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Vitamin D, sex hormones and anaemia in men – is there an influence of cholecalciferol supplementation on haemoglobin concentrations or regulation of sex hormones in male haemodialysis patients? A pilot study

Witamina D, hormony płciowe a niedokrwistość u mężczyzn – czy suplementacja cholekalcyferolu wpływa na stężenia hemoglobiny oraz regulację gospodarki hormonów płciowych u hemodializowanych mężczyzn? Badanie pilotażowe

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Słowa kluczowe
witamina D, testosteron, niedokrwistość, hemodializa

Summary

Introduction. Active metabolites of vitamin D, as well as androgens, may play a substantial role in the correction of anaemia caused by chronic kidney disease.

Aim. Aim of the study was to analyze the association among vitamin D metabolite – 25(OH)D, sex hormones and anaemia and assess the influence of vitamin D supplementation on haemoglobin concentrations, and sex hormones levels in male haemodialysis patients.

Material and methods. The study was performed in male patients on haemodialysis at the Diaverum Dialysis Unit at Cegłowska Street in Warsaw. In 18 eligible patients aged 35-65 who gave informed consent, out of the total of 81, concentrations of 25(OH)D were measured. All patients had vitamin D deficiency. Thus, in all patients included in the study cholecalciferol was supplemented for three months in the dose adjusted to the vitamin D deficiency. During the study the complete blood count, parameters of iron, calcium and phosphate homeostasis, and concentrations of sex hormones were measured.

Results. Normalization of 25(OH)D concentration was achieved in 11out of 16 patients who completed the study (68.75%). In 8 patients (50%) a significant increase in haemoglobin concentration was observed (P = 0.001). Baseline concentrations of 25(OH)D correlated strongly with total testosterone levels and weakly with haemoglobin concentrations. Moreover, free testosterone index (FTI) moderately correlated with haemoglobin concentrations. Substantial increase in androstenedione concentrations and a decrease in LH, but no significant changes in testosterone concentrations or FTI values were observed during cholecalciferol supplementation.

Conclusions. Cholecalciferol supplementation in vitamin D deficient men on haemodialysis effectively normalized its serum concentration. Strong correlation between 25(OH)D and testosterone concentrations may suggest pathophysiologic association, however, the influence of vitamin D supplementation on testosterone and haemoglobin levels seems clinically negligible.

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Streszczenie

Wstęp. Zarówno witamina D, jak i androgeny mogą mieć wpływ na przebieg niedokrwistości rozwijającej się wtórnie do przewlekłej choroby nerek (PChN).

Cel pracy. Ocena zależności pomiędzy stężeniami metabolitu witaminy D – 25(OH)D, poziomami hormonów płciowych a nasileniem niedokrwistości związanej z przewlekłą

chorobą nerek z jednoczesnym sprawdzeniem wpływu suplementacji witaminy D na stężenia hemoglobiny oraz hormonów płciowych u hemodializowanych mężczyzn.

Materiał i metody. Spośród 81 mężczyzn hemodializowanych w Stacji Dializ Diaverum przy ul. Cegłowskiej 80 w Warszawie u 18 pacjentów w wieku od 35 do 65 lat spełniających kryteria włączenia do badania, którzy wyrazili świadomą zgodę na udział w badaniu, oznaczono stężenia metabolitu 25(OH)D. Hipowitaminoza nie była kryterium włączenia do badania, natomiast u wszystkich zakwalifikowanych do badania mężczyzn zaobserwowano niedobór witaminy D. Wobec powyższego u wszystkich pacjentów przez okres trzech miesięcy suplementowano cholekalcyferol w dawce dostosowanej do stopnia niedoboru. W trakcie badania oznaczano parametry składu morfologicznego krwi obwodowej, gospodarki żelazowej, wapniowo-fosforanowej oraz stężenia hormonów płciowych.

Wyniki. Optymalne stężenie metabolitu 25(OH)D po zakończeniu suplementacji osiągnięto u 11 z 16 pacjentów (68,75%), którzy ukończyli badanie. U połowy pacjentów podczas suplementacji zaobserwowano znamienny wzrost stężenia hemoglobiny (P = 0,001). Początkowe stężenia 25(OH)D silnie korelowały ze stężeniami testosteronu całkowitego, słabo zaś ze stężeniami hemoglobiny. Ponadto, wartości indeksu wolnego testosteronu (FTI) wykazały umiarkowaną korelację ze stężeniami hemoglobiny. Podczas suplementacji zaobserwowano wzrost stężeń androstendionu i spadek LH, nie stwierdzono jednak istotnych statystycznie zmian w stężeniach całkowitego testosteronu i FTI.

Wnioski. Suplementacja cholekalcyferolu jest skutecznym sposobem na podwyższenie w ciągu trzech miesięcy stężenia 25(OH)D u 2/3 hemodializowanych mężczyzn. Silna korelacja pomiędzy stężeniami 25(OH)D oraz testosteronu sugeruje związek patofizjologiczny, nie wykazano jednak istotnego klinicznie wpływu trzymiesięcznej suplementacji witaminy D ani na poprawę stężeń hemoglobiny, ani testosteronu, obniżonych w przebiegu PChN.

INTRODUCTION

Chronic kidney disease, as well as haemodialysis therapy – one of its therapeutic options, has various debilitating consequences. Among them anaemia is one of the most prevalent and cumbersome – it contributes to increased morbidity and mortality. The etiology of anaemia in the course of the disease is multifactorial. It may result from a relative or absolute erythropoietin deficiency, increased eryptosis or circulating uremic toxins. Moreover, dialysis patients have negative iron balance caused by significant loss of this microelement during dialysis procedure, poor absorption from the gastrointestinal tract and sequestration in the mononuclear phagocyte system as a result of elevated hepcidin concentration, and simultaneous increased utilization of iron due to erythropoiesis stimulating agents (ESAs) (1). However, more and more data suggest that vitamin D and sex hormones deficiency may participate in the pathogenesis of anaemia in chronic kidney disease not. The vitamin deficiency in haemodialysis patients is not less present than in general population.

Active metabolites of vitamin D regulate diverse metabolic processes, including calcium and phosphate homeostasis, cell growth and differentiation and immunologic responses. Vitamin D receptor (VDR) and 1α-hydroxylase CYP27B1, an extrarenal enzyme responsible for conversion of 25(OH)D to an active metabolite 1,25$(OH)_2$D, were also discovered in the bone marrow (2). High local 1,25$(OH)_2$D concentration in the erythroid marrow is supposed to directly stimulate the erythroid progenitor cells – by paracrine regulation – to proliferate (3). Thus, low levels of vitamin D frequently observed in CKD patients may negatively influence effective erythropoiesis.

Androgens are another group of endogenous factors that for decades have been suspected of playing a substantial role in erythropoiesis. There is the evidence for direct stimulating effect of androgens on the erythroid line progenitor cells, and for indirect androgen regulation of erythropoiesis via hepcidin and erythropoietin (4, 5). The frequency of male hypogonadism, described as low levels of total and free testosterone, raises along with the stage of CKD – from 17% in patients with CKD G1 to 57% in patients with end- stage renal failure (6). Moreover, apart from association with anaemia, low testosterone levels are related to increased mortality due to cardiovascular diseases (7). The past trials gave conflicting results of anaemia treatment with androgens. Thus current KDIGO 2012 recommendations discourage the androgen administration as an adjuvant therapy to erythropoiesis stimulating agents (8).

To emphasize the complex interplay among various endogenous factors regulating erythropoiesis it is worth noticing that vitamin D may play a role in maintaining hormonal homeostasis in men. Vitamin D receptor and enzymes activating native form of vitamin D were found in numerous cells of the male genitourinary tract and pituitary gland, where their influence on androgenesis through paracrine activity is currently under investigation (9, 10). In several studies an association between vitamin D and male hormone levels was found (11, 12).

AIM

The aim of this study was to assess the existence of an association between concentrations of the circulating metabolite of vitamin D – 25(OH)D, sex hormones and anaemia in male haemodialysis patients, and if androgen or haemoglobin concentrations may rise during supplementation of native vitamin D.

MATERIAL AND METHODS

The authors declare that the study has been conducted according to the principles of the Declaration of Helsinki, and it was approved by the Bioethics Committee at the Center of Postgraduate Medical Education in Warsaw.

The study involved 18 patients with end-stage renal failure. The inclusion criteria were: male sex, age 35-65, and duration of haemodialysis therapy for at least two months. Patients with malignant tumours, liver failure, hypo- or hyperadrenocorticism, primary or secondary hypopituitarism, advanced aortic, or common iliac arteries atherosclerosis, and Leriche's syndrome, were excluded from the study. Baseline values of the markers of calcium-phosphate homeostasis, complete blood count, markers of iron homeostasis and sex hormones concentrations were measured in each patient. Three stages of vitamin D deficiency were set according to the concentrations of 25(OH)D: < 10 ng/ml – severe deficiency, 10-20 ng/ml – moderate deficiency, 20-30 ng/ml – mild deficiency (tab. 1).

Table 1. Levels of vitamin D deficiency and adjusted dose of vitamin D.

25(OH)D level [ng/ml]	Number of patients	Weekly dose of vitamin D [IU]
< 10	9	40 000
10-20	8	20 000
20-30	1	15 000

In patients with vitamin D deficiency, cholecalciferol – a form of native vitamin D – was supplemented orally in doses adjusted to the stage of the deficiency – in severe deficiency 40 000 IU per week, in moderate deficiency 20 000 IU per week, and in mild deficiency 15 000 IU per week. For three consecutive months, weekly doses of cholecalciferol were administered after the first weekly dialysis sessions, supervised by qualified nurses. During the supplementation period, complete blood count, calcium-phosphate and iron balance were monitored and sex hormones measured. Haemoglobin increase by not less than 0.5 g/dl at the end of the third month of cholecalciferol supplementation was set as the endpoint of the study. The results were analyzed with the use of Student t-test, ANOVA and Pearson's analysis of correlation. The significance level was set to 0.05.

RESULTS

Baseline patients' characteristics with primary values of the markers of calcium-phosphate homeostasis, sex hormones concentrations, albumin, vitamin B_{12} and folic acid levels are summarized in table 2.

Baseline values of complete blood count, reticulocyte indices and iron balance are shown in table 3.

Table 2. Patients' baseline characteristics, baseline markers of calcium-phosphate homeostasis, sex hormones concentrations, albumin, vitamin B_{12} and folic acid levels.

Parameter	Mean ± SD/n (%)
Age [years]	52.94 ± 10.15
Etiology of renal failure: diabetes arterial hypertension glomerulonephritis other	 4 (22.22%) 2 (11.11%) 6 (33.33%) 6 (33.33%)
Duration of haemodialysis therapy [months]	43.44 ± 57.24 (5-216)
Calcium concentration [mmol/l]	2.16 ± -0.58
Phosphate concentration [mmol/l]	1.28 ± 0.58
PTH concentration [pg/ml]	325.96 ± 213.75
25(OH)D concentration [ng/ml]	11.43 ± 4.96
Bone alkaline phosphatase [% total BAP]	51.59 ± 7.89
Estradiol [pg/ml]	33.27 ± 17.79
Progesterone [ng/ml]	0.31 ± 0.18
LH [IU/L]	12.69 ± 11.52
FSH [IU/L]	10.05 ± 7.73
PRL [ng/ml]	17.6 ± 14.83
Androstenedione [nmol/l]	7.74 ± 5.08
DHEA [ng/ml]	975.58 ± 839.11
SHBG [nmol/l]	55.46 ± 21.67
Testosterone [ng/ml]	5.63 ± 2.08
Free testosterone index [%]	37.53 ± 11.68
Albumin [g/dl]	4.21 ± -0.31
Vitamin B_{12} [pg/ml]	410.15 ± 178.1
Folic acid [ng/ml]	10.81 ± 13.12

Table 3. Baseline values of complete blood count, reticulocyte indices and iron balance.

Parameter	Mean ± SD
Erythrocytes [mln/μl]	3.35 ± 0.63
Haemoglobin [g/dl]	10.33 ± 1.45
Haematocrit [%]	31.72 ± 4.16
MCV [fl]	95.65 ± 7.84
MCH [pg]	31.12 ± 2.67
MCHC [g/dl]	32.52 ± 1.06
Reticulocytes [%]	1.30 ± 0.79
TIBC [μg/dl]	233.95 ± 44.04
Iron [μg/dl]	61.23 ± 35.57
UIBC [μg/dl]	172.49 ± 59.36
Ferritin [ng/dl]	815.16 ± 692.31
Transferrin [mg/dl]	183.89 ± 32.19
Macrocytic erythrocytes [% total erythrocytes]	7.31 ± 7.29
Microcytic erythrocytes [% total erythrocytes]	0.61 ± 0.58
Hyperchromic erythrocytes [% total erythrocytes]	0.24 ± 0.21
Hypochromic erythrocytes [% total erythrocytes]	9.34 ± 7.81
MCVr [fl]	120 ± 6.56
CHCMr [g/dl]	26.67 ± 1.36
CHr [pg]	32.06 ± 2.58
LFR [% total reticulocytes]	91.31 ± 7.26
MFR [% total reticulocytes]	7.76 ± 6.31
HFR [% total reticulocytes]	0.93 ± 1.02
Weekly ESA demand [IU]	4941.18 ± 2561.02
Iron supplementation [mg/month]	147.06 ± 62.43

Analysis of baseline values

All patients included in the study had vitamin D deficiency. Half of the patients had severe vitamin D deficiency and each patient had anaemia. It is also worth noticing that only one patient had mild deficiency with baseline 25(OH)D value over 20 ng/ml. None of the patients had either vitamin B_{12} or folic acid deficiency. Moreover, iron supplementation and ESA treatment was conducted according to KDIGO recommendations.

Low (< 30%) free testosterone index was observed in 5 patients. Merely 3 patients had normal levels of pituitary gland hormones: LH, FSH and prolactin. Isolated increased LH levels (in 6 patients) and concomitant elevated levels of LH and FSH (in 5 patients) were the most frequent abnormalities. Hyperprolactinaemia was observed in 4 patients (22.22%).

Baseline 25(OH)D correlated weakly with haemoglobin, LH, FSH, prolactin, SHBG, androstenedione, estradiol and progesterone concentrations. A strong correlation was found between 25(OH)D concentrations and total testosterone levels (r = 0.46).

Sex hormones levels (total testosterone, androstenedione, estradiol, progesterone) correlated weakly with haemoglobin, reticulocyte parameters and markers of iron metabolism. Moderate correlation was found between haemoglobin concentration and index of free testosterone (r = 0.4).

End-results analysis

The cholecalciferol supplementation was introduced in 18 patients, of whom 16 completed the study. One patient had suspicion of a gastrointestinal bleeding and was referred for further investigation. One patient was excluded from the study due to a severe urinary tract infection.

Following the three months of supplementation significant increase of 25(OH)D by a mean 23.37 ng/dl (SD = 11.67 ng/dl) was observed in all 16 patients (p < 0.001). In 11 patients the 25(OH)D concentration reached normal values (68.75%) (tab. 4).

Table 4. Mean baseline 25(OH)D values and mean concentrations of 25(OH)D observed after cholecalciferol supplementation according to the level of vitamin D deficiency.

Baseline deficiency level	Mean baseline 25(OH)D value ± SD	Mean 25(OH)D ± SD after cholecalciferol supplementation
< 10	7.11 ± 1.68	35.5 ± 12.62
10-20	15.37 ± 1.88	31.15 ± 7.5
20-30	21.5 (N/A)	44.2 (N/A)

Three cases of mild, intermittent, and asymptomatic hypercalcaemia occurred during cholecalciferol supplementation, followed by a decrease in cholecalciferol dose. 8 patients reached the study endpoint – the statistically significant increase in haemoglobin concentrations (p = 0.001). In this group a decreased demand for erythropoiesis stimulating agents was noted in 4 patients (25%) and lower demand for iron supplementation was observed in 6 patients.

At the end of the third month of supplementation, an increase in free testosterone index was observed in 6 patients (37.5%), androstendione levels rose in 12 patients (75%), LH concentrations decreased in 9 patients (56.25%) and FSH in 7 patients (43.75%).

DISCUSSION

Although in Polish general population vitamin D deficiency is frequent, in haemodialysis patients it is even more prevalent. Our study proves that in this subpopulation even young active men are at risk of vitamin D deficiency with all its consequences.

Vitamin D and anaemia in chronic kidney disease

Erythropoiesis is an extremely intricate process that on each stage is dependent on various growth factors and is regulated by diverse auto- and paracrine substances. Active vitamin D metabolites and androgens through their nuclear receptors are potent transcription factors, thus, they may participate in proliferation and differentiation of haematopoietic cells. (12, 13). *In vitro* studies have shown that $1,25(OH)_2D$ stimulates various processes in blood cells: induces cell differentiation in the myelocyte lineage, maturation of progenitor cells to osteoclasts, and regulates synthesis of interleukins and surface antigens in blood cells (13). Moreover, in a positive feedback loop, calcitriol increases proliferative response of haematopoietic cells to erythropoietin stimulation (14).

In contradiction to previous trials (15, 16), in our study no statistically significant correlation was found between 25(OH)D and haemoglobin concentration. However, in half of the patients during cholecalciferol supplementation haemoglobin concentrations rose, what deserves verification in a larger study.

Vitamin D and regulation of sex hormones

Recent data suggest that vitamin D may play a role in various processes in the male genitourinary tract, such as androgenesis or spermatogenesis, however, precise regulatory mechanisms have not been discovered. In VDR knock-out mice hypergonadotropic hyponadism resolves, suggesting a potential regulatory impact of vitamin D on the hypothalamus-pituitary-testes axis (17). Moreover, VDR expression takes place in Sertoli cells, spermatogonia, epididymic epithelium and seminal vesicles (18). In several studies VDR was also found in Leydig cells, that are directly engaged in androgenesis (19, 20). In the present study a strong correlation between 25(OH)D and total testosterone concentration was shown, that might confirm physiologic association. We observed an elevation in androstenedione concentrations and a decrease in LH levels, nevertheless, no statistically significant increase in total or free testosterone concentrations was reached

during cholecalciferol supplementation, which may be a consequence of a short duration of this pilot study.

Androgens and anaemia in chronic kidney disease

Androgen deprivation therapy frequently leads to anaemia, while androgen supplementation raises haemoglobin levels, sporadically even up to polycythaemic values. This action of androgens is attributed to the stimulating effect on erythropoietin production, transcriptional regulation of haematopoietic growth factors and decrease in hepcidic synthesis (5, 21). In our study we found a moderate correlation between free testosterone index and haemoglobin. However, whether changes in free testosterone levels during vitamin D supplementation may contribute to better anaemia treatment in haemodialysis men remains an unsolved issue.

CONCLUSIONS

Cholecalciferol, a form of native vitamin D may be used for effective normalization in vitamin D deficiency in haemodialysis patients. Strong correlation between 25(OH)D and testosterone concentrations may suggest common pathophysiologic association, however, the influence of vitamin D supplementation on testosterone and haemoglobin levels in the clinical aspect seems rather minor.

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