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ORIGINAL

Vitamin D has no significant associations with high sensitivity C reactive protein and tumor necrosis factor-alpha in adults with prediabetes

La vitamina D no tiene asociaciones significativas con la proteína C reactiva de alta sensibilidad y el factor de necrosis tumoral alfa en adultos con prediabetes

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Abstract

Objective: Both vitamin D deficiency (VDD) and meta-inflammation are common in patients with prediabetes. The aim of this study was to see the association of vitamin D with inflammatory markers: high-sensitivity C reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α) in adults with prediabetes.
Methods: This cross-sectional study included 115 newly detected adults with prediabetes [age (years): 36.37±10.06, m/f: 23/92, BMI (kg/m^2): 28.81±4.34]. Clinical information was collected and blood was taken in a fasting state to measure vitamin D by high-performance liquid chromatography, hs-CRP by turbidimetric/immunoturbidimetric, and TNF-α by ELISA method.
Results: About 46.09% had VDD (<20 ng/mL), 33.04% had vitamin D insufficiency (20-29.9 ng/mL) and 60.87% had high cardiovascular risk (hs-CRP ≥3.0 mg/L) and 92.17% had high inflammatory status (TNF-α ≥2.53 pg/mL). Both hs-CRP and TNF-α levels were statistically similar across the vitamin D status (NS for both). Regression analyses showed that both hs-CRP and TNF-α had no predictive associations with either vitamin D level or status in adults with prediabetes (NS for both).
Conclusion: Vitamin D had no significant association with hs-CRP and TNF-α in adults with prediabetes.

Key words: vitamin D, high sensitivity C reactive protein, tumor necrosis factor-alpha, prediabetes.

Resumen

Objetivo: Tanto la deficiencia de vitamina D (VDD) como la metainflamación son comunes en pacientes con prediabetes. El objetivo de este estudio fue ver la asociación de la vitamina D con los marcadores inflamatorios: proteína C reactiva de alta sensibilidad (hs-CRP) y factor de necrosis tumoral-alfa (TNF-α) en adultos con prediabetes.
Métodos: Este estudio transversal incluyó a 115 adultos recién detectados con prediabetes [edad (años): 36,37±10,06, m/f: 23/92, IMC (kg/m^2): 28,81±4,34]. Se recogió información clínica y se extrajo sangre en ayunas para medir la vitamina D por cromatografía líquida de alto rendimiento, la hs-CRP por turbidimetría/inmunoturbidimetría y el TNF-α por el método ELISA.
Resultados: Alrededor del 46,09% tenía VDD (<20 ng/mL), el 33,04% tenía insuficiencia de vitamina D (20-29,9 ng/mL) y el 60,87% tenía alto riesgo cardiovascular (hs-CRP ≥3,0 mg/L) y el 92,17% tenía un estado inflamatorio alto (TNF-α ≥2,53 pg/mL). Tanto los niveles de hs-CRP como de TNF-α no mostraron diferencias estadísticamente significativas según el estado de la vitamina D. Los análisis de regresión mostraron que tanto la hs-CRP como el TNF-α no tenían asociaciones predictivas con el nivel o el estado de la vitamina D en adultos con prediabetes (NS para ambos).
Conclusión: La vitamina D no tuvo una asociación significativa con la PCR-as y el TNF-α en adultos con prediabetes.

Palabras clave: vitamina D, proteína C reactiva de alta sensibilidad, factor de necrosis tumoral-alfa, prediabetes.

Introduction

The health care burden of diabetes mellitus (DM) is progressively increasing due to its rising prevalence throughout the whole world[1]. One of the strategies is to prevent its progression from its preceding stage-prediabetes. Both of the conditions are characterized by chronic low grade inflammation, β cell dysfunction and insulin resistance. Several circulating cytokines including tumor necrosis factor α (TNF-α) and C reactive protein (CRP) are found to be elevated in DM and prediabetes[2,3]. This meta-inflammation may contribute to insulin resistance in muscle and adipose tissue leading to the development of glucose intolerance, endothelial dysfunction, and ultimately atherosclerotic cardiovascular disease[4].

TNF-α is secreted from the monocyte-macrophage system as well as from adipose tissue. The expression in adipocytes contributes to insulin resistance via negative effects on insulin signaling and induction of lipolysis[5]. On the other hand, it also promotes the synthesis of CRP from the liver which is an acute phase protein and marker of inflammation. High-sensitivity CRP (hs-CRP) measures a very low level of inflammation and it is one of the best predictors of cardiovascular risk[6].

Recent studies also suggest that most of the cells of the immune system express receptors for vitamin D. This is hypothesized that vitamin D may act as an immune modulator and interfere with systemic inflammation and reduce insulin resistance[7]. Besides, vitamin D may play a role in insulin secretion from pancreatic β-cells[8]. Vitamin D deficiency (VDD) is very common even in areas with plenty of sunshine[9]. Its treatment is also easy and cost-effective. Although controversial, it was observed in several meta-analyses that vitamin D might prevent DM progression from prediabetes[10,11]. One of the possible mechanisms may be a reduction of inflammatory markers by vitamin D supplementation[12]. However, data regarding the association between vitamin D and inflammatory markers in adults with prediabetes are controversial. Moreover, there is very limited data from Bangladeshi population regarding their association in adults with prediabetes. The aim of this study was to see the association of vitamin D with hs-CRP and TNF-α in adults with prediabetes.

Methods

This cross-sectional study included 115 newly detected adults with prediabetes consecutively by convenient sampling from the department of Endocrinology of a University hospital over a period of one and half years (January, 2018 to June 2019). The sample size was calculated from the following formula: $n= [Z^2 \times p \times (1-p)] \div d^2$. Here, Z= 1.96 at a 95% confidence level, p= prevalence of VDD in prediabetes= 0.7325, and d= at 10% margin of error= 0.1.[13] The minimum number of samples to be studied was 76. As facilities permitted, 115 participants with prediabetes were included. Patients with a history of intake of vitamin D or calcium within 120 days of enrollment, and taking any medications that may alter vitamin D metabolism (glucocorticoids, oral contraceptives, anticonvulsants, anti-Koch, etc.) were excluded. Similarly, patients having known endocrine disorders (hyperthyroidism, hyperparathyroidism, Cushing syndrome, etc.) or malabsorption syndrome affecting vitamin D metabolism, pregnant and lactating mother, any acute or chronic disorders related to inflammation (history of fever, autoimmune disease, chronic heart failure, chronic kidney disease, chronic liver disease, malignancy, polycystic ovary syndrome, etc.) were also excluded. Prior to beginning, the approval of the study protocol and ethical clearance was taken from the institutional review board of the University. Informed written consent was taken from all the study participants.

Patients' relevant history and physical findings were collected in a semi-structured questionnaire. Venous blood was collected in a fasting state, centrifuged, and preserved in -200C until assay. Vitamin D (25-hydroxyvitamin D3) was measured by high-performance liquid chromatography, hs-CRP by turbidimetric/immunoturbidimetric method (Abbott Architect Plus), and TNF-α by ELISA (DRG Instruments GmbH, Germany) method. All the collected data were immediately verified by a senior author, and there were no missing data.

Prediabetes was diagnosed according to American Diabetes Association, 2018 criteria for non-pregnant adults[14]. Vitamin D status was classified by the Endocrine Society's clinical practice guideline, 2011 into sufficiency, insufficiency, and deficiency by vitamin D levels of 30 and 20 ng/mL respectively[15]. The hs-CRP status was categorized into low, moderate, and high cardiovascular risk with the cut-off value of one and three mg/L respectively[16]. The cut-off to define inflammatory status by TNF-α was 2.53 pg/mL.[17] General and central obesity were categorized according to Asian criteria.[18] Smoking status and physical activity level were defined as per the Mayo clinic and the international physical activity questionnaire respectively.[19,20]

Data were analyzed by SPSS software version 22.0. Qualitative data were expressed in frequency (percentages). The distribution of quantitative variables was checked by the Shapiro-Wilk test. Normally distributed data were expressed in mean±standard deviation (SD) and skewed data were expressed in median (inter-quartile range, IQR). Comparison among the different statuses of vitamin D was done by Pearson's chi-square or One-way ANOVA or Kruskal Wallis one-way ANOVA test as appropriate. Correlations of vitamin D with hs-CRP and TNF-α were done by Spearman's correlation test. Multivariate linear regression analysis was done to see the predictive association of hs-CRP

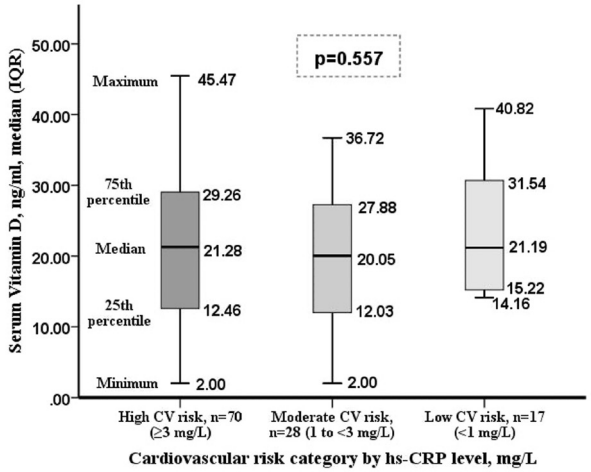
and TNF-α with vitamin D. At last, univariate multinomial logistic regression analysis was done to see the predictive associations of hs-CRP and TNF-α categories with vitamin D status (deficiency vs. not deficiency).

Results

The characteristics of the study population according to vitamin D status are shown in **table I**. Around half of the patients with prediabetes had VDD and one-third had vitamin D insufficiency according to Endocrine Society's criteria. All the baseline characteristics as well as hs-CRP and TNF-α were statistically similar among the vitamin D status (NS for all).

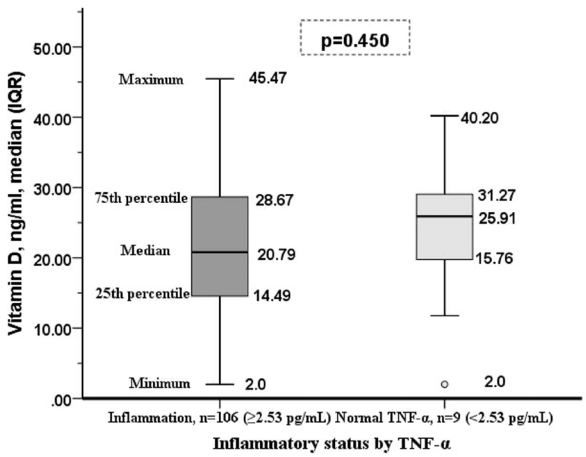
Around 60.87% of the study participants had high CV risk according to hs-CRP levels (cut-off of 3.0 mg/L) and 92.17% had high inflammatory status by TNF-α levels (cut-off of 2.53 pg/mL). However, Vitamin D levels were statistically similar according to both categories (NS for both) (**Figure 1** and **Figure 2**).

Figure 1: Serum vitamin D levels according to cardiovascular risk category by hs-CRP levels.



Kruskal-Wallis one way ANOVA test was done

Figure 2: Serum vitamin D levels according to TNF-α category.



Mann-Whitney U test was done

Table I: Characteristics of the study population with vitamin D status (N=115).

Variables	Vitamin D status			p
	Deficiency	Insufficiency	Sufficiency	
Frequency (%)	53 (46.09)	38 (33.04)	24 (20.87)	
Age, years	37.32±10.22	35.03±9.38	36.42±10.89	0.566
Female sex	38 (71.7)	32 (84.2)	22 (91.7)	0.106
Smoking status				
Current smoker	3 (5.7)	2 (5.3)	1 (4.2)	0.966
Past smoker	8 (15.1)	4 (10.5)	2 (8.3)	
Nonsmoker	42 (79.2)	32 (84.2)	21 (87.5)	
Physical activity level				
Low	24 (45.3)	15 (39.5)	17 (70.8)	0.061
Moderate	27 (50.9)	23 (60.5)	7 (29.2)	
High	2 (3.8)	0 (0.0)	0 (0.0)	
Family history of DM	38 (71.7)	19 (50.0)	13 (54.2)	0.080
Daily sunlight exposure between 11 am to 1 pm (minutes)	0.0 (0.0, 27.5)	10.0 (0.0, 30.0)	0.0 (0.0, 13.75)	0.183
Sunlight exposure of body surface area ≥20%	11 (20.8)	10 (26.3)	5 (20.8)	0.840
Use of sunscreen	2 (3.8)	2 (5.3)	1 (4.2)	1.00
Vitamin D containing food intake at least 1 serving/day				
Egg >3 days/week	17 (32.1)	9 (23.7)	4 (16.7)	0.317
Large fish >3 days/week	23 (43.4)	20 (52.6)	9 (37.5)	0.492
BMI category				
Optimal (18.5 - 22.9)	3 (5.7)	2 (5.3)	3 (12.5)	0.405
Overweight (23.0 - 24.9)	10 (18.9)	3 (7.9)	2 (8.3)	
Obese (≥25)	40 (75.5)	33 (86.8)	19 (79.2)	
Centrally obese (M ≥90, F ≥80)	48 (90.6)	36 (94.7)	22 (91.7)	0.802
Systolic BP, mm-Hg	118.49±14.40	115.32±13.87	120.13±17.07	0.415
Diastolic BP, mm-Hg	80.09±10.08	79.29±8.85	82.25±11.41	0.517
hs-CRP, mg/L	4.17 (1.73, 9.60)	3.23 (1.97, 6.53)	3.65 (1.43, 6.68)	0.752
TNF-α, pg/mL	16.40 (8.58, 42.25)	12.75 (7.10, 30.72)	8.94 (6.49, 22.47)	0.105

Data were expressed in mean±SD or median (IQR) or frequency (%) as appropriate
One-way ANOVA or Kruskal-Wallis one-way ANOVA or chi-square test was done as appropriate

Vitamin D significantly and inversely correlated with TNF-α [r= -0.204, p=0.029]. However, in multivariate linear regression analysis, none of the independent variables including hs-CRP and TNF-α had a predictive association with vitamin D (NS for both) (**Table II**).

Univariate logistic regression analysis also showed insignificant predictive associations of hs-CRP and TNF-α categories with vitamin D status (NS for both) (**Table III**).

Discussion

This cross-sectional study included 115 adults with prediabetes to see the association of vitamin D with inflammatory markers: hs-CRP and TNF-α. This study did not find any significant association between vitamin D with either hs-CRP or TNF-α in adults with prediabetes.

We found that around half of the adults with prediabetes had VDD. The association of low vitamin D levels with prediabetes was found in several large population-based studies[21,22]. Inadequate sunlight exposure time, exposure of the body surface area to sunlight along with low intake of vitamin D containing food intake might be the possible causes of VDD in our study population. Similarly, we also found more than 90% of patients had high inflammatory status and more than 60% had high CV risk. Although, the role of low-grade inflammation as a precipitating factor of the development of DM is questionable, their association is almost a constant finding[23].

We did not find a significant association of both hs-CRP and TNF-α with vitamin D among adults with prediabetes. Zhang et al. (2017) also did not find any significant association between vitamin D and TNF-α in adults with prediabetes. However, they found a significant inverse association between vitamin D with hs-CRP[24]. On the other hand, Beilfuss et al. (2017) did not find a significant change in hs-CRP levels by vitamin D supplementation over 5 years[25]. The conflicting result indicates that several confounding factors may be responsible for the alteration of these inflammatory markers or that the association has no significant importance[25].

Although observational studies found significant associations of vitamin D with inflammation, randomized trials mostly failed to show improvement in inflammatory status. One author suggested that this might be due to the fact that VDD is a marker of ill health rather than the cause of inflammation. Rather, inflammation may cause VDD[26]. Another possibility is that the association is found in diseases with higher inflammatory status rather than a relatively low-grade inflammatory status associated with prediabetes or in patients with low vitamin D levels[27,28].

This study has several limitations. The sample size was small and we could not take the control population.

In conclusion, despite a higher proportion of VDD and inflammatory status, there was no significant association of vitamin D with inflammation in adults with prediabetes.

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Ethical approval: The ethical clearance of the study protocol was taken from the institutional review board of Bangabandhu Sheikh Mujib Medical University (Protocol no: BSMMU/2018/4827, date: 06/05/2018)

Conflict of interest

The authors declare no conflict of interest

Table II: Correlation and linear regression analysis of vitamin D as dependent variable.

Determinants of 'r'	Spearman's correlation		Multivariate linear regression	
	r	p	β	p*
hs-CRP, mg/L	-0.062	0.510	-0.078	0.435
TNF-α, pg/mL	-0.204	**0.029**	-0.178	0.068
Constant			B=15.997	0.188

r, correlation co-efficient; β, linear regression co-efficient
*adjusted for age, BMI, WC, systolic and diastolic blood pressure

Table III: Multinomial logistic regression analysis of vitamin D status as dependent variable.

Independent variable	Groups	Univariate regression analysis	
		Odds ratio (95% CI)	p
hs-CRP	Low risk (<1 mg/L)	1	
	Moderate risk (1 to <3 mg/L)	1.203 (0.411, 3.522)	0.736
	High risk (≥3 mg/L)	1.429 (0.423, 4.826)	0.566
	Constant	B= -0.357	0.469
TNF-α	Normal (<2.53 pg/mL)	1	
	Inflammatory status (≥2.53 pg/mL)	1.786 (0.424, 1.517)	0.429
	Constant	B= -0.693	0.327

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