Relationship between vascular endothelial function and vitamin D and parathyroid hormone levels in women with arterial hypertension

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KEY WORDS
arterial hypertension, endothelial dysfunction, parathyroid hormone, vitamin D

INTRODUCTION
There are few studies indicating a relationship between vitamin D, parathyroid hormone (PTH), and parameters of endothelial function.

OBJECTIVES
The aim of the study was to establish the relationship between vascular endothelial function and the level of vitamin D and PTH in women with arterial hypertension (AH).

PATIENTS AND METHODS
It was a cross-sectional study of 141 women with AH stage II aged 50.8 ± 6.0 years. We determined the serum levels of total 25-hydroxyvitamin D (25(OH)D), PTH, endothelin 1, as well as nitrates and nitrites. Endothelial function was measured by impedance rheography. Endothelium-dependent vasodilatation was measured by a reactive hyperemia test. Endothelium-dependent vasodilatation of less than 12% was considered as endothelial dysfunction (ED).

RESULTS
Vitamin D deficiency was observed in 53 women (37.6%); insufficiency, in 42 (30.1%); and the optimal level, in 46 (32.3%). ED was determined in 49.6% of the patients. The serum PTH level was 36.6 ± 20.1 pg/ml, and it was above the upper limit of the reference range only in 5.5% of the cases. We showed significant correlations between PTH and systolic blood pressure \( R = 0.28; P = 0.003 \), PTH and 25(OH)D \( R = -0.27; P = 0.025 \), and 25(OH)D and SBP \( R = -0.23; P = 0.034 \). In the group with ED, the serum level of endothelin 1 was higher compared with the group without ED (0.57 ± 0.18 vs. 0.49 ± 0.21 pg/ml; \( P = 0.032 \)) and that of nitrates and nitrites was lower compared with the group without ED (15.2 [11.1–29.9] vs. 22.8 [16.9–33.0] µmol/l; \( P = 0.0005 \)). In the group without ED, PTH inversely correlated with endothelin 1 \( R = -0.27; P = 0.043 \). In the group with ED, PTH inversely correlated with nitrates and nitrites \( R = -0.48; P = 0.003 \).

CONCLUSIONS
In women with AH and without ED, PTH affected the production of a vasoconstrictor, endothelin 1, while in those with ED, PTH was associated with a lower production of vasodilators, nitrates and nitrites, by the vascular endothelium.

INTRODUCTION
The vascular endothelium is important in maintaining vascular homeostasis, and endothelial dysfunction (ED) plays a key role in the pathogenesis of arterial hypertension (AH). Currently, the role of vitamin D in the development of cardiovascular diseases is being extensively studied, and a decrease in the level of vitamin D is considered to be a potential significant risk factor for AH.1-4 In addition to potential effects on the renin–angiotensin–aldosterone system and regulation of vascular smooth muscle contractility, a link between vitamin D and AH has also been hypothesized to be mediated by other direct effects on the vascular endothelium and smooth muscle. Experimental studies have shown that, in spontaneously hypertensive rats, 1,25-dihydroxyvitamin \( D_3 \) (1,25(OH)\( D_3 \)) reduced endothelium-dependent contractions of the aorta by decreasing cytosolic-free calcium concentrations in endothelial cells.5 1,25(OH)\( D_3 \) exerts
its vasculoprotective effects by decreasing endothelial adhesion molecules, by increasing the activity of endothelial nitric oxide synthase, and through its anti-inflammatory properties. In line with these findings, vitamin D deficiency is associated with ED.

It is known that parathyroid hormone (PTH) levels are inversely correlated with 25-hydroxyvitamin D (25(OH)D) concentrations, and another potential mechanism of AH is thought to be associated with the effect of PTH, including increased serum calcium levels, renin activity, endothelin levels, and myocardial hypertrophy. In a prospective study, endothelium-dependent flow-mediated dilation of the brachial artery was impaired in patients with primary hyperparathyroidism compared with control subjects. Other investigators demonstrated that successful parathyroidectomy improves this parameter of ED. Thus, there are some data indicating a relationship between vitamin D, PTH, and parameters of endothelial function but they are insufficient to draw firm conclusions. Therefore, the aim of the present study was to establish a relationship between vascular endothelial function and the serum level of vitamin D and PTH in women with AH.

PATIENTS AND METHODS

It was a cross-sectional study including 141 women with AH stage II (mean age, 50.8 ± 6.0 years). The diagnosis, stage, and risk of AH were established on the basis of the 2013 European Society of Hypertension / European Society of Cardiology Guidelines for the management of AH. Consecutive patients attending the outpatient clinics of Grodno hospitals no. 1–6 and diagnosed with AH stage II risk II or III by general medicine physicians or cardiologists were enrolled into the study if they fulfilled the inclusion criteria and signed written informed consent. The inclusion criteria were the presence of essential AH stage II, and age of up to 65 years. The exclusion criteria were as follows: the presence of diseases leading to secondary AH (for example endocrine or renal diseases), administration of glucocorticosteroids, chronic renal disease, ischemic heart disease, diabetes mellitus, chronic rhythm and conduction disturbances (atrial fibrillation, frequent extrasystoles, etc.), known malignancy, and other severe concomitant diseases that might affect the examined parameters. In all subjects, height and body weight were measured and body mass index (BMI) was calculated using the following formula: weight (kg) / height (m²). Blood samples for laboratory tests were obtained by venipuncture from the antecubital vein using minimal stasis after 12 hours of fasting. Blood was collected between November 2012 and February 2013. Complete blood count, urine analysis, glucose, urea, and creatinine levels were measured in all patients using standard laboratory methods. The results were normal in all patients. Standard 12-lead electrocardiography was performed in each participant.

Office blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP]) was measured twice in a sitting position on the upper arm using a standard cuff after a 10-minute rest, as recommended by the World Health Organization. The research protocol was reviewed and accepted by the local ethics committee.

We determined total 25(OH)D levels (25(OH)D3 and 25(OH)D2), serum PTH levels using the DRG reagent (Germany, Marburg), and endothelin 1 levels using the IBL International GmbH reagent (Germany, Hamburg) by means of immunoenzymatic assays according to the manufacturers’ instructions. According to the international standards, the serum total 25(OH)D level was considered sufficient if exceeding 30 ng/ml, insufficient if between 20 and 30 ng/ml, and deficient if lower than 20 ng/ml.

Using the spectrophotometric method and Griss reagent, we measured the total level of end-metabolites of nitric oxide—nitrates and nitrites—in blood plasma, which reflected the production of vasodilators by the vascular endothelium.

Forearm vascular endothelial function was evaluated using the «IMPECARD-M» software (Cardiology Centre, Minsk, Belarus) and a reactive hyperemia test. Using impedance rheography, we measured the blood flow rate in both forearms at rest (after 10–15 minutes in a prone position) at baseline and within 5 minutes after cuff decompression at a pressure exceeding the baseline by 50 mmHg. Reactive hyperemia was considered as endothelium-dependent vasodilation (EDV)—an index of a relative change from the baseline in the maximum rate of blood flow (Δdz/dtmax) within the 1st, 2nd, 3rd minutes after the end of occlusion. EDV was considered to be preserved if Δdz/dtmax exceeded 12%, while Δdz/dtmax at a range of −2% to 12% denoted ED stage I; that at a range of −2% to −15%, ED stage II, and that of −15% or less, ED stage III.

Statistical analysis was performed using the STATISTICA 7.0 software package. The Shapiro–Wilk test was used for data with normal distribution, expressed as mean values ± standard deviation, and for data with nonnormal distribution, expressed as median and interquartile range. The t test was used to compare between 2 groups of variables with normal distribution, and the Mann–Whitney test to compare between 2 independent groups of variables with nonnormal distribution. To assess the correlations between the variables, the nonparametric Spearman rank correlation analysis (R) was used. Diagnostic significance was assessed using the receiver operating characteristic (ROC) curve analysis with 95% confidence intervals (CIs). To visualize the results and group multiple characteristics into general classes, the cluster analysis was used. The null hypothesis was rejected at a P value of 0.05 or less for each of the tests.
The physical examination and laboratory test results of women with AH stage II are presented in Table 1. Of all women, 25 did not receive any drug therapy. Of 116 women who received antihypertensive therapy, 69 (40.5%) took angiotensin-converting enzyme inhibitors and 47 (40.5%) received a combined therapy of 2 drugs. We did not find any significant differences in the studied parameters (SBP, DBP, total 25(OH)D, PTH, endothelin 1, nitrates and nitrites, and Δdz/dtmax) between patients receiving various types of antihypertensive therapy. Vitamin D deficiency was reported in 53 women (37.6%); insufficiency, in 42 (30.1%); and the optimal vitamin D level, in 46 (32.3%). The optimal serum level of total 25(OH)D was observed more often in patients with AH stage II risk II than in those with AH stage II risk III (52.4% and 26.4%, respectively; \( P = 0.02 \)).

We observed significant negative correlations between the serum level of total 25(OH)D and weight \( \left( R = -0.24; P = 0.025 \right) \), BMI \( \left( R = -0.23; P = 0.03 \right) \), SBP \( \left( R = -0.23; P = 0.034 \right) \), and PTH \( \left( R = -0.27; P = 0.025 \right) \), and a positive correlation between PTH and SBP \( \left( R = 0.28; P = 0.003 \right) \). We did not find any significant correlations between total 25(OH)D and age \( \left( R = 0.13; P = 0.23 \right) \).

EDV was preserved in 50.4% of the cases, and the signs of ED stages I–III were observed in 49.6% of the cases. The signs of ED in women with AH risk III occurred significantly more often than in those with AH risk II \( \left( n = 114 \text{ vs. } n = 27; P < 0.0001 \right) \). In women with AH risk II, EDV was preserved in 81.5% of the cases compared with 43.0% of the cases with AH risk III. In patients with AH risk II, the disturbances in EDV were present in 18.5% of the patients with ED stage I, while in patients with AH risk III, the signs of ED stage I were observed in 22.8% of the cases, ED stage II, in 15.8%, and ED stage III, in 18.4%. We observed a significant positive correlation between smoking and serum endothelin 1 \( \left( R = 0.27; P = 0.02 \right) \). There were no significant correlations between smoking and Δdz/dtmax \( \left( R = -0.18; P = 0.07 \right) \) or between smoking and serum levels of nitrates and nitrites \( \left( R = 0.08; P = 0.48 \right) \).

EDV positively correlated with the serum level of nitrates and nitrites \( \left( R = 0.51; P = 0.00001 \right) \) and inversely with the serum level of endothelin 1 \( \left( R = -0.27; P = 0.02 \right) \), which allowed us to make a detailed assessment of vascular endothelial function. On the other hand, EDV \( \left( \Delta dz/dt \right) \) inversely correlated with SBP \( \left( R = -0.43, P = 0.000001 \right) \) and PTH \( \left( R = -0.31, P = 0.0002 \right) \). It remains unclear whether elevated blood pressure is a risk factor for ED or whether it is ED that predisposes to AH. We performed a cluster analysis as presented in Figure 1. The studied variables constituted one cluster. The most closely associated variables were EDV and the serum level of nitrates and nitrites \( \left( R = 0.51 \right) \), while the weakest association was found for SBP (Figure 1) and DBP.

Women with AH were divided into 2 groups depending on endothelial vasomotor function: without ED (group 1, \( n = 71 \)) and with ED (group 2, \( n = 70 \)). In group 1, EDV was 34% (23–53) and in group 2, 5% (±18 to 5). In group 1, there were 7 smokers and in group 2, 4. We did not show any significant correlations between smoking and the parameters of endothelial function either in group 1 or group 2. In group 2, the serum level of endothelin 1 was higher compared with that in group 1 (0.57 ±0.18 vs. 0.49 ±0.21 pg/ml;
in 69% of the cases was the serum level of nitrites and nitrates and endothelium-dependent vasodilation.

The type of correlations differed between groups 1 and 2 (Table 2). In a cluster analysis, if subjects with AH did not have signs of ED, the studied parameters in the cluster of the vascular endothelium were weakly related to each other (EDV and endothelin 1, \( R = –0.26 \); EDV and the serum level of nitrates and nitrites, \( R = 0.32 \)). However, the cluster of endothelial function was within a closer range to 25(OH)D and PTH, the farthest member of the cluster was SBP (Figure 3) or, similarly, DBP. Moreover, the contribution of the vascular endothelium into both SBP and DBP was minimal. When the cluster relationship between 25(OH)D, PTH, and the cluster of vascular endothelial function was disturbed, it contributed to ED (Figure 4). Therefore, the association of endothelial function parameters with SBP (\( R = –0.55 \) with nitrates and nitrites; \( R = –0.47 \) with \( \Delta dz/dt_{\text{max}} \)) and DBP (\( R = –0.45 \) with nitrates and nitrites; \( R = –0.31 \) with \( \Delta dz/dt_{\text{max}} \)) became more pronounced.

### Table 2: Significant correlations in women with arterial hypertension with and without endothelial dysfunction

<table>
<thead>
<tr>
<th>Parameters</th>
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<th></th>
<th></th>
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</tr>
<tr>
<td>PTH and endothelin 1</td>
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<tr>
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<td>0.36</td>
</tr>
<tr>
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<td>–</td>
<td>–0.55</td>
</tr>
<tr>
<td>SBP and EDV</td>
<td>–</td>
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<td>–0.47</td>
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<td>PTH and nitrates/nitrites</td>
<td>–</td>
<td>–</td>
<td>–0.48</td>
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Abbreviations: see Table 1 and Figure 1

\( P = 0.032 \), and the serum level of nitrates and nitrites was lower compared with that in group 1 (15.2 [11.1–29.9] vs. 22.8 [16.9–33.0] \( \mu \text{mol/l} \); \( P = 0.0005 \)). Thus, in women with ED, the production of vasoconstrictors (particularly of endothelin 1) increased, while the production of vasodilators, (ie, nitric oxide) decreased.

The serum levels of endothelin 1 as well as nitrates and nitrites as variables that allow to make the diagnosis of ED were assessed using the ROC curve analysis (Figure 2). The area under the ROC curve was used to compare the serum endothelin 1 level with the data on the presence or absence of ED (\( \Delta dz/dt_{\text{max}} \) equal to or lower or higher than 12%) (AUC, 0.644; 95% CI: 0.521–0.768; \( P = 0.032 \)). The optimal cut-off point that defined the presence of ED in 76% of the cases was the serum endothelin 1 level of 0.53 pg/ml and higher, with a specificity of 62%, which allowed to exclude the presence of ED in patients with AH stage II (Figure 2A).

The type of correlations differed between groups 1 and 2 (Table 2). In a cluster analysis, if subjects with AH did not have signs of ED, the studied parameters in the cluster of the vascular endothelium were weakly related to each other (EDV and endothelin 1, \( R = –0.26 \); EDV and the serum level of nitrates and nitrites, \( R = 0.32 \)). However, the cluster of endothelial function was within a closer range to 25(OH)D and PTH, the farthest member of the cluster was SBP (Figure 2) or, similarly, DBP. Moreover, the contribution of the vascular endothelium into both SBP and DBP was minimal. When the cluster relationship between 25(OH)D, PTH, and the cluster of vascular endothelial function was disturbed, it contributed to ED (Figure 4). Therefore, the association of endothelial function parameters with SBP (\( R = –0.55 \) with nitrates and nitrites; \( R = –0.47 \) with \( \Delta dz/dt_{\text{max}} \)) and DBP (\( R = –0.45 \) with nitrates and nitrites; \( R = –0.31 \) with \( \Delta dz/dt_{\text{max}} \)) became more pronounced.
**DISCUSSION** In previous studies, we did not find any differences in the incidence of vitamin D deficiency, insufficiency, or optimal serum levels with regard to cardiovascular disorders and when compared with healthy individuals. Both the mean vitamin D concentration in the examined group of women with AH stage II, and the incidence of its optimal level (32.3% vs. 4%) was higher than, for example, in the general population of elderly women in Poland, while the mean level of PTH was very similar (36.6 ±20.1 vs. 32.9 ±14.1 and 34.0 ±19.4 pg/ml). Moreover, there was a negative association between the vitamin D concentration and PTH. Previously, we demonstrated a high incidence of primary hyperparathyroidism (29%) in elderly patients with ischemic heart disease, in those with high prevalence of 25(OH)D deficiency (86%). In the present study, women were younger than in the study of Napiórkowska et al., but, in line with the Polish studies, we did not find any relationship between vitamin D and age. On the other hand, there were some studies that reported an inverse relationship between vitamin D and age. In another population-based study, women (but not men) with increased serum PTH levels had significantly higher SBP and DBP. According to the Longitudinal Aging Study Amsterdam in older men and women, higher serum PTH levels were associated with higher SBP and DBP. However, in the present study, the serum level of PTH above the upper limit of the reference range (13.9–75.1 pg/ml) was observed only in 5.5% of the cases. In our study, we determined a positive correlation between PTH and SBP (R = 0.28; P = 0.003). We described a relationship between PTH and DBP in this cohort of women in a previous study, in which we reported that the variability in DBP depended on a combined action of blood phosphate and PTH levels (F = 3.5; P = 0.01), while they did not cause a significant effect on their own. Moreover, the highest DBP values were correlated with high serum PTH levels associated with hyperphosphatemia. To our knowledge, this is the first study to suggest a relationship between PTH and systolic pressure in women with AH associated with a normal but not elevated serum PTH level. We also determined that the serum PTH level inversely correlated with total 25(OH)D concentration; therefore, suppression of PTH could be obtained by an additional intake of vitamin D, which must contribute to a reduction in SBP.

We determined a negative correlation between SBP and serum 25(OH)D levels (R = −0.23; P = 0.034), which corresponds to the results of other studies. A number of studies reported negative correlations with both SBP and DBP. While others, such as ours, reported a negative correlation only with SBP or DBP. Previously, we determined associations between the serum 25(OH)D level and SBP, thus SBP higher than 140 mmHg corresponded to a 25(OH)D level of 17 nmol/l and lower. In our study, when the serum 25(OH)D level was 72 nmol/l and higher SBP and DBP were caused by the effect of vitamin D and PTH on vascular endothelial function, which was already demonstrated by experimental studies. ED plays an important role in maintaining SBP and DBP values, and endothelial function should be normalized in individuals with AH and ED. We performed a detailed assessment of endothelial function, which enabled us to obtain, for the first time, an optimal cut-off point for such biochemical markers as endothelin 1 and nitrates.

**FIGURE 3** Dendrograms of the cluster of systolic blood pressure in patients without endothelial dysfunction

**FIGURE 4** Dendrograms of the cluster of systolic blood pressure in patients with endothelial dysfunction

Abbreviations: see TABLE 1 and FIGURE 1
and nitrates, thus allowing us to make a conclusion about the presence of ED in women with AH stage II with high specificity.

The type of correlations differed between the groups with and without ED. It is known that PTH affects the secretion of endothelin 1, while, on the other hand, endothelin may affect PTH secretion. Lakatos et al. demonstrated that serum endothelin 1 level was increased in patients with primary and secondary hyperparathyroidism. We found a negative correlation between PTH and endothelin 1 in patients without vasomotor ED, which suggested that PTH affected the production of this vasoconstrictor by the vascular endothelium. On the other hand, in patients with ED, there was a negative correlation between PTH and the serum level of nitrates and nitrates, which suggested the effect of PTH on the production of these vasodilators by the vascular endothelium.

In our study, there was a small number of smokers in the groups with and without ED, but continued smoking is known to further enhance endothelial damage by increasing the plasma level of asymmetric dimethylarginine and by inhibition of fibrinolysis.

Thus, normalizing the serum vitamin D level will regulate the activities of PTH and reducing the level of PTH will contribute to nitric oxide production by the endothelium because, in ED, there is a moderate negative correlation between PTH and nitrates and nitrates, while increased nitric oxide production itself will lead to a decrease in SBP and DBP.

We have reported for the first time that normal serum PTH levels were positively correlated with SBP and that serum 25(OH)D levels were negatively correlated with SBP. We have also been the first to determine an optimal cut-off point for such biochemical markers of endothelial function as endothelin 1 and nitrates and nitrates, which allowed us to identify the presence of ED in women with AH stage II with high specificity. The mechanisms whereby vitamin D and PTH affected SBP were likely to reflect a number of pathogenetic associations between the parameters of endothelial function and the serum level of vitamin D and PTH in women with AH; however, those associations were different in women with vasomotor ED. In patients with ED, PTH affected the production of endothelin 1, while in those with ED, PTH affected the production of nitrates and nitrates.

**CONTRIBUTION STATEMENT**

LY conceived the idea for the study. LY and VS contributed to the design of the research. All authors were involved in data collection data analysis. VS coordinated funding for the project. All authors edited and approved the final version of the manuscript.

**REFERENCES**


ARTYKUŁ ORYGINALNY

Związek między funkcją śródbłonka naczyniowego a poziomem witaminy D i parathormonu u kobiet z nadciśnieniem tętniczym

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dysfunkcja śródbłonka, nadciśnienie tętnicze, parathormon, witamina D

STRESZCZENIE

WPROWADZENIE Niewiele badań wskazuje na związek między witaminą D, parathormonem (PTH) i parametrami czynności śródbłonka.

CELE Celem badania było ustalenie związku między funkcją śródbłonka naczyniowego a poziomem witaminy D i parathormonu u kobiet z nadciśnieniem tętniczym (NT).

PACJENTI I METODY Przeprowadziliśmy badanie przekrojowe obejmujące 141 kobiet z NT stopnia II w wieku 50,8 ±6,0 lat. Ocenialiśmy stężenia w surowicy 25-hydroksy-witaminy D (25(OH)D), PTH, endoteliny 1 oraz azotanów i azotynów. Czynność śródbłonka mierzono za pomocą reografii impedancyjnej. Wazodylatację zależną od śródbłonka mierzono za pomocą testu przekrwienia reaktywnego. Wazodylatację zależną od śródbłonka mniejszą niż 12% uznawano za dysfunkcję śródbłonka (endothelial dysfunction – ED).

WYNIKI Znaczny niedobór witaminy D stwierdzono u 53 kobiet (37,6%), niedobór mniejszego stopnia u 42 (30,1%), a optymalne stężenie u 46 (32,3%). ED stwierdzono u 49,6% chorych. Stężenie PTH w surowicy wynosiło 36,6 ±20,1 pg/ml i było powyżej górnej granicy zakresu referencyjnego tylko w 5,5% przypadków. Wykazałyśmy istotne korelacje między PTH i ciśnieniem skurczowym (R = 0,28; p = 0,003), PTH i 25(OH)D (R = –0,27; p = 0,025) oraz 25(OH)D i ciśnieniem skurczowym (R = –0,23; p = 0,034). W grupie z ED stężenie endoteliny 1 w surowicy było większe (0,57 ±0,18 vs 0,49 ±0,21 pg/ml; p = 0,032), a azotanów i azotynów mniejsze (15,2 [11,1–29,9] vs 22,8 [16,9–33,0] µmol/l, p = 0,0005) niż w grupie bez ED. W grupie chorych bez ED PTH korelował ujemnie z endoteliną 1 (R = –0,27; p = 0,043). W grupie z ED PTH wykazywał ujemną zależność z azotanami i azotynami (R = –0,48; p = 0,003).

WNIOSKI PTH wpływał na produkcję substancji wazokonstrykcyjnej – endoteliny 1 u kobiet z NT bez ED, natomiast w obecności ED PTH wiązał się z mniejszą produkcją wazodylatorów – azotanów i azotynów – przez śródbłonkek naczyniowy.