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Serum 25(OH)D and VEGF in diabetes mellitus type 2: gender-specific associations

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ABSTRACT

Background: Vitamin D insufficiency has been defined as serum 25-hydroxyvitamin D (25(OH)D) levels below 30 ng/mL and is common among patients with diabetes mellitus (DM) type 2 and the elderly.

Aim & Objectives: Our aim was to investigate clinically meaningful associations implicating low serum levels of 25(OH)D and vascular endothelial growth factor (VEGF) levels in DM type 2.

Methods: Serum 25(OH)D and VEGF levels were determined in 40 patients with DM type 2 and vitamin D insufficiency. Their correlation with markers of advanced diabetic disease (amputation, diabetic foot, proliferative diabetic retinopathy, insulin dependence) as well as with serum biochemical parameters was examined. Subanalyses were performed on men and women.

Results: Compared with males, female patients exhibited lower 25(OH)D levels ($p<0.0001$) but higher serum VEGF ($p=0.018$). There was a trend towards an inverse vitamin D - VEGF association. Subanalysis on women showed low serum 25(OH)D levels strongly associated with amputation ($p=0.003$). High serum VEGF levels were associated with amputation ($p=0.038$), and marginally with diabetic foot ($p=0.058$), insulin dependence ($p=0.084$) and proliferative diabetic retinopathy ($p=0.086$). Higher serum 25(OH)D levels were associated with serum uric acid ($p=0.007$), calcium ($p=0.042$) and albumin levels ($p=0.033$). Subanalysis on men demonstrated positive correlation between 25(OH)D levels, albumin ($p=0.004$) and calcium levels ($p=0.060$, borderline association).

Conclusion: The association between low serum 25(OH)D levels and amputation in women may be inscribed into the wider context portraying vitamin D insufficiency as a poor prognostic factor. Vitamin D insufficiency may exert gender-specific effects in the context of DM type 2.

Keywords: Vitamin D, VEGF, diabetes, amputation

Introduction

Vitamin D insufficiency is a prevalent condition worldwide; nearly one billion people have vitamin D insufficiency, among which 40-100% of U.S. and European elderly individuals [1]. Various definitions for vitamin D insufficiency have appeared in the literature; the best established one pertains to serum levels below 30 ng/mL[2]. A recent meta-analysis has demonstrated that low vitamin D levels in middle-aged and elderly populations

represent a risk factor for type 2 diabetes mellitus (DM), cardiovascular disease and metabolic syndrome[3].

Vascular endothelial growth factor (VEGF) represents another pathway implicated in the pathophysiology of DM. VEGF is known to stimulate all aspects of endothelial function, namely proliferation, migration and permeability[4].

This study aims to investigate whether serum 25(OH)D levels are correlated with VEGF serum levels and associated with clinical consequences of type 2 DM, such as amputation, diabetic foot, insulin dependency, proliferative diabetic retinopathy (PDR), as well as serum biochemical parameters in a well defined group of patients with vitamin D insufficiency. Since gender may represent a modifier of serum vitamin D[5], separate analyses were performed on males and females, to reveal any gender-specific associations.

Materials and Methods

We selected 40 consecutive DM type 2 patients (age range: 47 to 84) with 25(OH)D insufficiency (<30 ng/mL). Hypercalcemia, intake of vitamin D for osteoporosis in dietary supplements, or end-stage renal failure were exclusion criteria. All participants were interviewed at baseline by the same investigator. They also underwent indirect biomicroscopy in order to identify those patients with proliferative diabetic retinopathy (PDR). Blood samples were obtained for serum 25(OH)D, serum calcium, HBA1c, uric acid, albumin and VEGF measurement. To measure 25(OH)D the chemiluminescence, non-radioactive variant, Liaison method was used. To quantify the serum VEGF measurement we used the Invitrogen's VEGF Human ELISA Kit.

Due to deviation from normality, non-parametric statistics were performed. Separate analyses were performed on men and women. Concerning continuous variables, patients have been categorized into those <median and those >median for purely descriptive purposes in the Tables. The association of serum 25(OH)D and VEGF with binary variables was evaluated with Mann-Whitney-Wilcoxon test for independent samples (MWW). The association between 25(OH)D, VEGF with continuous and ordinal variables was evaluated with Spearman's rank correlation coefficient. Statistical analysis was performed with STATA 8.0 (Stata Corp, College Station, TX, USA).

Results

Compared with males, females demonstrated lower serum 25(OH)D levels (7.91±3.41 vs. 17.95±7.46 ng/ml, p<0.0001, MWW), but higher serum VEGF levels (335.5±150.8 vs. 236.1±144.2 pg/ml, p=0.018, MWW). There was a trend towards an inverse 25(OH)D-VEGF association, which did not reach statistical significance, most probably due to the relatively small sample size (Spearman's rho = -0.252, p=0.121).

At the subanalysis on women (Table 1), lower serum 25(OH)D levels were strongly associated with amputation (p=0.003). High serum VEGF levels were associated with amputation (p=0.038), and at a borderline level with diabetic foot (p=0.058), insulin dependence (p=0.084) and PDR (p=0.086). Higher serum 25(OH)D levels were strongly associated with serum uric acid (p=0.007), calcium (p=0.042) and albumin (p=0.033) levels. At the subanalysis on men (Table 2) higher serum 25(OH)D levels were associated with serum albumin (p=0.004) and serum

calcium levels (p=0.060, borderline association).

Discussion

Low serum 25(OH)D levels were strongly associated with amputation of the diabetic foot in women with DM; this may be inscribed into the wider context portraying vitamin D insufficiency as a poor prognostic factor[6,7]. At this direction, a recent study on veterans highlighted the association between vitamin D deficiency (defined as <20 ng/ml) and amputation, pointing to the value of traditional risk factors as effect mediators[7]. The parallel increase in serum VEGF is worth mentioning, reflecting the hypoxic and inflammatory conditions leading to amputation. Nevertheless, an alternative aspect may not be ruled out, as mobility impairment and more advanced disease may lead to less time spent outside and thus less sun exposure, which could in turn result in lower serum 25(OH)D levels.

There was a trend towards an inverse 25(OH)D - VEGF association, the reason of which remains unclear. This association may entail an indirect aspect; as mentioned above, lower serum 25(OH)D levels were associated with amputation and may thus denote hypoxic conditions. Hypoxia is in turn a well established stimulus for VEGF expression[4]. On the other hand, concerning direct VEGF-vitamin D links, data remain controversial. In vitro vitamin D may enhance VEGF expression in vascular smooth muscle cells[8]; vitamin D reduces however VEGF expression in various human cancer cells[9]. Noticeably, borderline direct correlations of serum VEGF levels with a series of serious diabetic advance phenomena emerged in women, namely: diabetic foot, insulin dependence, PDR As far as it concerns PDR, increased serum VEGF levels may indicate an additional systemic aspect to its well established local role to the evolution of diabetic retinopathy[10].

The strong direct association of 25(OH)D serum levels with uric acid levels seemed rather surprising, as Bonakdaran et al have demonstrated a null association between uric acid and vitamin D in diabetic patients[11]; further studies are needed to evaluate gender-specific issues, since our finding was confined to women. Given that vitamin D increases calcium absorption, the direct association with serum calcium was expected[2]. The demonstrated binding of vitamin D to albumin may well explain the relationship with serum albumin[12]. Lower 25(OH)D levels in females were also rather expected as previous, large prospective studies have emphasized a suboptimal vitamin D status in diabetic, post-menopausal women[13].

A variety of limitations of this study need however to be addressed. The small sample size did not allow a multivariate approach incorporating additional, potentially meaningful factors modifying the levels of serum 25(OH)D, such as BMI and exercise. Given the cross-sectional design of this study, it should be declared that no firm evidence is provided that improving vitamin D status will reduce the risk for amputation in people with DM type 2. Nevertheless, it seems that routine screening for vitamin D insufficiency may provide meaningful information and could be considered for diabetic care. Interventional studies are needed to evaluate whether vitamin D long-term supplementation could reduce morbidity in diabetic population with awareness of side effects.

Conflict of Interest: The authors declare no conflict of interest.

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Table 1: Subanalysis in women (values are mean±SD)

Parameters	25(OH)D (ng/ml)	P	VEGF (pg/ml)	P
Amputation		**0.003**^{MWW}		**0.038**^{MWW}
Yes(n=9)	5.69±2.64		424.8±163.9	
No (n=9)	10.14±2.56		246.1±59.1	
Diabetic foot		0.441^{MWW}		**0.058**^{MWW}
Yes (n=15)	7.72±3.55		360.5±151.7	
No (n=3)	8.86±3.00		210.1±62.0	
Insulin dependence		0.460^{MWW}		**0.084**^{MWW}
Yes(n=13)	8.09±3.58		368.8±165.6	
No (n=5)	7.46±3.24		248.7±35.9	
PDR		0.214^{MWW}		**0.086**^{MWW}
Yes (n=3)	5.16±0.67		447.9±127.3	
No (n=15)	8.46±3.47		313.0±148.5	
Years since DM		0.806^{S}		0.667^{S}
diagnosis	8.34±3.71		324.7±163.0	
<median(median=17)	7.48±3.23		346.2±146.6	
≥median(median=17)				
HBA1c (%)		0.833^{S}		0.496^{S}
<median(median=7.25%)	7.56±2.77		383.2±172.4	
≥median(median=7.25%)	8.27±4.09		287.7±116.0	
Serum Ca2+(mg/dl)		**0.042**^{S}		0.419^{S}
<median(median=9.4)	6.35±2.81		383.2±177.0	
≥median(median=9.4)	9.48±3.36		287.8±108.9	
Uric Acid (mg/dl)		**0.007**^{S}		0.898^{S}
<median(median=5.7)	5.75±1.39		335.2±205.0	
≥median(median=5.7)	8.74±3.62		335.5±134.9	
Albumin (g/dl)		**0.033**^{S}		0.316^{S}
<median(median=3.9)	6.52±2.26		381.7±170.5	
≥median(median=3.9)	8.61±3.75		312.3±142.2	

^{MWW}: p-value derived from Mann-Whitney-Wilcoxon test for independent samples
^{S}: p-value derived from Spearman's rank correlation coefficient

Table 2: Sub-analysis in men (values are mean±SD)

Parameters	25(OH)D (ng/ml)	P	VEGF (pg/ml)	P
Amputation		0.237[MWW]		0.833[MWW]
Yes(n=11)	16.00±7.61		233.1±159.2	
No (n=11)	19.90±7.13		238.8±137.0	
Diabetic foot		0.217[MWW]		0.929[MWW]
Yes (n=18)	16.94±7.08		240.5±154.3	
No (n=4)	22.53±8.49		217.5±105.6	
Insulin dependence		0.463[MWW]		0.434[MWW]
Yes(n=13)	16.82±7.49		259.2±166.0	
No (n=9)	19.59±7.55		205.3±110.6	
PDR		0.411[MWW]		0.654[MWW]
Yes (n=5)	15.00±7.81		253.5±144.5	
No (n=17)	18.82±7.37		232.0±148.3	
Years since DM		0.984[S]		0.954[S]
diagnosis	17.21±6.17		224.8±118.7	
<median(median=18.5)	18.70±8.81		248.6±173.8	
≥median(median=18.5)				
HBA1c (%)		0.375[S]		0.594[S]
<median(median=6.9%)	19.67±7.50		242.7±162.1	
≥median(median=6.9%)	16.53±7.45		230.1±133.6	
Serum Ca2+(mg/dl)		**0.060**[S]		0.691[S]
<median(median=9.6)	16.01±5.57		254.6±153.3	
≥median(median=9.6)	19.57±8.64		219.3±140.7	
Uric Acid (mg/dl)		0.254[S]		0.146[S]
<median(median=5.8)	14.28±3.92		251.0±128.7	
≥median(median=5.8)	20.05±8.29		226.9±157.4	
Albumin (g/dl)		**0.004**[s]		0.620[S]
<median(median=4.5)	13.82±7.12		215.0±143.9	
≥median(median=4.5)	21.40±6.03		251.9±148.7	

[MWW]: p-value derived from Mann-Whitney-Wilcoxon test for independent samples
[S]: p-value derived from Spearman's rank correlation coefficient