Relationship of Vitamin D Deficiency to Cardiovascular and Metabolic Parameters in Women with Polycystic Ovary Syndrome

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Abstract

Objective To determine if vitamin D deficiency (25-hydroxyvitamin D3 (25(OH)D3)), exacerbated the cardiovascular and metabolic characteristics in women with polycystic ovary syndrome (PCOS).Design Comparative cross-sectional analysis.

Methods Demographic and metabolic data from women aged 18-40 years from the Qatar Biobank (QBB) (78 diagnosed with PCOS, 641 controls).

Results Vitamin D deficiency (median (range)) was seen in both normal 14.0 (124) ng/ml and the PCOS cohorts 14.0 (62) ng/ml and did not differ between them. Whilst PCOS subjects were heavier with a more metabolic profile (greater systolic and diastolic blood pressure, higher levels of C-reactive protein, androgens, insulin and glycosylated hemoglobin (HbA1c) and decreased high density lipoprotein (HDL) levels, with endothelial dysfunction as determined by pulse wave velocity, there was no correlation (Pearson coefficient) of any these parameters with vitamin D for either the control or PCOS population.

Conclusion Vitamin D deficiency was equally prevalent in women with and without PCOS and was not correlated to insulin resistance, metabolic or cardiovascular parameters, suggesting that vitamin D deficiency is not associated with the PCOS phenotype.

Background

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders; in a recent systematic analysis, the prevalence was estimated to be between 6% (National Institute of Health (NIH) criteria) to 10% (Rotterdam and Androgen excess society guidelines) (1), though is likely higher in the Middle East, estimates being 12% by NIH criteria (2). PCOS affects 6–20% of reproductive-aged women (3–5) and is associated with increased androgen levels causing hirsutism and acne (6, 7). PCOS patients often present with obesity, irregular periods and infertility (6, 7) and there is a higher prevalence of both impaired glucose tolerance and type 2 diabetes (5, 8).

Vitamin D deficiency is very common in women with PCOS, with 67–85% having serum concentrations of 25-hydroxyvitamin D_3 ($25(OH)D_3$) < 20 ng/ml, and levels have been reported to correlate with obesity and increased insulin resistance, as well as testosterone and dehydroepiandrosterone

sulphate (DHEAS) levels (9–12). Studies have revealed that vitamin D replacement therapy may have beneficial effects on insulin resistance and steroidogenesis of estradiol and progesterone in obese women with PCOS (13, 14). In Qatar, approximately 61.3% of females have vitamin D deficiency (15); therefore, we hypothesized that vitamin D deficiency would correlate with, and may exacerbate, the insulin resistance and cardiovascular parameters in PCOS.

Methods

The goal of the Qatar BioBank (QBB) is to harness information and data on approximately 60,000 participants, 18 years of age and older (2). This study was carried out in accordance with the recommendations of the Qatar Biobank and by the Ministry of Health in Qatar with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the QBB IRB and by the Ministry of Health in Qatar. 750 women between the ages of 18 to 40 years were identified, of whom 719 subjects had complete data for analysis. PCOS status of these subjects was based on the NIH criteria with complete information that included total testosterone and sex hormone binding globulin (SHBG). All PCOS subjects had thyroid function tests, prolactin, dehydroepiandrosterone sulfate (DHEAS) and 17-beta hydroxyprogesterone to exclude other confounding diagnoses and no subject was taking any medication (2).

Baseline parameters including height, weight, waist circumference, insulin levels, glucose levels, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), testosterone, Free Androgen Index (FAI), C-reactive protein (CRP), alanine aminotransferase (ALT) and vitamin D were measured in all subjects. Body mass index (BMI) was calculated based on World Health Organization (WHO) guidelines. Vicorder (SMT medical GmbH & Co, Germany) measurements for pulse wave velocity, as a non-invasive assessment of arterial stiffness (a determinant of cardiovascular risk), were performed according to the manufacturer’s instructions.

Blood samples for serum and plasma were collected and immediately processed (within 5 min), centrifuged for 10 minutes at 3000 rpm, and stored frozen at -80 °C pending analysis. Analyses for thyroid stimulating hormone (TSH), prolactin, insulin, testosterone, C-reactive protein (CRP), DHEAS,

and sex hormone binding globulin (SHBG) were conducted in the Chemistry Laboratory at Hamad Medical Corporation, Doha, Qatar, and measured by an immunometric assay with fluorescence detection on a DPC Immulite 2000 analyzer using the manufacturer's recommended protocol. The Abbott testosterone method was performed within manufacturer's specification throughout the sample collection (within run coefficient of variation (CV) 3.1%, within laboratory CV 3.6% at 2.6 nmol/L). The free androgen index (FAI) was calculated as the total testosterone x 100/SHBG. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2μU/ml, the coefficient of variation was 6%, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyzer (Beckman-Coulter), using the manufacturer's recommended protocol; the coefficient of variation for the assay was 1.2% at a mean glucose value of 5.3 mmol/L during the study period. The insulin resistance was calculated using the HOMA method [HOMA-IR = (insulin x glucose)/22.5]. Serum vitamin D levels were quantified using the Roche immunoassay platform (Roche Penzberg, German); the coefficient of variation for the assay was 17% at a mean vitamin D value of 23.4 nmol/L during the study period. All methods of analysis were performed in accordance with the relevant guidelines and regulations with appropriate quality control.

Data were supplied to Weill Cornell Medicine Qatar (WCMQ) biostatistics unit from the QBB in an anonymous coded manner that had been approved by the WCMQ IRB. The demographic and biochemical data for all women between the ages of 18–40 were supplied in a coded anonymous manner.

Statistical analysis

The level of significance was set at 5%. Data trends were visually and statistically evaluated for normality. Independent T-tests were applied on normally distributed data, while non-parametric tests (Mann Whitney U) were applied on data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov Test. Statistical analysis was performed using SPSS for Windows, version 24.0. Correlations between vitamin D and the differing parameters were undertaken with Pearson

coefficient, and a P value for each of the parameters is summarized in Table 2. This test was used to assess whether there is an additive linear association between lower vitamin D levels and cardiovascular disease/ androgenicity in females with PCOS in Qatar.

Results

Data for PCOS subjects were compared to the control group without PCOS in their baseline parameters (Table 1) showing that vitamin D did not differ between PCOS and controls and the medians were both in the deficient range, 14 (62) versus 14 (124), respectively. Significant differences in androgens, insulin resistance and cardiovascular risk parameters were seen. Pearson correlation showed no correlation (p values, PCOS and control, respectively) between: vitamin D and BMI (0.3, 0.9), HOMA-IR (0.9, 0.6), insulin (0.9, 0.6), glucose (0.8, 0.5), testosterone (0.5, 0.8), FAI (0.8, 0.7), CRP (0.4, 0.5), ALT (0.9, 0.8) or pulse wave velocity (0.6, 0.7) (Table 2).

579/641 (90%) of controls were vitamin D deficient (< 20 ng/ml [< 50 nmol/l]) compared to 72/98 PCOS subjects (92%). When the analysis was restricted to vitamin D deficiency alone there were no changes in the correlations with any of the parameters measured. When 62 vitamin D replete controls were compared to the 579 deficient/insufficient controls, there were no differences in any of the measured parameters, in accord with that of the Pearson correlations and as expected.

Discussion

The novelty of this study is that it is the first to look at the association of PCOS with vitamin D deficiency in this Qatari population. The results showed that vitamin D levels were equally deficient in both healthy control women and in women with PCOS in Qatar, and did not differ between the groups. Vitamin D deficiency was defined as a 25(OH)D_3 level below 20 ng/ml (50 nmol/liter), and vitamin D insufficiency as a 25(OH)D_3 level of 21–29 ng/ml (52.5–72.5 nmol/liter) (16). However, there was no correlation of vitamin D levels with metabolic or cardiovascular risk indices. The comparable level of vitamin D between the normal and PCOS women is discordant to other studies where vitamin D is often much lower in subjects with PCOS (17); however, those studies were not undertaken in the Middle East where the practice of whole body coverage is the norm and low sunlight exposure is universal.

In this study, vitamin D did not correlate with BMI, insulin resistance, inflammatory markers or cardiovascular indices. This is in accord with a recent vitamin D interventional trial that showed that liver markers of fibrosis were affected, but not other hormonal parameters (18), and is in accord with a recent meta-analysis (19). Our results are also in accord with those found in slim PCOS patients where there was no association with insulin resistance (20). Conversely, others have reported that vitamin D correlates with obesity and increased insulin resistance, testosterone and dehydroepiandrosterone sulphate (DHEAS) levels (9–12). The results of the meta-analyses of vitamin D supplementation have not been consistent, with one reporting that there was no evidence that vitamin D improved hormonal or glycemic profiles in PCOS (21) whilst another reported vitamin D supplementation reduced total testosterone but that serum free testosterone was unaffected (22).

The low vitamin D levels are of concern as there is an extensive literature suggesting that a vitamin D level below 50 nmol/liter is associated with increased cardiovascular events. For example, data from the National Health and Nutrition Examination Survey has shown that 25-hydroxyvitamin D levels were inversely related to the following cardiovascular risk factors: blood pressure $>$ 140/90 mm Hg, blood glucose $>$ 125 mg/dL (6.95 mmol/L), and BMI of 30 kg/ m^2 or greater (23). Similarly, the Framingham Offspring Study cohort showed that there was a 62% higher risk of cardiovascular events in patients whose 25-hydroxyvitamin D level was $<$ 15 ng/mL (38 nmol/L), compared with those whose level was 15 ng/ mL or greater (24). It remains unclear whether vitamin D intervention improves the metabolic parameters (17, 25).

Differences in phenotype and clinical symptoms of PCOS related to the clinical, hormonal, and metabolic characteristics among various ethnic backgrounds have been described, including in Hispanics, African Americans, Asians, and Indians (26, 27). Differences in women of Middle East origin with PCOS have been compared to Caucasian women (27); Caucasian women with PCOS were reported as having a more adverse cardiovascular profile with increased insulin sensitivity (28). It is unclear from the literature what the impact of chronic vitamin D deficiency would have on the ethnic differences seen for cardiovascular risk in PCOS.

The strength of this study was that it was performed in a general population of the same

homogeneous ethnicity and with relatively large numbers. Limitations of this study include that this was a cross-sectional retrospective study and the subjects were not matched for age and adiposity; therefore, a prospective interventional design would have been more powerful. Ideally, a comparison of vitamin D deficient versus vitamin D replete PCOS subjects would have been undertaken; however, at the bivariate level of analysis, there was no significant linear association between the metabolic indicators with vitamin D.

In conclusion, our hypothesis that vitamin D deficiency would be associated with a greater increase in insulin resistance and androgen levels was not found, with no correlation seen between vitamin D and any parameter. Future definitive interventional studies are needed to confirm whether vitamin D is related to changes in these parameters in this Middle Eastern population.

Table 1. Demographic details for 719 women between the ages of 18 to 40 years, comparing normal subjects with women defined as having polycystic ovary syndrome by NIH criteria (free androgen index greater than 4.5 and/ or a total testosterone greater than 2.7 nmol/l, with irregular menses).

Table 2. Correlations between vitamin D and metabolic parameters within control (N = 641) and PCOS (n = 78) populations.

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the recommendations of the Qatar Biobank and by the Ministry of Health in Qatar with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the QBB IRB and by the Ministry of Health in Qatar.

Consent for publication

All authors consent to the publication of this manuscript.

Competing interests statement

I declare that the authors have no competing interests as defined by Nature Publishing Group, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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Data availability:

All underlying data associated with this study will be made available by the corresponding author upon reasonable request.

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Tables

Table 1. Demographic details for 719 women between the ages of 18 to 40 years, comparing normal subjects with women defined as having polycystic ovary syndrome by NIH criteria (free androgen index greater than 4.5 and/ or a total testosterone greater than 2.7nmol/l, with irregular menses).

	Control (N=641) Mean (SD)	PCOS (N=78) Mean (SD)	P-value[A]
Age (years)	29.54 (6.05)	27.78 (5.68)	0.015*
High Density lipoprotein (mmol/l)	1.57 (0.36)	1.32 (0.3)	<0.001*
Systolic blood pressure (mmHg)	104.71 (10.97)	108.69 (10.92)	0.003*
Diastolic blood pressure (mmHg)	68.83 (9.33)	72.64 (8.06)	0.001*
	Median (Range)	**Median (Range)**	**P-value[B]**
Weight (Kg)	65.4 (109.1)	78.8 (72.3)	<0.001*
Waist (cm)	77.0 (82.0)	88.0 (57.0)	<0.001*
Cholesterol (mmol/l)	4.7 (5.4)	4.6 (3.8)	0.143
Body mass index	25.9 (40.7)	30.5 (24.1)	<0.001*
HbA1c (%)	5.2 (9.4)	5.4 (6.6)	<0.001*
FAI	1.54 (3.87)	5.87 (15.43)	<0.001*
Insulin (µIU/ml)	8.0 (599.0)	13.0 (110.0)	<0.001*
Insulin resistance (HOMA-IR)	1.8 (263.8)	2.6 (32.1)	<0.001*
Low density lipoprotein (mmol/l)	2.8 (5.0)	2.9 (3.2)	0.652
Triglycerides	0.82 (3.70)	1.03 (2.61)	<0.001*
Testosterone (nmol/l)	1.1 (2.3)	1.7 (5.8)	<0.001*
C-reactive protein (mmol/l)	5.0 (51.0)	5.0 (36.0)	<0.001*
Vitamin -D (ng/ml)	14.0 (124.0)	14.0 (62.0)	0.971
Pulse wave Velocity (m/s)	9.3 (38.3)	9.5 (10.6)	0.076

*P-value < 0.05

A:Comparisons conducted using Independent T-test

B:Comparisons conducted using Mann-Whitney test

Table 2. Correlations between vitamin D and metabolic parameters within control (N=641) and PCOS (n=78) populations.

	Controls (n=641)		PCOS (n=78)	
	Pearson Correlation	P-value	Pearson Correlation	P-value
BMI kg/m2	0.001	0.982	-0.101	0.326
HOMA-IR	-0.022	0.584	0.014	0.89
Triglycerides	0.035	0.379	0.049	0.636
Insulin (µIU/ml)	-0.023	0.561	0.015	0.884
Glucose (mg/dl)	0.028	0.495	0.022	0.834
Testosterone) (nmol/l	0.01	0.798	-0.066	0.526
FAI	0.014	0.731	-0.027	0.794
CRP (mmol/dl)	0.032	0.448	-0.085	0.432
ALT (U/l)	0.012	0.757	-0.009	0.931