

THE CORRELATION OF METABOLIC AND RENAL BIOMARKERS WITH VITAMIN D STATUS IN POSTMENOPAUSAL WOMEN

KORELACIJA RENALNIH I METABOLIČKIH BIOMARKERA SA NIVOOM VITAMINA D KOD ŽENA U POSTMENOPAUZI

Aleksandra Klisic¹, Irena Radoman Vujačić², Jelena Kostadinovic³, Ana Ninic⁴

¹Primary Health Care Center, University of Montenegro-Faculty of Medicine, Podgorica, Montenegro

²Clinical Center of Montenegro, Department of Internal medicine, University of Montenegro – Faculty of Medicine, Podgorica, Montenegro

³Clinical Hospital Center Bezanijska kosa, Belgrade, Serbia

⁴Department for Medical Biochemistry, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Summary

Background: To our knowledge, the mutual involvement of a variety of metabolic and renal biomarkers and vitamin D (determined as 25-hydroxyvitamin D [25(OH)D]) in postmenopausal women has not been examined yet. Therefore, we aimed to explore such a relationship by a thorough statistical multimarker approach.

Methods: A total of 150 (diabetes and cardiovascular disease-free) postmenopausal women were included. Anthropometric and biochemical parameters were measured. The fatty liver index (FLI) and Homeostasis model assessment of insulin resistance (HOMA-IR) were calculated. Univariate and multivariate binary logistic regression analyses were used to test the predictions of cardiometabolic markers for [25(OH)D] status. Principal component analysis (PCA) was applied to explore the effect of examined biomarkers on [25(OH)D] status.

Results: Univariate binary regression analysis revealed positive associations between FLI and [25(OH)D] [OR=1.036 (1.020–1.052), P<0.001] and HOMA-IR with [25(OH)D] [OR=2.385 (1.601–3.551), P<0.001]. Multivariate logistic regression analysis with backward selection enabled us to find the best model consisted of two markers, i.e. FLI [OR=1.032 (1.016–1.048), P<0.001] and glucose [OR=2.167 (1.001–4.693), P=0.050] associated with the first tertile of [25(OH)D].

Kratak sadržaj

Uvod: Prema našem saznanju zajednički uticaj različitih metaboličkih i renalnih biomarkera na nivo vitamin D (određenog kao 25-hidroksivitamin D [25(OH)D]) kod žena u postmenopauzi još uvijek nije ispitan. Stoga je cilj istraživanja bio da se ispita povezanost ovih biomarkera primenom temeljne statističke analize koja uključuje više navedenih biomarkera.

Metode: Ukupno 150 žena u postmenopauzi (koje nemaju dijabetes, ni kardiovaskularna oboljenja) je uključeno u istraživanje. Mereni su antropometrijski i biohemijski parametri. Indeks masne jetre (FLI) i indeks insulinske rezistencije (HOMA-IR) su izračunati. Univarijantna i multivarijantna binarna logistička regresija su primenjene da bi se odredili kardiometabolički prediktori nivoa [25(OH)D].

Rezultati: Univarijantna binarna regresiona analiza je pokazala pozitivnu korelaciju između FLI i [25(OH)D] [OR=1.036 (1.020–1.052), P<0,001] i HOMA-IR sa [25(OH)D] [OR=2.385 (1.601–3.551), P<0,001]. Multivarijantna logistička regresiona analiza je pokazala najbolji model sa 2 markera, tj. FLI [OR=1.032 (1.016–1.048), P<0,001] i glukoza [OR=2.167 (1.001–4.693), P=0,050] koji su korelirali sa prvom tercilom vrednosti [25(OH)D]. PCA je izdvojila 2 značajna faktora: Renal function-related faktor i Metabolic syndrome-related faktor. Renal function-related faktor [tj. kreatinin, mokraćna

Address for correspondence:

Aleksandra Klisic, MD PhD
Center for Laboratory Diagnostics, Primary Health Care Center,
University of Montenegro-Faculty of Medicine, Podgorica,
Montenegro
Trg Nikole Kovacevica 6, 81000 Podgorica, Montenegro
Phone and Fax: +382 20 481 999
e-mail: aleksandrklisic@gmail.com

PCA extracted 2 significant factors: Renal function-related factor and Metabolic syndrome-related factor. Renal function-related factor [i.e. creatinine, uric acid, retinol-binding protein 4 (RBP4) and cystatin C] explained 29% of the variance. Also, Metabolic syndrome-related factor (i.e. HOMA-IR, FLI, hsCRP and fibrinogen) explained 29% of the variance of the tested parameters. Moreover, binary logistic regression analysis revealed significant positive predictive ability of both factors, i.e. Renal function-related factor and Metabolic syndrome-related factor ($P=0.044$ and $P<0.001$, respectively) towards [25(OH)D] status.

Conclusion: The joint involvement of renal function biomarkers and metabolic syndrome-related features (i.e. insulin resistance, inflammation and non-alcoholic fatty liver disease) can differentiate postmenopausal women with lower [25(OH)D] levels from other women.

Keywords: postmenopausal, vitamin D deficiency, cardiometabolic risk, inflammation, obesity

Introduction

It is established that vitamin D (obtained from a diet or through the exposure of the skin to the sun) is metabolized by two important steps of hydroxylation, i.e. in the liver (25-hydroxyvitamin D [25(OH)D]) and in the kidney (1,25-dihydroxyvitamin D [1,25(OH)2D]) (1). The major form of vitamin D in circulation is 25-hydroxyvitamin D (25[OH]D) (1).

In recent decades, great attention has been paid to the examination of the vitamin D level in a variety of inflammatory disorders in the human population, such as obesity, polycystic ovary syndrome, type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD), etc. (2–6).

However, despite the great number of investigations related to this topic, the findings are still inconclusive. While some cross-sectional studies showed an inverse correlation between glucose homeostasis indices and other metabolic syndrome components, as well as inflammation parameters (2, 7) with vitamin D, the others did not (8–10). Similarly, discordant results were shown regarding the effect of vitamin D supplementation on cardiometabolic risk factors (3, 11–15).

Compared to women of reproductive age, women who experience postmenopause are faced with hormonal changes due to estrogen deficiency (16, 17). Moreover, metabolic disturbances such as insulin resistance due to re-distributed adipose tissue towards visceral compartments with concomitant central obesity, dyslipidemia and hypertension (all of which are well-known components of metabolic syndrome) are present in postmenopausal period. Liver steatosis as a hepatic manifestation of metabolic syndrome is highly prevalent, as well (16). Recently, the association between NAFLD and chronic kidney disease (CKD) with vitamin D level has also been confirmed due to their common metabolic pathways (18, 19).

kiselina, retinol-vezujući protein 4 (RBP4) i cistatin C] su objasnili 29% varijanse. Takođe, Metabolic syndrome-related faktor (tj. HOMA-IR, FLI, hsCRP i fibrinogen) su objasnili 29% varijanse ispitivanih parametara. Staviše, binarna logistička regresiona analiza je pokazala pozitivnu prediktivnu sposobnost oba faktora, tj. Renal function-related faktora i Metabolic syndrome-related faktora ($P=0,044$ i $P<0,001$) za nivo [25(OH)D].

Zaključak: Zajedničko dejstvo renalnih biomarkera i markera metaboličkog sindroma (tj. insulinska rezistencija, inflamacija i nealkoholna steatoza jetre) mogu diferencirati žene u postmenopauzi sa nižim nivoom [25(OH)D] u odnosu na ostale žene.

Ključne reči: žene u postmenopauzi, deficit vitamina D, kardiometaoblički rizik, inflamacija, gojaznost

As far as we know, the joint involvement of metabolic and renal biomarkers and vitamin D (determined as [25(OH)D]) in postmenopausal women has not been investigated yet. Since that vitamin D becomes biotransformed in the liver and the kidney, it would be expected that diseases that affect the liver/kidney might influence the level of vitamin D in circulation. If such is the case, vitamin D supplementation might play a beneficial role in the prevention and/or delay of such cardiometabolic disorders in postmenopausal women.

Therefore, we aimed to examine a relationship of metabolic and renal biomarkers with [25(OH)D]) in postmenopausal women by a thorough statistical multimarker approach called Principal Component Analysis (PCA), as reported in our previous studies related to cardiometabolic disorders (20–22).

Materials and Methods

Study population

After obtaining the approval of the Institutional Ethics Committee and written informed consent of the participants, and following the principles of the Declaration of Helsinki, the research included consecutively a cohort of 150 postmenopausal women free of known cardiometabolic diseases.

Each woman who voluntarily accepted participation in the study was given a questionnaire that included demographic data, history of diseases, medications use, smoking and alcohol consumption history. On the same day clinical examinations (i.e. anthropometric and blood pressure measurements) were provided, as described previously (16).

Women were included in the study if reported to be postmenopausal (i.e., if reported the absence of menstruation for more than 1 year), with no using any

medications or hormone replacement therapies, with no history of cardiometabolic, malignant and autoimmune diseases.

Exclusion criteria were as follows: smoking, ethanol consumption >20 g/day, high sensitivity C-reactive protein (hsCRP) ≥ 10 mg/L, diabetes mellitus, liver disease other than steatosis, hyperthyroidism, hypothyroidism, cardiovascular and kidney diseases and medications used in the last 6 months. Also, women that reported irregular menstrual bleeding for less than 1 year or premenopausal women were excluded from the research.

Non-alcoholic fatty liver disease is assessed by fatty liver index (FLI), an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and gamma-glutamyl transferase (GGT), as the best validated score of hepatic steatosis in the general population (23).

Biochemical analyses

The blood samples were provided in the morning after at least 8 hours of an overnight fast. After blood collection the samples were left to clot for 30 minutes and centrifuged at 3000 rpm for 10 minutes. Fasting glucose was measured immediately whereas the other obtained sera samples were aliquoted and kept at -80°C , until analyses of total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), TG, aspartate aminotransferase (AST), alanine aminotransferase (ALT), GGT, uric acid and creatinine. All these parameters were determined using standardized enzymatic procedures, spectrophotometrically on Roche Cobas 400, Mannheim, Germany.

Insulin levels were determined by chemiluminescent immunometric assay (Immulite 2000, Siemens, Muenchen, Germany). HOMA-IR was calculated: $\text{HOMA-IR} = \text{Fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{IU/L}) / 22.5$ (16).

Retinol-binding protein 4 (RBP4), hsCRP and cystatin C levels were measured with a nephelometric assay (Behring Nephelometer Analyzer, Marburg, Germany).

The levels of serum [25(OH)D] were determined by electrochemiluminescence (Cobas 6000/e601, Roche Diagnostics, Mannheim, Germany). Although, the whole population of patients had low vitamin D according to references values (less than 75 nmol/L) we have arbitrary decided to present the first tertile of the vitamin D values (less than 37.19 nmol/L) as risky and compared it with the other two tertiles.

Statistical analysis

Statistical software SPSS version 21.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Two-tailed P values less than 0.05 were considered significant.

Variables with Gaussian distribution were shown as mean \pm standard deviation and compared by the Student *t*-test. Variables that did not follow Gaussian distribution were shown as median (interquartile range) and compared by the Mann-Whitney *U*-test. The correlation of [25(OH)D] and other clinical data were tested by the Spearman's correlation analysis and the results were given as a correlation coefficient (ρ). The associations of [25(OH)D] with FLI and HOMA-IR/glucose were assessed with univariate and multivariate binary logistic regression analysis with backward selection criterion. Data from these analyses were given as odds ratio (OR) and 95% Confidence Interval (CI). The explained variation in [25(OH)D] levels was given by Nagelkerke R^2 value. Further data analysis involved Principal Component Analysis (PCA) with varimax rotation to reduce the number of examined variables to a smaller number of factors. Eigenvalues greater than 1.0 was used as a criterion to determine which factor should be extracted. Markers with factor loadings greater and equal to 0.5 were used for factor interpretation. PCA allowed us to calculate scores for factors. Scores were included as the independent variables in the following binary logistic regression analysis to determine potential significant predictors for lower [25(OH)D] levels.

Results

Table I shows the clinical characteristics of postmenopausal women. BMI, WC, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, glucose, insulin, HOMA-IR, uric acid, cystatin C, hsCRP, fibrinogen and FLI were higher in women in the 1. tertile of [25(OH)D] levels than women in the 2. and 3. tertile of [25(OH)D] levels. On the contrary, only HDL-c levels were lower in women in the 1. tertile of [25(OH)D] levels.

Further, we wanted to determine the correlation between [25(OH)D] levels and examined markers in all women (Table II). Spearman's correlation analysis demonstrated a significant negative correlation between [25(OH)D] and BMI, WC, SBP, DBP, TG, glucose, insulin, HOMA-IR, uric acid, cystatin C, hsCRP, fibrinogen and FLI. [25(OH)D] correlated positively with HDL-c.

Our further intention was to search for in-depth associations of [25(OH)D] with FLI and HOMA-IR. Therefore, we conducted a binary logistic regression analysis (Table III). Both variables (i.e., FLI and HOMA-IR) were significantly positively associated with [25(OH)D] levels.

Table I General characteristics of tested population.

Variable	1. tertile of [25(OH)D] levels < 37.19 nmol/L	2. and 3. tertile of [25(OH)D] levels ≥ 37.19 nmol/L	P
Postmenopausal women, N	50	100	
Age, years	56±5	57±5	0.593
BMI, kg/m ²	28.6±4.5	25.4±3.8	<0.001
WC, cm*	98 (88–103)	86 (78–94)	<0.001
SBP, mmHg*	139 (120–155)	126 (107–145)	0.005
DBP, mmHg*	90 (84–95)	82 (70–94)	0.002
TC, mmol/L	6.4±1.1	6.5±1.0	0.488
HDL-c, mmol/L	1.6±0.4	1.8±0.4	0.009
LDL-c, mmol/L	4.3±1.0	4.3±1.0	0.804
TG, mmol/L*	1.4 (1.1–2.3)	1.2 (0.9–1.7)	0.039
Glucose, mmol/L*	5.4 (5.1–6.0)	5.2 (5.0–5.5)	0.005
Insulin, μIU/L*	8.4 (6.2–12.0)	5.5 (4.2–7.6)	<0.001
HOMA-IR*	2.0 (1.5–3.1)	1.2 (1.0–1.8)	<0.001
GGT, IU/L*	13 (9–19)	11 (9–14)	0.055
Total bilirubin, μmol/L*	7.1 (6.0–8.5)	7.8 (6.4–10.4)	0.160
Uric acid, μmol/L	286±62	250±63	0.001
Creatinine, μmol/L	56±6	55±7	0.431
Cystatin C, μmol/L	0.80±0.10	0.75±0.10	0.002
RBP4, g/L	42.08±8.67	40.46±9.24	0.303
HsCRP, mg/L*	1.3 (0.8–2.4)	0.7 (0.4–1.7)	0.002
Fibrinogen, g/L*	3.9 (3.5–4.4)	3.6 (3.3–4.1)	0.045
FLI*	41 (23–71)	21 (8–37)	<0.001
[25(OH)D], nmol/L	29 (23–34)	48 (41–58)	<0.001

Normally distributed variables are presented as mean ± standard deviation and compared by the Student *t*-test.

* Skewed distributed variables are presented as median and interquartile range and compared by the Mann-Whitney *U*-test. Categorical variables are presented as absolute frequencies.

Univariate binary regression analysis revealed positive associations of FLI and HOMA-IR with [25(OH)D] demonstrated by following OR 1.036 and 2.385, respectively. Furthermore, the multivariate logistic regression analysis with backward selection enabled us to find the best model consisting of two markers, i.e., FLI ($P < 0.001$) and glucose ($P = 0.05$) associated with the first tertile of [25(OH)D]. Adjusted R^2 for the best model was 0.242, which means that 24.2% of variation in lower [25(OH)D] values could be explained by this model. On the contrary, HOMA-IR was found to be the single marker for lower [25(OH)D], because backward selection did not segregate any best model for lower [25(OH)D].

PCA was applied to biochemical data to find their relationship with [25(OH)D] status. Sample

adequacy was confirmed with the Keiser-Meier-Olkin measure (KMO index = 0.783). Bartlett's test of sphericity was significant ($P < 0.001$). After initial analysis total bilirubin was excluded because it was the only marker of factor 3. The factors were given in *Table IV*. This PCA extracted 2 significant factors with a total percent of the explainable variation of 58% of the investigated parameters. The Renal function-related factor explained 29% of the variance and it was associated with positive loading of creatinine, RBP4, cystatin C and uric acid. The Metabolic syndrome-related factor explained 29% of the variance and it was associated with positive loading of hsCRP, HOMA-IR, FLI and fibrinogen. By using scores derived from PCA, a univariate binary logistic regression analysis was conducted. We demonstrated

Table II Spearman’s correlation coefficients of [25(OH)D] and clinical data.

Variable	ρ	P
Age, years	0.020	0.811
BMI, kg/m ²	-0.377	<0.001
WC, cm	-0.379	<0.001
SBP, mmHg	-0.257	0.002
DBP, mmHg	-0.236	0.004
TC, mmol/L	0.016	0.850
HDL-c, mmol/L	0.189	0.021
LDL-c, mmol/L	-0.062	0.454
TG, mmol/L	-0.197	0.016
Glucose, mmol/L	-0.252	0.002
Insulin, μIU/L	-0.389	<0.001
HOMA-IR	-0.423	<0.001
GGT, IU/L	-0.125	0.126
Total bilirubin, μmol/L	0.062	0.448
Uric acid, μmol/L	-0.285	<0.001
Creatinine, μmol/L	-0.043	0.600
Cystatin C, μmol/L	-0.224	0.006
RBP4, g/L	-0.120	0.144
HsCRP, mg/L	-0.272	0.001
Fibrinogen, g/L	-0.184	0.024
FLI	-0.383	<0.001

Data are given as coefficients of correlation Rho (ρ)

Table III Odds ratios (OR) after univariate and multivariate binary logistic regression analysis with backward selection in the best-fit model for FLI and HOMA-IR/glucose predicting abilities towards the first tertile of [25(OH)D] values, i.e. lower [25(OH)D] levels.

Predictors	β β (SE)	Wald	Unadjusted OR (95%CI)	P	Nagelkerke R ²
FLI	0.075 (0.008)	21.099	1.036 (1.020–1.052)	<0.001	0.211
HOMA-IR	0.869 (0.203)	18.305	2.385 (1.601–3.551)	<0.001	0.200
Model 1	β β (SE)	Wald	Adjusted OR (95%CI)	P	Nagelkerke R ²
FLI	0.032 (0.008)	15.968	1.032 (1.016–1.048)	<0.001	0.242
Glucose	0.773 (0.394)	3.849	2.167 (1.001–4.693)	0.050	

Table IV Factors extracted by principal component analysis with percent of variability and variables’ loadings in relationship with [25(OH)D] levels.

Factors	Variables (loadings)	Factor variability
Renal function-related factor	Creatinine (0.769) RBP4 (0.706) Cystatin C (0.695) Uric acid (0.660)	29%
Metabolic syndrome-related factor	HsCRP (0.799) HOMA-IR (0.746) FLI (0.700) Fibrinogen (0.515)	29%

the positive predictive ability of both factors for the first tertile of [25(OH)D] levels, i.e. lower [25(OH)D] levels. The Renal function-related factor was positively associated with the first tertile of [25(OH)D] levels [OR=1.455 (1.009–2.056), P=0.044]. Increased Renal function-related marker values were associated with a 1.455 greater probability for lower [25(OH)D] levels. This first factor explained variation in [25(OH)D] levels by 3.9% which was demonstrated by Nagelkerke R² of 0.039. As well, the Metabolic syndrome-related factor was positively associated with the first tertile of [25(OH)D] levels [OR=2.273 (1.536–3.362), P<0.001]. Increased Metabolic syndrome-related factor values were associated with a 2.273 greater probability for lower [25(OH)D] levels. The second factor explained variation in [25(OH)D] levels by 17.4% which was demonstrated by Nagelkerke R²=0.174.

Discussion

This study is the first that investigated the involvement of a variety of cardiometabolic biomarkers in relation to [25(OH)D] status among diabetes-free and cardiovascular-free postmenopausal women. Moreover, this study is the first to apply a thorough statistical approach, such as PCA to gain deeper insight into the relationship between [25(OH)D] status and metabolic and kidney biomarkers.

We have shown the relationship between lower serum [25(OH)D] and HOMA-IR and FLI (as a proxy of NAFLD), respectively. We have also reported that increased Renal function-related marker values and Metabolic syndrome-related factor values were associated with a 1.455 and 2.273 greater probability of lower [25(OH)D] levels, respectively.

Previous reports have shown conflicting findings regarding the association between low serum vitamin D levels and NAFLD (5, 25, 26). The possible dis-

crepancies between studies might in part be attributed to differences in diagnostic procedures of NAFLD, differences in sample size, variety of associated comorbidities, as well as different ages and ethnicities of examined populations.

The associations between vitamin D deficiency and NAFLD, as well as with CKD might in part be explained by the process of hydroxylation of vitamin D that occurs both in the liver and the kidney (24). Those findings suggest that vitamin D deficiency may be one of the links between NAFLD and CKD since these two diseases are interrelated and share common metabolic pathways (18, 19).

Zhang et al. (5) have shown that vitamin D supplementation led to the improvement of insulin resistance and liver steatosis through the process of vitamin D receptor upregulation and hepatocyte nuclear factor 4 overexpression.

We have recently shown the anti-inflammatory and antioxidative properties of vitamin D (27), as well as a decrease in the level of glycated hemoglobin after 3 and after 6 months of vitamin D supplementation in patients with type 2 diabetes mellitus, although the HOMA-IR did not significantly change during the follow-up period (4).

The properties of vitamin D in insulin-sensitivity modulation were recently confirmed by the inhibition of the expression of peroxisome proliferator-activated receptor γ (PPAR- γ) and reducing peripheral insulin resistance (28). Vitamin D activates its receptors in pancreatic islet β -cells and favors insulin secretion which further confirms the beneficial properties of vitamin D (29). It was also found that vitamin D favors glucose transporter 4 (GLUT-4) expression in adipose tissue cells and upregulates insulin receptor substrate-1 (IRS-1), thus enabling the uptake of glucose in the muscle cells (30). Moreover, vitamin D diminishes inflammation by the reduction of lipid droplets output from the adipose tissue, thus preventing liver steatosis since *de novo* lipogenesis and fatty acid oxidation in the liver are attenuated (30).

We have also found the association between [25(OH)D] and each metabolic syndrome component. Our results of higher BMI and WC in the first tertile of [25(OH)D] (i.e. lower vitamin D levels) are in accordance with the study of Metheniti et al. (2) who showed low [25(OH)D] levels in extremely obese young females. However, unlike our study, they also found an inverse correlation between [25(OH)D] levels and an adipokine RBP4.

Similarly, discrepant results were shown regarding the association between inflammation markers and serum [25(OH)D] levels (7–9). We have shown a negative correlation between hsCRP and fibrinogen, respectively with vitamin D. Some studies also reported an inverse correlation between hsCRP and serum [25(OH)D] levels (7), whereas the others failed to find the mentioned relationship (8, 9).

The anti-inflammatory potential of vitamin D has been confirmed by investigations on cell cultures (31). The ability of macrophages to secrete pro-inflammatory cytokines (CRP, Tumor necrosis factor- α , Interleukin-6), as well as the promotion of monocyte transformation to macrophages, are diminished by vitamin D (31). Also, vitamin D lowers the level of inflammation in the adipose tissue by attenuating the activation of nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways and disabling the transcription of pro-inflammatory genes (32).

We have also reported the inverse association between [25(OH)D] and cystatin C and uric acid, respectively which is in line with one recent study (33), and opposite to the other (34).

Previous investigations have shown the association between vitamin D deficiency and kidney disease progression (35, 36) which may be in part attributed to the renin-angiotensin-aldosterone system (RAAS) by vitamin D (37). Concomitantly, the downregulation of RAAS could lower blood pressure (37). One animal study showed that [1,25(OH)2D] favors suppression of the biosynthesis of renin, whereas vitamin D deficiency stimulates its synthesis (38). Also, the protective renal effects of vitamin D were shown by lowering levels of transforming growth factor- β and SMAD3 proteins, which are related to oxidative stress and fibrosis in kidneys (39). Derakhshanian et al. reported (40) that besides a reduction in glucose levels and an increase in insulin secretion, higher serum vitamin D levels can prevent diabetic nephropathy by diminishing fructose-6-phosphate aminotransferase (GFAT) synthesis which is the key enzyme of the hexosamine pathway in the kidney tissue.

Jorde et al. (9) did not report a correlation of serum [25(OH)D] levels neither with BMI, nor with hsCRP in nonsmoking females with BMI ≥ 28 kg/m², presuming that vitamin D has more enhanced properties in the presence of cardiometabolic diseases, when the immune response is stimulated. However, we included in our study normal weight and overweight/obese postmenopausal women free of known cardiometabolic diseases and we reported significant correlation between cardiometabolic biomarkers and serum [25(OH)D] levels, as well as the relationship between HOMA-IR, glucose and FLI, respectively, and serum [25(OH)D] levels. Additionally, the joint involvement of renal function biomarkers and metabolic syndrome-related features in relation to serum [25(OH)D] level in postmenopausal women was confirmed.

It is important to mention several limitations of this study. Namely, its cross-sectional design does not allow us to confirm the causal link between serum [25(OH)D] levels and examined cardiometabolic biomarkers. We were limited for the measurement of

the level of sex hormones in postmenopausal women. Moreover, we included in this research only Montenegrin postmenopausal women, thus the obtained results cannot be generalized to other populations and ethnicities. On the other hand, we included a narrow age range of women who were non-smokers, who were not taking any medications or vitamin D supplements in the last several months, and who were diabetes-free and cardiovascular disease-free. Hence, we aimed to limit the potential confounding factors such as hormonal variability, smoking, medications, co-morbidities, and the influence of vitamin D supplements on the serum [25(OH)D] levels (3, 9, 11, 41). Additionally, we included a variety of biomarkers that may reflect cardiometabolic disturbances in the postmenopausal period of women, as some of the previous studies reported (16, 17, 42), and applied a deeper statistical approach (20–22) in relation to serum [25(OH)D] status in the examined cohort.

Conclusion

Ours is the first study that examined the mutual involvement of renal function biomarkers and metabolic syndrome-related features (i.e. inflammation,

insulin resistance and non-alcoholic fatty liver disease) in relation to serum [25(OH)D] level in postmenopausal women. The PCA analysis revealed that these two factors (i.e. kidney and metabolic) can differentiate women with higher [25(OH)D] levels from those women with lower [25(OH)D] levels. Future researches are necessary to explore the causal link between chronic kidney disease, non-alcoholic fatty liver disease and [25(OH)D] deficiency in postmenopausal women.

Funding

This research was partially funded by a grant from the Ministry of Science, Montenegro and the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-68/2022-14/200161.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

References

- Windrichova J, Broz P, Fuchsova R, Topolcan O, Pecan L, Mayer O, Kucera R. Comparison of four routinely used vitamin D automated immunoassays. *J Med Biochem* 2021; 40 (3): 277–85.
- Metheniti D, Sakka S, Dracopoulou M, Margeli A, Papassotiriou I, Kanaka-Gantenbein C, Chrousos GP, Pervanidou P. Decreased circulating 25-(OH) Vitamin D concentrations in obese female children and adolescents: positive associations with Retinol Binding Protein-4 and Neutrophil Gelatinase-associated Lipocalin. *Hormones (Athens)* 2013; 12(3): 397–404.
- Akbari M, Ostadmohammadi V, Lankarani KB, Tabrizi R, Kolahdooz F, Heydari ST, Kavari SH, Mirhosseini N, Mafi A, Dastorani M, Asemi Z. The Effects of Vitamin D Supplementation on Biomarkers of Inflammation and Oxidative Stress Among Women with Polycystic Ovary Syndrome: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Horm Metab Res* 2018; 50(4): 271–9.
- Cojic M, Kocic R, Klisic A, Kocic G. The effects of vitamin D supplementation on metabolic and oxidative stress markers in patients with type 2 diabetes: A 6-Month follow up randomized controlled study. *Frontiers Endocrinol* 2021; 12: 610893. doi: 10.3389/fendo.2021.610893.
- Zhang H, Shen Z, Lin YM, Zhang J, Zhang YW, et al. Vitamin D receptor targets hepatocyte nuclear factor 4 and mediates protective effects of vitamin D in nonalcoholic fatty liver disease. *J Biol Chem* 2020; 295(12): 3891–905.
- Roksandic Milenkovic M, Klisic A, Ceriman V, Kotur Stevuljevic J, Savic Vujovic K, Mirkov D, Gajic M, Ilic B, Dimic N, Samardzic N, Jovanovic D. Oxidative stress and inflammation parameters-novel biomarkers for idiopathic pulmonary fibrosis. *Eur Rev Med Pharmacol Sci* 2022; 26(3): 927–34.
- Kuloğlu O, Gür M, Şeker T, Kalkan GY, Kırım S, Şahin DY, Harbaloğlu H, Türkoglu C, Acele A, Elbasan Z, Özaltun B, Çaylı M. Serum 25-hydroxyvitamin D level is associated with aortic distensibility and left ventricle hypertrophy in newly diagnosed type 2 diabetes mellitus. *Diab Vasc Dis Res* 2013; 10(6): 546–9.
- Haidari F, Zakerkish M, Karandish M, Saki A, Pooraziz S. Association between Serum Vitamin D Level and Glycemic and Inflammatory Markers in Non-obese Patients with Type 2 Diabetes. *Iran J Med Sci* 2016; 41(5): 367–73.
- Jorde R, Sneve M, Torjesen PA, Figenschau Y, Gøransson LG, Omdal R. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. *Cytokine* 2010; 50(2): 175–80.
- Kostecka D, Schneider-Matyka D, Barczak K, Starczewska M, Szkup M, Ustianowski P, Brodowski J, Grochans E. The effect of vitamin D levels on lipid, glucose profiles and depression in perimenopausal women. *Eur Rev Med Pharmacol Sci* 2022; 26(10): 3493–505.
- Ağar M, Güngör K, Güngör ND, Kavrut M, Madenli AA. Vitamin D supplementation inhibits NF-κB signaling pathway in lean and obese women with PCOS. *Eur Rev Med Pharmacol Sci* 2022; 26 (11): 3973–7.

12. Yu Y, Tian L, Xiao Y, Huang G, Zhang M. Effect of Vitamin D Supplementation on Some Inflammatory Biomarkers in Type 2 Diabetes Mellitus Subjects: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Ann Nutr Metab* 2018; 73(1): 62–73.
13. Jamka M, Woźniewicz M, Walkowiak J, Bogdański P, Jeszka J, Stelmach-Mardas M. The effect of vitamin D supplementation on selected inflammatory biomarkers in obese and overweight subjects: a systematic review with meta-analysis. *Eur J Nutr* 2016; 55(6): 2163–76.
14. Dinca M, Serban MC, Sahebkar A, Mikhailidis DP, Toth PP, Martin SS, Blaha MJ, Blüher M, Gurban C, Penson P, Michos ED, Hernandez AV, Jones SR, Banach M; for Lipid Blood Pressure Meta-analysis Collaboration LBPAC Group. Does vitamin D supplementation alter plasma adipokines concentrations? A systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res* 2016; 107: 360–71.
15. Zuk A, Fitzpatrick T, Rosella LC. Effect of vitamin D3 supplementation on inflammatory markers and glycemic measures among overweight or obese adults: a systematic review of randomized controlled trials. *PLoS One* 2016; 11: e0154215. doi: 10.1371/journal.pone.0154215
16. Klisic A, Kavacic N, Jovanovic M, Soldatovic I, Gligorovic-Barhanovic N, Kotur-Stevuljevic J. Bioavailable testosterone is independently associated with fatty liver index in postmenopausal women. *Arch Med Sci* 2017; 5(13): 1188–96.
17. Klisic A, Kotur-Stevuljevic J, Kavacic N, Martinovic M, Matic M. The association between follicle stimulating hormone and glutathione peroxidase activity is dependent on abdominal obesity in postmenopausal women. *Eat Weight Disord-St* 2018; 23(1): 133–41.
18. Byrne CD, Targher G. Nafld as a driver of chronic kidney disease. *J Hepatol* 2020; 72: 785–801.
19. Mantovani A, Petracca G, Beatrice G, Csermely A, Lonardo A, Schattenberg JM, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident chronic kidney disease: an updated meta-analysis. *Gut* 2022; 71(1): 156–62.
20. Klisic A, Kavacic N, Ninic A, Kotur-Stevuljevic J. Oxidative stress and cardiometabolic biomarkers in patients with non-alcoholic fatty liver disease. *Sci Rep* 2021; 11: 18455. <https://doi.org/10.1038/s41598-021-97686-6>.
21. Klisic A, Kavacic N, Kotur-Stevuljevic J, Ninic A. Serum soluble transferrin receptor levels are independently associated with homeostasis model assessment of insulin resistance in adolescent girls. *Arch Med Sci* 2021; doi: <https://doi.org/10.5114/aoms/132757>.
22. Klisic A, Kavacic N, Vujcic S, Spasojevic-Kalimanovska V, Kotur-Stevuljevic J, Ninic A. Factorial analysis of the cardiometabolic risk influence on redox status components in adult population. *Oxid Med Cell Longev* 2021; ID: 6661940. <https://doi.org/10.1155/2021/6661940>
23. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; 6: 33. doi: 10.1186/1471-230X-6-33.
24. Heda R, Yazawa M, Shi M, Bhaskaran M, Aloor FZ, Thuluvath PJ, Satapathy SK. Non-alcoholic fatty liver and chronic kidney disease: Retrospect, introspect, and prospect. *World J Gastroenterol* 2021; 27(17): 1864–82.
25. Anty R, Hastier A, Canivet CM, Patouraux S, Schneck AS, Ferrari-Panaia P, et al. Severe vitamin D deficiency is not associated with liver damage in morbidly obese patients. *Obes Surg* 2016; 26(9): 2138–43.
26. Saberi B, Dadabhai AS, Nanavati J, Wang L, Shinohara RT, Mullin GE. Vitamin D levels do not predict the stage of hepatic fibrosis in patients with nonalcoholic fatty liver disease: a PRISMA compliant systematic review and meta-analysis of pooled data. *World J Hepatol* 2018; 10(1): 142–54.
27. Cojic M, Kocic R, Klisic A, Cvejanov-Kezunovic Lj, Kavacic N, Kocic G. A novel mechanism of vitamin D anti-inflammatory/antioxidative potential in type 2 diabetic patients on metformin therapy. *Arch Med Sci* 2020; 16(5): 1004–12.
28. McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. *Med Hypotheses* 2003; 61(5–6): 535–42.
29. Khan RJ, Gebreab SY, Riestra P, Sims M, Gaye A, Xu R, Davis SK. Associations between Vitamin D and Cardiovascular Disease Risk Factors in African Americans Are Partly Explained by Circulating Adipokines and C-Reactive Protein: The Jackson Heart Study. *J Nutr* 2016; 146(12): 2537–43.
30. Barchetta I, Cimini FA, Cavallo MG. Vitamin D and Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD): An Update. *Nutrients* 2020; 12(11): 3302. doi: 10.3390/nu12113302.
31. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. Vitamin D and inflammation. *Joint Bone Spine* 2010; 77(6): 552–7.
32. Szymczak-Pajor I, Drzewoski J, Śliwińska A. The Molecular Mechanisms by Which Vitamin D Prevents Insulin Resistance and Associated Disorders. *Int J Mol Sci* 2020; 21(18): 6644. doi: 10.3390/ijms21186644
33. Zhang YY, Qiu HB, Tian JW. Association Between Vitamin D and Hyperuricemia Among Adults in the United States. *Front Nutr* 2020; 7: 592777. doi: 10.3389/fnut.2020.592777.
34. Alkhatatbeh MJ, Smadi SA, Abdul-Razzak KK, Saadeh NA. High Prevalence of Vitamin D Deficiency and Correlation with Cystatin-C and Other Cardiovascular and Renal Risk Biomarkers in Patients with Type 2 Diabetes Mellitus Complicated with Hypertension. *Curr Diabetes Rev* 2021; 17(1): 81–90.
35. Melamed ML, Astor B, Michos ED, Hostetter TH, Powe NR, Muntner P. 25-hydroxyvitamin D levels, race, and the progression of kidney disease. *J Am Soc Nephrol* 2009; 20(12): 2631–9.
36. de Boer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination

- Survey (NHANES III). *Am J Kidney Dis* 2007; 50(1): 69–77.
37. Riek AE, Oh J, Darwech I, Worthy V, Lin X, Ostlund RE Jr, Zhang RM, Bernal-Mizrachi C. Vitamin D3 supplementation decreases a unique circulating monocyte cholesterol pool in patients with type 2 diabetes. *J Steroid Biochem Mol Biol* 2018; 177: 187–92.
38. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002; 110(2): 229–38.
39. Plum LA, Zella JB. Vitamin D compounds and diabetic nephropathy. *Arch Biochem Biophys* 2012; 523: 87–94.
40. Derakhshanian H, Djazayeri A, Javanbakht MH, Eshraghian MR, Mirshafiey A, Zarei M, Alvandi E, Djalali E, Djalali M. The Effect of Vitamin D on Cellular Pathways of Diabetic Nephropathy. *Rep Biochem Mol Biol* 2019; 7(2): 217–22.
41. Yuste C, Quiroga B, de Vinuesa SG, Goicoechea MA, Barraca D, Verdalles U, Luño J. The effect of some medications given to CKD patients on vitamin D levels. *Nefrologia* 2015; 35(2): 150–6.
42. Klisic A, Kotur-Stevuljevic J, Kavaric N, Matic M. Relationship between cystatin C, retinol-binding protein 4 and Framingham risk score in healthy postmenopausal women. *Arch Iran Med* 2016; 19(12): 845–51.

Received: July 16, 2022

Accepted: January 26, 2023