal variations in cutaneous VD production. Median VD was 8.36 ng/mL. Two subjects were VD insufficient, while all other subjects were VD deficient. There was no difference in VD concentration between ED patients and controls. However, patients with both diabetes and ED had lower VD levels than all other subjects. Controls had higher levels of parathyroid hormone (PTH) than ED patients, although the levels were within the normal reference range. PTH was an independent risk factor, lowering ED risk by 4% with every unit increase. Overall, no association between VD and ED was found, except in patients with both diabetes and ED, who had lower VD levels compared to all other subjects. PTH might have a protective effect on erectile function.

Keywords: *vitamin D; parathyroid hormone; IIEF-5 questionnaire; erectile dysfunction; vitamin D deficiency.*

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Introduction

Penile erection is a neurovascular phenomenon that implies undisturbed interaction and coordination of the central and peripheral nervous system and a specialized tissue of penile corpora cavernosa and its vascular endothelium, all under unimpaired hormonal control[1,2]. The vascular endothelium of the corpora plays a key role in achieving an erection, while its injury results in endothelial dysfunction (END) — the main factor in the pathogenesis of erectile dysfunction (ED)[3]. ED is a persistent inability of a man to achieve and/or maintain penile erection sufficient for sexual activity[4]. It is a highly prevalent disorder worldwide, with prevalence that increases with age and has been reported in up to 69.2% of men[5,6,7]. Causes of ED can be vascular (coronary artery disease [CAD], arterial hypertension [AH], diabetes mellitus [DM]), neurogenic (stroke, multiple sclerosis), hormonal (hyperprolactinemia, hypogonadism, dysthyroidism), anatomical (hypo- and epispadias, Peyronie's disease), psychogenic and drug-induced (neuroleptics, antidepressants, thiazide diuretics)[8].

ED was historically considered a benign condition, however, not only does ED impair quality of life, but it also bears an increased risk of impending cardiovascular disease (CVD)[9], because of END as their common pathological mechanism. Furthermore, ED itself significantly increases the risk of all-cause mortality. It is estimated that the first symptoms of CAD develop 2-3 years after the initial onset of ED, while a complete manifestation of CVD with incidents like heart attack or stroke occurs after 3-5 years[10]. The aforementioned intervals not only emphasize ED as a predictor of serious medical conditions, but also allow interventions to diminish the influence of CVD risk factors, which overlap with ED risk factors (obesity, lack of exercise, metabolic syndrome, smoking). ED precedes CVD because of the smaller caliber of penile vessels, which are impaired by the same systemic process as larger coronary and cerebral vessels, but manifest symptoms earlier[11].

VD is primarily synthesized through sun exposure of the skin, via ultraviolet B (UVB) radiation. Natural food sources of VD are scarce[12] and a VD intake analysis in the United States of America revealed that none of the food sources were sufficient to meet the recommended daily needs[13]. Although cutaneous VD production is influenced by skin pigmentation, air pollution and age[14], the key factor influencing a persons' VD status is the zenith angle of the sun, which alters with changes in latitude and altitude, seasonal periods and time of day[14-16]. In areas below and above 35° latitude, including Croatia (42°-46° north latitude), little if any VD is endogenously produced from October till March due to a sharper UVB angle, regardless of the duration of sun exposure. For the remainder of the year, only several minutes of UVB exposure are required for adequate VD synthesis[16,17]. Calcidiol (25-hydroxycholecalciferol, 25[OH]D) is a major circulating VD metabolite and the best indicator of VD concentration adequacy; hence its usage in determining patients' VD status[16]. Calcitriol (1,25-dihydroxycholecalciferol, $1,25[OH]_2D$) is a biologically active form of VD, but it is not suitable for VD measurement because of its strict homeostatic regulation and short circulating half-life[14,16,17]. The "classic" role of VD is in regulating mineral metabolism in the bone, as well as maintaining the homeostasis of serum calcium and phosphorus concentrations. Recently, it has been shown that VD has a wide variety of extraskeletal effects, directly linked to CVD, autoimmune disorders and cancer[16,18-24].

Vitamin D deficiency (VDD) is defined as a serum 25-hydroxyvitamin D (25(OH)D) concentration below 20 ng/mL, VD insufficiency as a 25(OH)D level of 21-29 ng/mL and VD sufficiency as 25(OH)D levels above 30 ng/mL[14]. VDD has previously been associated with CVD and several ED risk factors, such as atherosclerosis[25-29], AH[30-32] and DM[33-40], which all cause endothelial impairment of all blood vessels, including penile vasculature, as well as with some nonvascular disorders linked both to ED and VDD[41]. One recent study[42] found a direct link between the presence of ED and VDD, and another[43] associated lower VD levels with ED in patients with type 2 DM. DM bears an increased risk for both CVD and ED, regardless of DM type. Better glycemic control results in a less frequent or delayed onset of microvascular complications of CVD[35]. The mentioned process of END predisposes the occurence of ED in diabetics 10-15 years earlier[7] and overall 3-4 times more frequently than in normoglycemic men[36,41]. Multiple beneficial roles of VD in DM have been demonstrated[19]. Oral supplementation of VD increased insulin secretion and improved insulin resistance[37,38].

With regard to the aforementioned pathophysiology of END and interplay between common risk factors of both CVD and ED, and most importantly because of the already proven influence of VD on the same risk factors, we set to determine VD levels in patients with ED and to test the hypothesis that ED patients have a lower serum 25(OH)D concentration in comparison with healthy subjects, as well as that the intensity of ED correlates with 25(OH)D levels.

Methods

Patients

Sixty subjects were included in the cross-sectional research: 32 ED patients and 28 age-matched controls without ED. According to the G-power calculation, with $\alpha = 0.05$, power of 0.8 and effect size of 0.95, the sample size of overall 38 subjects was to be met, which was in accordance with the size of our sample. The study was conducted at the Department of Urology, University Hospital Center Osijek, Osijek, Croatia and was approved by the Ethics Committee of University Hospital Center Osijek and the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek. Patients with ED visiting our outpatient clinic were enrolled in the study consecutively, as were the control subjects without ED, who visited the clinic for different reasons. Exclusion criteria comprised anatomical ED (penile fracture, Peyronie's disease), postoperative ED (e.g. after radical prostatectomy or colorectal operation), neurological ED (brain tumors, spinal injuries, stroke), prostate cancer and symptoms of urogenital infection. Subjects taking medications affecting erectile function (tricyclic antidepressants, neuroleptics, beta blockers, phosphodiesterase type 5 inhibitors [PDE-5 inhibitors]) and those on VD supplementation therapy were also excluded.

Methods

After obtaining a written informed consent, 60 male patients were enrolled. Patients' characteristics were obtained through an in-house questionnaire, while clinical data were collected from the clinical exam and patients' charts. The simplified 5-item version of IIEF-5 was used as a diagnostic tool for ED evaluation[44] and to further divide the research subjects into different ED categories. In accordance with their IIEF-5 score, the patients were categorized as having no ED (ED 0; IIEF-5 score 22-25), mild ED (ED 1; score 17-21), mild to moderate ED (ED 2; score 12-16), moderate ED (ED 3; score 8-11) or severe ED (ED 4; score ≤ 7).

Blood samples for the analysis of 25(OH)D concentration and other laboratory parameters of interest (complete blood count, hemoglobin, urea, creatinine, uric acid, electrolytes, fasting glucose, hemoglobin A1c [HbA1c], cholesterol and triglycerides, liver enzymes, bilirubin, prostate specific antigen [PSA], parathyroid hormone [PTH], testosterone, prolactin, follicule stimulating hormone [FSH], luteinizing hormone [LH], thyroid stimulating hormone [TSH], triiodothyronine [T3] and thyroxine [T4]) were collected from the participants' cubital vein using the usual procedure. All blood samples and freshly collected clean catch urine samples were analyzed at the Department of Clinical Laboratory Diagnostics, University Hospital Center Osijek. Sampling for all 60 subjects took place over a short period of time (February and March 2015) to avoid the aforementioned seasonal variations in cutaneous VD production.

VD serum concentration was measured with high-performance liquid chromatography (HPLC) using the Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) and a Chromsystems reagent (Chromsystems Instruments & Chemicals GmbH, München, Germany), following the manufacturers' instructions. Testosterone, prolactin, FSH and LH concentrations were tested using a chemiluminiscent microparticle immunoassay (CMIA) on an ARHITECT i1000SR device (Abbott Diagnostics, Lake Forest, USA), and PSA was analyzed with an electrochemiluminescence immunoassay (ECLIA) on a COBAS 6000 device (Roche Diagnostics GmbH). Serum PTH, TSH, T3 and T4 concentrations were measured with a chemiluminescence immunoassay (CLIA) using a UniCel DxI600 device (Beckman Coulter Inc., Brea, USA).

Statistical analysis

Statistical analysis was performed using the SPSS statistical program (version 15.0, SPSS Inc. Chicago, IL, USA). Numeric data were reported as a median and interquartile range (IQR) and normality

of distribution was examined with the Kolmogorov-Smirnov test. Nominal data were shown as absolute and relative frequencies. The difference between two independent samples was calculated with the Mann-Whitney test and between more than two independent samples with the Kruskal-Wallis test, while the Mann-Whitney test was used for *post-hoc* analysis. The chi-square and Fisher exact tests were used to display differences for categorical data. Correlations were assessed using Spearman's rho coefficient and a multivariate analysis was performed by binary logistic regression using the Hosmer-Lemeshow goodness-of-fit test, where covariates were chosen at a $P < 0.1$ level of significance. For predictor variables, the odds ratio (OR) and 95% confidence interval (CI) were calculated. A two-sided *P*-value of < 0.05 was considered statistically significant.

Results

The median IIEF-5 score for all 60 subjects was 18.5 points; 32 ED patients had an IIEF-5 score of 10, while 28 control subjects had a median IIEF-5 score of 23 points. Some men had ED symptoms for up to 16 years and the average time from the onset of ED to seeking medical help was 1.5 years. Overall, the median age of the subjects was 55.5 years (range 42-74 years), with no significant difference between the studied groups. None of the subjects had symptoms of genitourinary infection, nor was there significant leukocyturia present.

Over one third of the subjects (37%) had two or more comorbidities present (AH, DM, cardiac arrhythmias, posttraumatic stress disorder [PTSD]), which was more frequent in the ED group ($P = 0.007$). Almost half of the subjects (48%) had AH as the most common comorbidity, while 28% had DM, with 29% of them being insulin-dependent. Subjects with DM had worse erectile function compared to non-diabetics (median IIEF-5 score 15; IQR 5.5-22 vs. median IIEF-5 score 22; IQR 10-24), $P = 0.028$. Moreover, men with DM also had lower VD levels (median 6.09 ng/mL; IQR 3.5-9.6 vs. median 8.72 ng/mL; IQR 5.8-12.7), $P = 0.048$. Finally, men with both DM and ED (12 subjects) had lower VD levels (median 5 ng/mL; IQR 3.4-8.3 vs. median 8.74 ng/mL; IQR 5.9-12), $P = 0.034$ and worse erectile function (median IIEF-5 score 8.5; IQR 5-16.5 vs. median IIEF-5 score 22; IQR 11-24), $P = 0.001$, but did not differ in

Table 1. Clinical characteristics of subjects without ED (ED 0) and those with any degree of ED; (N = 60)*

Characteristic	**ED 0 (n = 28)**	**ED (n = 32)**	**total**	***P-value***
Age (median [IQR†]) (years)	54 (49 – 60.8)	56.5 (52 – 61)	55.5 (50.3 – 61)	0.295‡
BMI§ (median [IQR])	28.1 (26.3 – 30.3)	28.4 (26.2 – 32.6)	28.2 (26.3 – 31.5)	0.589‡
IIEF-5‖ score (median [IQR])	23 (22 – 24.8)	10 (7 – 14.8)	18.5 (9.3 – 23)	<0.001‡
Smokers (n)	6	13	19	0.165¶
Alcohol consumers (n)	19	17	36	0.297¶
Physically active (n)	12	5	17	0.024¶
Arterial hypertension (n)	10	19	29	0.077¶
Cardiac arrhythmias (n)	2	5	7	0.432¶
Diabetes mellitus (n)	5	12	17	0.15¶
Insulin therapy (n)	0	5	5	0.055¶
PTSD** (n)	1	8	9	0.029¶
Depressive episodes (n)	0	2	2	0.494¶
≥ 2 comorbidities (n)	5	17	22	0.007¶

*ED – erectile dysfunction; †IQR – interquartile range; ‡Mann-Whitney test; §BMI – body mass index (kg/m^2); ‖IIEF-5 – International Index of Erectile Function-5 questionnaire; ¶Fisher exact test; **PTSD – posttraumatic stress disorder.

age (P=0.579) from the rest of the subjects. Figure 1 illustrates the difference in VD between men with both DM and ED and other subjects.

Subjects with AH were older (median 59 years, IQR 54.5-62.5 vs. median 52 years, IQR 49-60; P=0.007) and had worse erectile function (median IIEF-5 score 10 points, IQR 7-22 vs. median score 22 points, IQR 13-24; P=0.001), while VD levels did not differ between AH and normotensive subjects. PTSD was more prominent in the ED group and physical activity in the control group. The clinical characteristics of the studied groups are shown in Table 1. In a multivariate analysis with AH, physical activity, two or more comorbidities present and PTSD as covariates, the only independent prognostic factor for ED was physical activity, lowering the risk of ED almost five times (Exp (B) 0.214; 95 % CI 0.053-0.868; P = 0.031).

Overall, median serum 25(OH)D concentration was 8.36 ng/mL. None of the subjects had sufficient VD levels of 30ng/mL or more. Two men had insufficient VD levels (21-29 ng/mL), while all other subjects had VDD (≤ 20 ng/mL). There was no difference in VD concentration between the ED and non-ED group. VD levels correlated positively with age (Spearman's rho = 0.298, P=0.021). The ED group had higher blood glucose and HbA1c in comparison with the control group. There was no difference in testosterone, prolactin, FSH, LH, PSA, TSH, T3 and T4 levels between the groups.

Non-ED subjects had higher PTH concentrations than ED patients (P=0.037). Rosner's Extreme Studentized Deviate two-sided test for multiple outliers was performed and two subjects with "extreme" PTH concentrations were excluded (one control subject with a PTH concentration of 471.4 pg/mL and one ED patient with a PTH concentration of 132.1 pg/mL), but the difference between the studied groups was even more pronounced (median and IQR for non-ED subjects 46.8 [33.4-60.6] vs. 38 [26.8-46.2] pg/mL for ED subjects, with P=0.031). Figure 2 illustrates the aforementioned difference in PTH

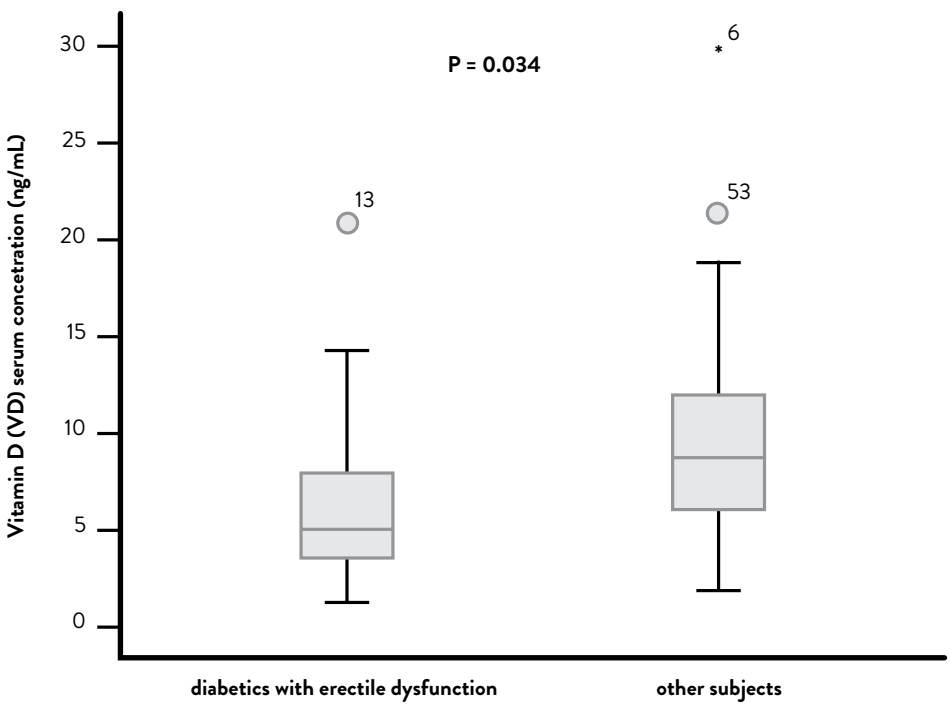

Figure 1. The difference in VD between diabetics with erectile dysfunction (n = 12) and other study subjects (n= 48).

Table 2. Multivariate analysis of subjects without ED (ED 0) [n = 27] and those with any degree of ED (n = 31) after the exclusion of subjects with outlier PTH† values; (N = 58)*

Characteristic	Exp (B)	95 % CI‡	*P*-value§
Physical activity	0.29	0.051 – 1.662	0.165
Arterial hypertension	9.173	1.015 – 82.938	0.049
PTSD‖	21.706	1.007 – 468.098	0.05
≥ 2 comorbidities	1.303	0.14 – 12.122	0.816
HbA1c¶	0.157	0.016 – 1.544	0.112
T4**	0.516	0.269 – 0.989	0.046
PTH	0.959	0.921 – 0.999	0.045
Leukocytes	1.687	0.947 – 3.004	0.076
AST††	0.953	0.902 – 1.007	0.089
Bilirubin	0.937	0.755 – 1.161	0.55
Glucose	2.62	0.915 – 7.499	0.073

*ED – erectile dysfunction; †PTH – parathyroid hormone; ‡CI – confidence interval; §Hosmer-Lemeshow goodness-of-fit test; ‖PTSD – posttraumatic stress disorder; ¶HbA1c – hemoglobin A1c; **T4 – thyroxine; ††AST – aspartate transaminase.

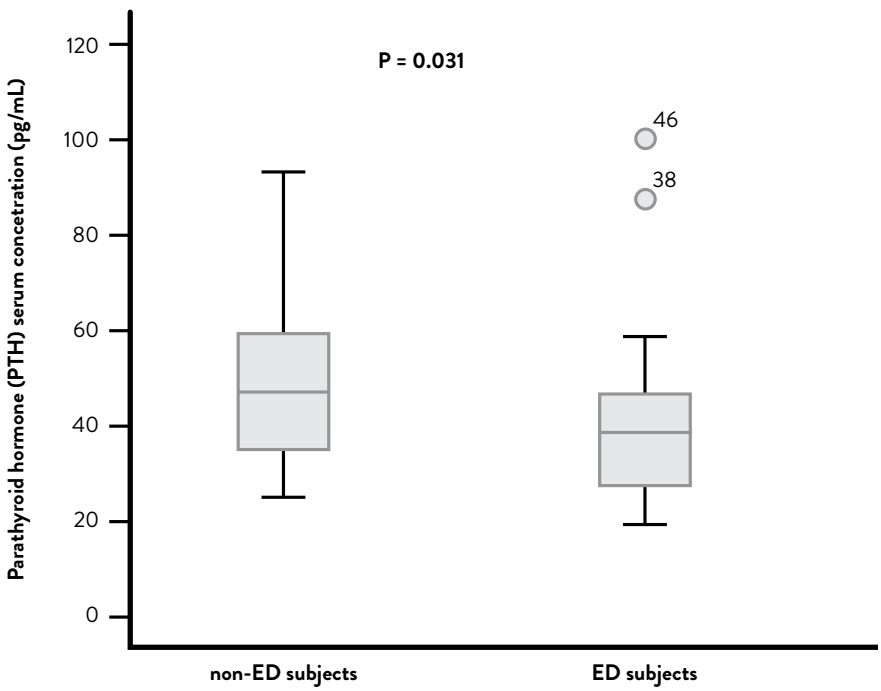

Figure 2. The difference in PTH between non-ED (n = 27) and ED subjects (n = 31).

between the studied groups, after the exclusion of subjects with outlier PTH values. A correlation analysis showed that PTH levels positively correlated with the IIEF-5 score and negatively with the intensity of ED, even more so after the exclusion of PTH outliers (Spearman's rho = 0.271, $P = 0.04$ and Spearman's rho = -0.333, $P = 0.011$, respectively), while there was no significant correlation between PTH and other variables. A multivariate analysis (after the exclusion of two PTH outliers) with AH, physical activity, two or more comorbidities present, PTSD, HbA1c, T4, PTH, bilirubin, blood glucose and white blood cell count as covariates, revealed independent risk factors for having ED, which is shown in Table 2. AH and PTSD increased the risk of ED, while the results for T4 concentrations were marginally significant. A significant reduction in the risk for ED was found for PTH concentration, with a 4% risk reduction for every PTH unit increase.

We further categorized the subjects according to their IIEF-5 score, which displayed ED severity. Apart from 28 non-ED subjects (ED 0), there were 6 subjects both in the mild (ED 1) and mild to moderate ED subgroup (ED 2), 8 in the moderate ED subgroup (ED 3) and 12 in the severe ED subgroup (ED 4). Overall, ED subgroups did not differ in age ($P = 0.068$), although a clear prevalence of older age in the severely affected subgroups (ED 3 and ED 4) was noted and in a *post-hoc* Mann-Whitney test proved to be significant between the ED 1 and ED, ED 1 and ED 4, and ED 2 and ED 4 subgroups. The clinical characteristics of the ED subgroups are shown in Table 3. There were no differences in serum VD concentration or other studied laboratory parameters between the ED subgroups.

Discussion

The median VD serum concentration of our studied population of 60 men was 8.36 ng/mL, with an IQR of 4.84-11.67 ng/mL and the lowest measured VD value being 1.18 ng/mL. According to the definition of VD hypovitaminosis[14], not one subject had sufficient VD levels, two were VD insufficient (21-29 ng/mL) and all the other men had VDD (≤ 20 ng/mL). A total of 39 subjects (65%) had 25(OH)D levels under

Table 3. Clinical characteristics of ED subgroups of different severity; (N = 60)*

Characteristic	ED 0 (n = 28)	ED 1 (n = 6)	ED 2 (n = 6)	ED 3 (n = 8)	ED 4 (n = 12)	*P*-value
Age (median [IQR†]) (years)	54 (49 – 60.8)	51 (49 – 57.8)‡	52.5 (48 – 57)§	60.5 (53.3 – 64)	60 (56 – 65.8)	0.068‖
IIEF-5¶ score (median [IQR])	23 (22 – 24.8)	18 (17 – 19.3)	13.5 (12 – 15)	10 (9 – 10.8)	5.5 (5 – 7)	<0.001‖
Smokers (n)	6	3	3	2	5	0.414**
Alcohol consumers (n)	19	2	3	6	6	0.406**
Physically active (n)	12	0	2	2	1	0.101**
Arterial hypertension (n)	10††	1‡‡	3	5	10	0.03**
Cardiac arrhythmias (n)	2	1	1	0	3	0.41**
Diabetes mellitus (n)	5	3	2	2	5	0.397**
Insulin therapy (n)	0§§	1	2	1	1	0.085**
PTSD‖‖ (n)	1	2	2	1	3	0.135**
Depressive episodes (n)	0	1	0	0	1	0.224**
≥ 2 comorbidities (n)	5¶¶	3	3	4	7	0.084**

* ED – erectile dysfunction; † IQR – interquartile range; ‡ $P = 0.029$ vs. ED 3, $P = 0.024$ vs. ED 4 (post-hoc Mann-Whitney test); § $P = 0.041$ vs. ED 4 (post-hoc Mann-Whitney test); ‖ Kruskal-Wallis test; ¶ IIEF-5 – International Index of Erectile Function-5 questionnaire; ** Pearson's χ^2 test; †† $P = 0.014$ vs. ED 4 (Fisher exact test); ‡‡ $P = 0.013$ vs. ED 4 (Fisher exact test); §§ $P = 0.027$ vs. ED 2 (Fisher exact test); ‖‖ PTSD – posttraumatic stress disorder; ¶¶ $P = 0.021$ vs. ED 4 (Fisher exact test).

10 ng/mL, while 16 of them (27%) had levels under 5 ng/mL. The researchers of VDD in the Croatian population have so far been focused on postmenopausal women, where Laktašić et al.[17] found a very high prevalence of VD hypovitaminosis, with research focused on men being less common. A favorable factor for the poor VD status measured in our research is certainly winter sampling, because little if any VD is endogenously produced during winter months, regardless of the amount of sun exposure. Nevertheless, the fact that none of the studied men had a VD level of 30 ng/mL or more, bearing in mind the multiple general health benefits of VD adequacy, is unsettling.

We found no difference in VD serum concentration between ED patients and non-ED controls. Adding the mild ED 1 to the ED 0 subgroup and comparing the newly formed group with more severely affected subjects did not result in a VD level difference, nor did a comparison between the most severely affected subjects (ED 3 and ED 4 subgroups) with the rest. However, when comparing subjects with DM and non-diabetics, men with DM had lower VD levels ($P = 0.048$) and, as expected, worse erectile function compared to non-diabetics ($P = 0.028$). Furthermore, men with both DM and ED (12 subjects) had lower VD levels ($P = 0.034$) and worse erectile function ($P = 0.001$), but did not differ in age ($P = 0.579$).

As previously mentioned, END may be the main mechanism that leads to ED in diabetics, but the role of specific DM complications, endocrine disorders and psychological factors is substantial and illustrates the multifactorial etiology of ED in that population[45]. Multiple beneficial roles of VD in DM have previously been demonstrated[19], either via oral supplementation of VD[37,38,46-49] or indirectly through amounts of UVB exposure[39,40]. The conclusion of a recent meta-analysis of 11 prospective studies correlates the incidence of type II DM inversely with serum 25(OH)D concentration and suggests a decrease of DM risk by 41% with 25(OH)D > 32 ng/mL compared to values < 19.5 ng/mL, but also highlights a lack of randomized trials on the subject[50]. Recently, Caretta et al.[43] investigated the link between ED and VDD in 92 DM type II men and found that subjects with 25(OH)D < 10 ng/mL had worse IIEF-5 scores compared to subjects with 25(OH)D > 20 ng/mL. Our aforementioned results of lower VD levels in men with both DM and ED are in accordance with previously published studies and illustrate that in a relatively homogeneous population of diabetic men there may be an association between VDD and ED.

However, the overall absence of difference in VD levels between ED patients and non-ED controls in our study is contrary to the results published in 2014 by Barassi et al.[42]. The methodological differences of the two studies may be held accountable for their different outcomes. Barassi et al. conducted their research on 143 ED patients, who were otherwise healthy, did not have any comorbidities (CVD, DM, AH) and were non-smokers. They performed serum sampling between May and September in two consecutive years, which also differs from our study where we tried to minimize the influence of UVB exposure on endogenous VD synthesis and therefore sampled in winter months of the same year, as explained above[16,17]. The results of their study showed a difference in VD levels between men with mild and severe ED, and also lower VD levels in arteriogenic ED compared to non-arteriogenic ED. In our research, however, there was a control group of healthy men without ED, so we did not perform dynamic color Doppler examinations, although this may define ED subtypes more precisely, because of the need for an intracavernous prostaglandin injection. Furthermore, their IIEF-5 test scores were categorized somewhat differently than in our research or the conventional categorization[44] and a radioimmunoassay procedure was used to assess VD serum concentration as opposed to HPLC used in our study. The stated differences make comparing the two studies difficult.

A PTH concentration analysis was included in our study because of its close metabolic connection with VD and calcium homeostasis. Somewhat surprisingly, we found that the control subjects without ED had higher PTH levels compared to ED patients, even though PTH levels were normal in general. PTH concentration correlated positively with IIEF-5 scores, meaning higher PTH related to a better erectile function. No significant correlations between PTH and age, BMI, VD and other variables were observed.

There were no chronic kidney disease (CKD) patients in our study population (median creatinine 83.5 μmol/L) and the groups did not differ in creatinine levels. Therefore, the influence of any secondary

hyperparathyroidism was eliminated, which was important bearing in mind its connection with ED in CKD patients via increased prolactin secretion[51,52]. Furthermore, to eliminate the impact of suspected primary hyperparathyroidism, a two-sided outlier test for multiple outliers was performed and two subjects with "extreme" PTH concentrations were detected (one non-ED control with PTH 471.4 pg/mL and one ED patient with PTH 132.1 pg/mL) and excluded from further analysis. Median PTH was accordingly 42.45 pg/mL (IQR 29.7-53.43) and, despite the exclusion of the outliers, the difference in PTH between the groups was even more pronounced ($P=0.031$; Figure 2) and PTH concentration still positively correlated with the IIEF-5 score.

The reason for higher PTH levels in control subjects without ED could be explained by the possible influence of other factors, like calcium intake. In one research[53] on the influence of calcium food intake on PTH and VD levels in over 2000 healthy individuals, the authors concluded that an increase in calcium intake can diminish the increase in PTH levels, which accompanies VDD, and could enable somewhat lower 25(OH)D concentrations for the maintenance of "ideal" serum PTH. Since we measured extremely low VD levels overall, higher PTH levels in non-ED subjects could be the result of a hypothetical increased calcium intake in the ED group, which relates to a lower PTH than in non-ED subjects. A very low VD concentration cannot therefore adequately suppress PTH without the help of food calcium intake which, unfortunately, has not been included in the current analysis.

A second possible explanation of higher, although generally normal, PTH levels in non-ED subjects is presumably preserved negative feedback between VD and PTH. Low VD concentrations, as measured in our research, result in a compensatory increase in PTH, which physiologically attempts to keep the calcium homeostasis intact by stimulating 1-alpha hydroxylation of calcidiol in kidneys and converting it into an active form of VD. Higher PTH in non-ED subjects could be due to the still intact negative VD-PTH feedback, while in ED men normal feedback might be impaired.

The average age of our study subjects was 55.5 years, with no difference between groups. Age is an independent risk factor for ED, which is not surprising, as the incidence of all CVD increases with age and with END as a common pathological mechanism. The Massachusetts Male Aging study epidemiologically confirmed this, with age being the most influential factor on ED occurrence[7]. Our results also show a negative correlation of age and IIEF-5 test results. One surprising finding in our study was a positive correlation of age and serum VD concentration, which was contrary to previously published data of older individuals presenting a poorer VD status. Skin atrophy and the resulting decreased availability of the epidermal vitamin D_3 precursor, 7-dehydrocholesterol, is the main reason of high VDD prevalence in older populations[16,17,54]. When interpreting our opposing results, one has to have in mind the specifics of the studied subjects, who were mostly from rural Slavonija and Baranja populations, where people of middle and older age spend most of their time outside, working in gardens, backyards and fields, allowing for more exposure to UVB radiation. Younger individuals, on the other hand, spend most of their time inside, especially during the last twenty years of the digital age. The mentioned observations should be, however, epidemiologically verified.

In conclusion, in a study pool of 60 men, where the overall VD level was very low and none of the studied men had sufficient VD levels, we found no association between VD status and erectile function, except in a subgroup of patients who had both DM and ED and who had significantly lower VD levels compared to all the other study subjects. The results illustrate how in a relatively homogeneous population of diabetic men there may be a direct link between VDD and ED, which should be tested through interventional studies on the impact of VD supplementation on a potential improvement of ED symptoms and compare the results with the current gold standard of ED therapy, i.e. PDE-5 inhibitors. Another finding in the study was higher PTH levels in non-ED than ED men, both with normal function of the parathyroid gland. Thus, PTH could have a protective role in preserving erectile function, but the causality and mechanism for that still remain unknown.

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Sažetak

EREKTILNA DISFUNKCIJA I KONCENTRACIJA VITAMINA D U KRVI POVEZANE ISKLJUČIVO KOD DIJABETIČARA

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U posljednje vrijeme prevalencija erektilne disfunkcije (ED) raste te se brojni rizični čimbenici dovode u vezu s ovim oboljenjem. Mjerili smo koncentraciju vitamina D (VD) u serumu 32 oboljela od ED-a i 28 kontrolnih ispitanika, uz pretpostavku nižeg VD-a u oboljelih od ED-a, što proizlazi iz prethodno dokazane povezanosti rizičnih čimbenika ED-a s nedostatkom VD-a. Isključeni su ispitanici s postoperativnim, anatomskim i neurogenim ED-om, rakom prostate i simptomima donjeg mokraćnog sustava. Upitnik *International Index of Erectile Function-5* korišten je za otkrivanje i razvrstavanje bolesnika s ED-om, dok je VD uzorkovan tijekom zimskih mjeseci kako bi se izbjegle sezonske promjene u kutanoj proizvodnji VD-a. Medijan VD-a iznosio je 8,36 ng/ml, dva ispitanika bila su nedostatnog, a svi ostali deficijentnog VD-a. Ispitanici i kontrole nisu se razlikovali u koncentraciji VD-a, ali su dijabetičari s ED-om imali niži VD u odnosu na ostale ispitanike. Zdravi su ispitanici imali veće koncentracije paratireoidnog hormona (PTH) u odnosu na ispitanike s ED-om, premda unutar referentnih vrijednosti, te je PTH bio nezavisni rizični čimbenik ED-a, snižavajući svakim svojim jediničnim povećanjem rizik ED-a za 4 %. Ukupno gledajući nije pronađena povezanost VD-a s ED-om, osim u dijabetičara s ED-om. PTH bi mogao imati zaštitni učinak na erektilnu funkciju.

Ključne riječi: *vitamin D; paratireoidni hormon; IIEF-5 upitnik; erektilna disfunkcija; nedostatak vitamina D*