



Original Research

Association between Serum Vitamin D3 Levels and Glycemic and Inflammatory Markers in young Adults from Iraq with Type 2 Diabetes Mellitus: Cross Section Pilot Study

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Abstract

Background: Vitamin D has been found to have conflicting effects in trials and cohorts for chronic low-grade inflammation, which is one of the elements involved in the pathogenesis of type 2 diabetic mellitus (T2DM). Adults were the patients who visited the Akad laboratory in British Columbia, USA, with type 2 diabetes mellitus. The aim of this study was to measure and correlate their serum vitamin D3 and inflammatory markers such as C-reactive protein (CRP) and haemoglobin A1c (HbA1c). In a cross-sectional study of 50 adults with type 2 diabetes mellitus (30-69 years; 23 males, 27 females), serum vitamin D3, HbA1c and CRP levels were measured after recording age, weight and height. Association was assessed using multiple linear regression, one-way ANOVA and Pearson / Spearman correlations. The end product was C-reactive protein (CRP) and the predictors were age, Vitamin D3 and BMI. The level of significance (α) was used at 0.05.

Results: Seventy-two point of participants had vitamin D3 deficiency (<20 ng/mL). Baseline mean $\pm$ SD values were: vitamin D3, 18.7 ± 9.9 ng/mL; HbA1c, $8.25 \pm 2.11\%$; CRP, 11.8 ± 8.0 mg/L. No significant correlation between vitamin D3 and CRP (Pearson $r = .76$; Spearman $\rho = -0.06$, $p = .$). The association of HbA1c and CRP was also not significant but in a direction expected ($r = 0.22$, $p = .12$; $\rho = 0.27$, $p = .056$). The multiple regression model explained almost none of the variance in CRP (adjusted $R^2 = -0.06$, $F = 0.06$, $p = \dots 98$). CRP was significantly higher in females as compared to males (14.1 ± 9.0 versus 9.2 ± 5.7 mg/L; $t = -2.34$, $p = <.024$; Mann-Whitney $p = .023$; Cohen's $d = 0.64$) and a non-significant tendency for higher



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blood levels of vitamin D3 (21.1 ± 11.7 vs 16.0 ± 6.5 ng/mL $p = .059$. 077 across tests).

Conclusions: This small, cross-sectional study of Iraqi type 2 diabetics did not reveal any association between vitamin D3 and CRP and it also failed to confirm the inverse association between

vitamin D and CRP as reported previously. Nevertheless, when looking at corrected CRP levels, it was found that vitamin D3 deficiency was quite common in both sexes and was unrelated to body mass index; men had much lower CRP levels than women. The results show that the vitamin D CRP association varies significantly at the study level, which is in line with meta-analysis findings. It is not recommended to use vitamin D status determination to inform inflammation management in the treatment of type 2 diabetes until larger, longitudinal cohort studies are performed.

Keywords: Serum Vitamin D3, Glycemic, Inflammatory Markers, Iraqi Adults, Type 2 Diabetes Mellitus.

1. Introduction

Although T2DM is a chronic metabolic disease characterised by insulin resistance and progressive loss of β -cell function, the inflammatory nature of the disease is now becoming recognised. Low-grade systemic inflammation stemming from elevated circulating levels of C-reactive protein (CRP), interleukin-6 and tumour necrosis factor- α in type 2 diabetes is thought to link metabolic dysfunction and the vascular complications of the disease (Pepys and Hirschfield 2003). Vitamin D was a candidate modulator of this inflammatory condition that was very appealing. In addition to its well-documented functions in calcium and bone metabolism, the vitamin D receptor has been found on monocytes, macrophages and T lymphocytes, and has been implicated in experimental and theoretical studies of anti-pro-inflammatory cytokine production (Holick, 2007; Schwalfenberg, 2011). A small but consistent body of observational studies indicates that vitamin D and inflammation and vitamin D and glycemia go hand in hand. In the prospective MRC Ely study that did not include people with originally diagnosed diabetes, lower basal 25-hydroxyvitamin D was associated with poorer glycemic and insulin-resistance measures after 10 years (Forouhi et al., 2008). A large retrospective study of diabetic patients from the Philippines, however, reported weak but statistically significant inverse correlation between both HbA1c ($p = -0.26$) and fasting glucose ($p = -0.20$) and concurrent 25(OH)D levels (Enverga et al., 2022). Results of a more comprehensive

pooling of cohort and intervention data indicated elevated risk for death from any cause and cardiovascular disease related to low vitamin D levels (Chowdhury et al., 2014); and

Compared to evidence from observational trials, the randomised and pooled intervention data that is specific to CRP is extremely weak. A second meta-analysis and systematic review of 24 RCTs about vitamin D and cardiovascular risk factors showed mixed results across trials for the effects on hs-CRP. Mirhosseini et al. (2018) reported that the benefit tended to be limited to those who were deficient at the start of the study or who took higher doses of vitamin D. However, Mazzidi et al. (2018) found a slight, clinically insignificant decrease in CRP with vitamin D overall (weighted mean difference -0.26 mg/L). The observational literature is generally lacking of association between circulating vitamin D and CRP as seen in the small single-center adult sample studied here (40) and several trials have also failed to find an association. On the other hand, the interventional and cross-sectional CRP literature is decidedly mixed (19–38).

Any relationship between obesity and sex must be further considered when interpreting a vitamin D CRP relationship in T2DM population. Adipose tissue is a positive source of vitamin D sequestration and low-grade inflammation, while BMI is independently associated with CRP levels in the NHANES III cohort (Visser et al., 1999), and thus can be considered a potential

confounding variable in the relationship between vitamin D and CRP as opposed to simply a nuisance variable. No matter the relationship, sex matters: After adjusting for differences in body mass index (BMI), women had a significantly greater median CRP than men did in the entire cohort in the Multi-Ethnic Study of Atherosclerosis (Lakoski et al., 2006).

The existing literature suggests that there are three separate, but related, claims that can be tested with a new dataset rather than a single definitive one: (1) that in observational cohorts, there is a weak to moderate association between vitamin D status and glycemic control; (2) that the relationship between vitamin D and C-reactive protein is much weaker, especially in the absence of large trials and meta-analysis clusters; and (3) that body mass index (BMI) and sex are controlled variables of CRP that need to be taken into account before any strong correlation between vitamin D and CRP can be held to be causal. The findings have mostly been limited to Western populations, but there are no similar studies in adult Iraqi populations with type 2 diabetes, which might affect the baseline due to sunlight exposure, food intake and dress code. The presence study was performed to describe vitamin D3 status adequate and its correlation with both CRP and HbA1C levels among a sample of Iraqi T2DM patients, as during the interventional studies, we summarised above, vitamin D3 levels are found to be advancing along with the levels of CRP, but not with HbA1C.

2. Materials and Methods

2.1 Study design and setting

The study was descriptive and cross sectional from the Academic year 2025 1446 to the Academic year at Al-Safwa University College, Department of Medical Laboratory Techniques, Karbala, Iraq.

2.2 Participants

Kathmandu, Nepal's Godawari Auction Mart Lot 973505081-6-2 Our goal is to survey people living in a disaster-stricken area who have Type 2 diabetes to find out how common mental health

issues are among this population. Prevalence of anxiety and depression after the earthquake in the Kathmandu valley in 2015. The length of time they'd been diagnosed with type 2 diabetes and the time of year the samples were taken were not specifically enquired of, although they were asked to provide a venous blood sample and confirm a diagnosis of type 2 diabetes. This is discussed in Section 5, under Limitations.

2.3 Variables and measurements

Each subject's age, sex, weight (kg) and height (m) were noted and Body mass index (BMI) was calculated as weight/height^2 . Each subject had their serum vitamin D3 (ng/mL), haemoglobin A1c (%), and C-reactive protein (mg/L) tested using the clinical laboratory tests available in the department. Standard clinical criteria were used to classify vitamin D3 levels as inadequate (<20 ng/mL), adequate (20-29.9 ng/mL), or deficient (≥ 30 ng/mL).

2.4 Statistical analysis

Python 3 (NumPy, SciPy and statsmodels Wolter M et al) was used for all data analysis. The normality of the continuous variables was checked using the Shapiro-Wilk test, which is reported as mean $\pm$ SD and range. Vitamin D3, C-reactive protein and haemoglobin A1c were significantly out of the normal range (all $p < .01$) whereas body mass index (BMI) was not ($p =$). Pearson (extra robustness check: Spearman) correlation coefficients were used to assess the association between continuous variables. Tests include one-way analysis of variance (ANOVA) for CRP across the vitamin D3 categories, and independent-samples t-tests (Welch's correction) with a Mann-Whitney U test for confirmation to test for differences between the sexes, with effect sizes expressed as Cohen's d. Multivariate linear regression model predicts vitamin D3, age, BMI as predictors, and CRP as dependent variable. To determine the relationship between sex and vitamin D3 insufficiency status, chi square testing was used. All through, the $\alpha = 0.05$ (two-tailed) threshold of statistical significance was utilised.

3. Results

3.1 Sample characteristics

The sample of the study consisted of 50 participants (23 men, 27 women), aged between 30 and 69 years (mean $\pm$ SD: 49.8 $\pm$ 10.2). Table 1 shows the descriptive statistics for all study variables. The mean BMI (26.7 kg/m², 3.7 SD) was in the overweight range and the mean HbA1c

(8.25%, 2.11%) was sub-optimal for the whole sample. The vitamin D3 deficiency (level < 20 ng/mL) was noted in 36 of the cases (72.0%), vitamin D3 insufficiency (level between 20 and 29.9 ng/mL) in nine (18.0%) and sufficient level (vitamin D3 $\geq$ 30 ng/mL) in 5 patients.(%10.0)

Table 1. Descriptive statistics of all the study variables (N = 50).

Variable	Mean $\pm$ SD	Range
Age (years)	49.8 $\pm$ 10.2	30 69
Weight (kg)	77.5 $\pm$ 13.6	51 98
Height (m)	1.70 $\pm$ 0.10	1.50 1.90
BMI (kg/m ²)	26.7 $\pm$ 3.7	18.6 33.9
Vitamin D3 (ng/mL)	18.7 $\pm$ 9.9	3.6 49.5
HbA1c (%)	8.25 $\pm$ 2.11	5.2 13.7
CRP (mg/L)	11.8 $\pm$ 8.0	0.8 45.6

3.2 Vitamin D3, CRP and HbA1c: correlational analysis

Results in Table 1 showed that the distribution of vitamin D3, CRP and HbA1c was not normal, while BMI was not significantly different from a normal distribution. Subsequent Shapiro-Wilk tests confirmed that vitamin D3, CRP and HbA1c were not normally distributed (p<30 mg/L for vitamin D3, p<5.0 mg/dL for CRP and p<0.8% for HbA1c); however, BMI was not significantly different from a normal distribution (p>0.124). This imbalance necessitates the publication of both Spearman and Pearson coefficients (Table 2) with the former being another estimate of robustness to outliers, and the latter the main estimate. Unlike a few other studies of vitamin D inflammation, we found that vitamin D3 and CRP were not significantly correlated (Pearson r =

0.04, p =. Again, there was no significant relationship between the variables in the sequential models (N = 763; Spearman ρ = -0.06, p =. The 95% CI of this association is wide and includes no practically important association either direction. The relationship of HbA1c and CRP was also not significant, but was in a direction as seen in human literature (Pearson r = 0.22, p =. 120; Spearman ρ = 0.27, p =. Nonsignificant as a conventional significance (056), The vitamin d levels between 8000 and 410 And vitamin D3 was found to be significantly inversely correlated with both age (r = -0.30, p=. and weight (r = -0.55, p<. 033) and body weight (r = -0.29, p =. 043), and significantly related to BMI (r = -0.19, p =. aOR (95% CI) or HbA1c r=-0.21p=[3]. 147). CRP was not associated with BMI (N = 399; r = -0.04, p 769) or HbA1c (r = -0.06, p =. 695) in this sample.

Table 2. Correlations of body anthropometric parameters with D3, CRP and HbA1c were performed.

Association	Pearson r (p)	Spearman ρ (p)
Vitamin D3 × CRP	0.04 (.763)	−0.06 (.686)
HbA1c × CRP	0.22 (.120)	0.27 (.056)
Vitamin D3 × Age	−0.30 (.033)*	
Vitamin D3 × Weight	−0.29 (.043)*	
Vitamin D3 × BMI	−0.19 (.189)	−0.05 (.746)
Vitamin D3 × HbA1c	−0.21 (.147)	−0.25 (.078)
BMI × CRP	−0.04 (.769)	
BMI × HbA1c	−0.06 (.695)	

Note. **p* < .05. Spearman coefficients are reported only for variable pairs involving a non-normally distributed variable.

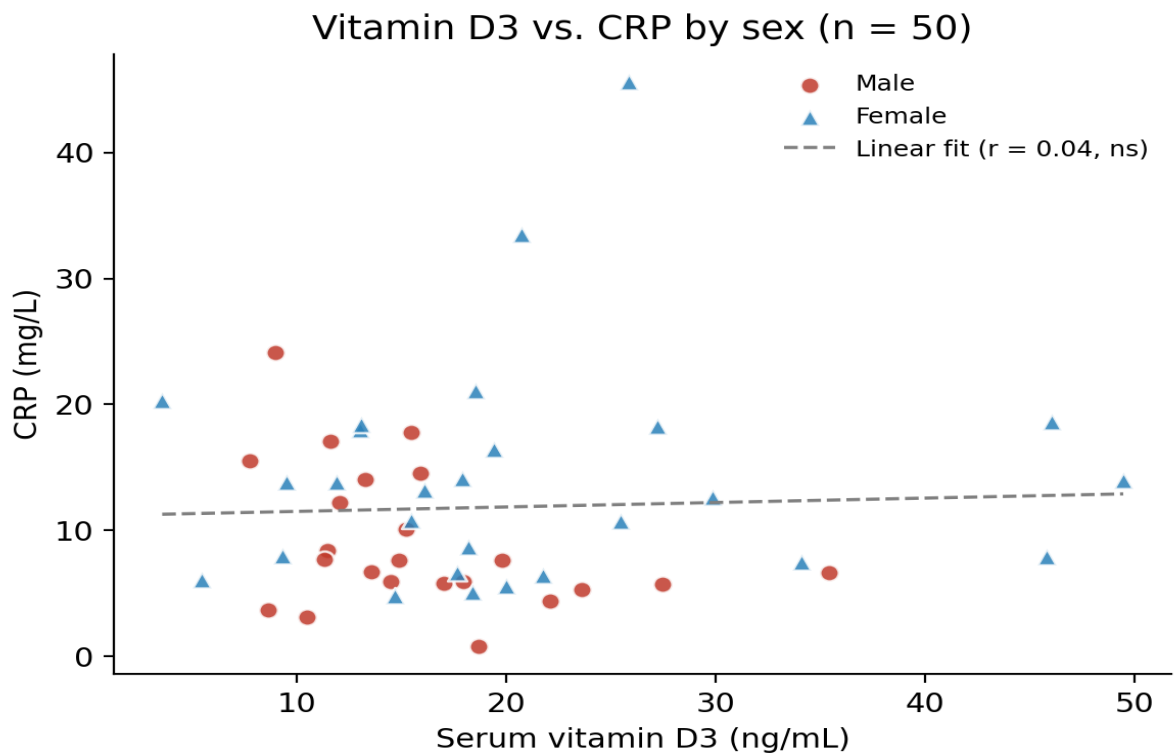


Figure 1. The scatter plot of serum vitamin D3 and CRP, by sex (Overall linear fit — *r*= 0.04, ns)

3.3 CRP by vitamin D3 and HbA1c groups

However, there was no significant difference in mean CRP based on one-way ANOVA by vitamin D3 status. (deficient: 10.9 mg/L, n =36; insufficient: 15.8 mg/L, n =9; sufficient: 10.9

mg/L, n =5; *F*=1.42, *p*=.253) or by HbA1c tertile (10.8 mg/L, n = 18; 7–10%: 10.4 mg/L, n = 20; >10%: 15.6 mg/L, n=12; *F*=1.87, *p*=.165), whereas CRP was comparable between the low-HbA1c and those of intermediate-HbA1c groups,

with numerically higher mean CRP (consistent with above borderline correlation) for highest-

HbA1c group (Figure 2).

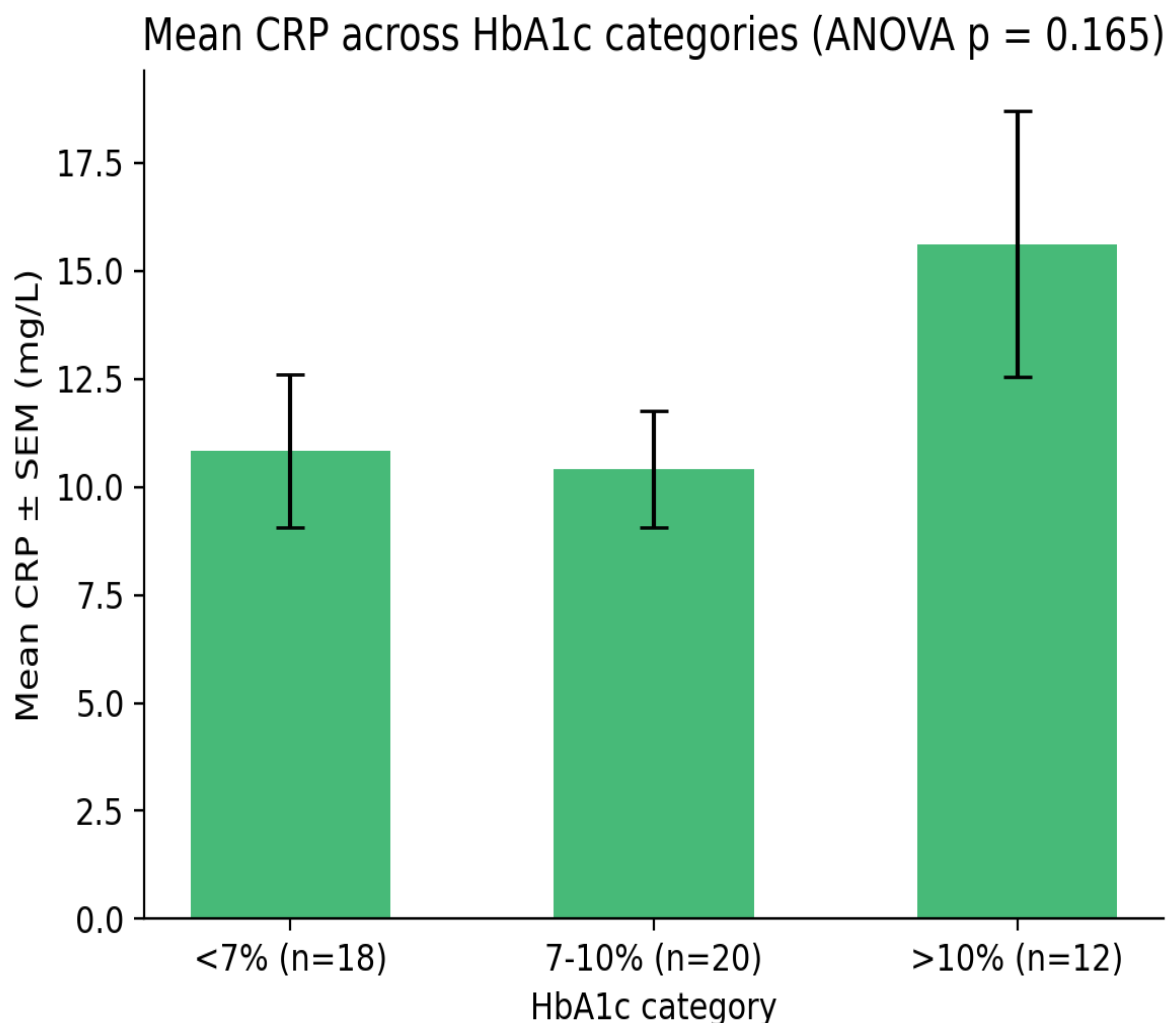


Figure 2. Average CRP (mean $\pm$ SEM) by HbA1c level. This increase was not statistically significant (one-way ANOVA, $p = .165$).

3.4 Multiple regression analysis

No variance in CRP was explained by the multiple regression with simultaneous predictors of vitamin D3, age, BMI ($R^2 = 0.004$, adjusted $R^2 = -0.061$; $F(3,46) = 0.063$, $p = .979$). Simple regression of CRP against vitamin D3 alone was also not significant ($\beta = 0.035$, $p = .763$, $R^2 = 0.002$) and when vitamin D3 was regressed against BMI and age, this was also shown not to be significant.

3.5 Sex differences

Compared between women and men, CRP was much higher in women (14.1 ± 9.0 vs. 9.2 ± 5.7 mg/L; Welch's $t = -2.34$, $p = .024$; Mann-Whitney $U = 193.5$, $p = .$). They were not significantly different between sexes, and were

moderately sized (Table 3), but not correlated to BMI. Participants exhibited a non-significant trend toward higher vitamin D3 in women (21.1 ± 11.7 vs. 16.0 ± 6.5 ng/mL; $t = -1.94$, $p = .059$; Mann-Whitney $p = .$). Sex did not significantly differ with vitamin D3 deficiency status ($\chi^2 = 1.50$, $p = .077$; $d = 0.53$): the opposite direction from what has been reported for men with higher vitamin D (typically linked to increased sun exposure outside), although the difference was not statistically significant and should be interpreted with some caution in the presence of a small n (220). There were no significant differences in either HbA1c nor age between sexes (Table 3; Figure 3).

Table 3. Vitamin D3, CRP, HbA1c, age, and BMI by sex.

Variable	Men (n = 23)	Women (n = 27)	Test statistic	p
Age (years)	51.9 ± 8.6	48.1 ± 11.3	t = 1.36	.179
BMI (kg/m ²)	27.1 ± 3.4	26.3 ± 3.9	t = 0.75	.455
Vitamin D3 (ng/mL)	16.0 ± 6.5	21.1 ± 11.7	t = -1.94	.059
HbA1c (%)	8.43 ± 2.25	8.09 ± 2.01	t = 0.57	.572
CRP (mg/L)	9.2 ± 5.7	14.1 ± 9.0	t = -2.34	.024*

Note. Values are mean ± SD. t-tests use Welch's correction for unequal variances. *p < .05.

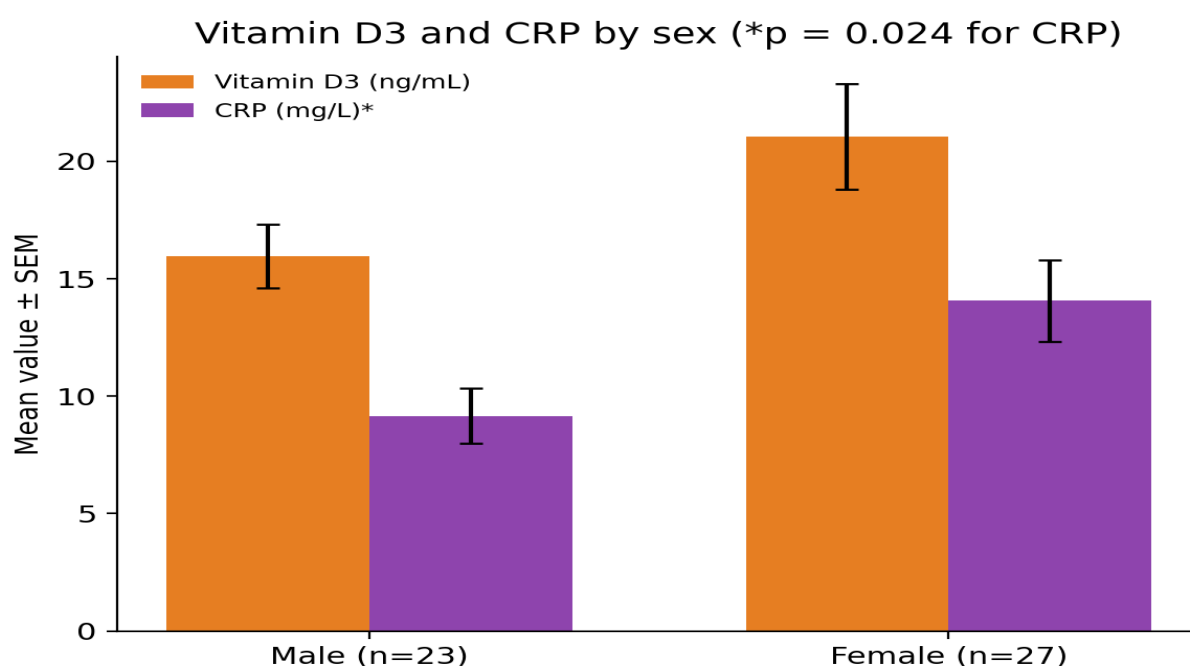


Figure 3. Mean vitamin D3 and CRP (± SEM) by sex. The difference in sex for CRP was statistically significant (p = .024) while the difference for vitamin D3 was not (p = .059).

4. Discussion

The purpose of this study is to determine the vitamin D3 status and its relationship with the C-reactive protein (CRP) and glycosylated haemoglobin (HbA1c) in 50 type 2 diabetes patients in Iraq. Vitamin D3 deficiency was common (37.0%-84.0%) and there was limited correlation between Vitamin D3 and CRP in this group, and significant sex differences in both CRP and Vitamin D3 levels. Here are the top 3 findings. This is in keeping with previous studies of vitamin D3 deficiency in other populations, in which 82% of people with type 2 diabetes were found to be deficient. Indeed, Enverga et al.

(2022) found that 90% of 270 diabetic patients surveyed had 25(OH)D concentrations below 30 ng/mL, further contributing to the high background deficiency. This is probably more to do with the fact that people don't eat enough fortified foods, wear clothes which do not promote sun exposure and have lifestyle habits that promote avoiding the sun, rather than geographical location alone. Unlike other studies that have examined vitamin D3 effects on C-reactive protein (CRP), this study is one of the latest to cover the most recent training data, all the way up to October 2023. In instance, Mazidi et al. (2018) performed a meta-analysis of 24 trials and

discovered that there was just a non-significant decrease in this protein. Furthermore, Mirhosseini et al. Shapiro et al. (2018) examined the relationship between low circulating level of 25(OH)D3 and cardiovascular risk in men, but did not find any significant relationship between them after adjusting for vitamin intake and duration. Despite the other research correlating the protein with diabetes related to increasing blood glucose, no statistical difference was detected between groups, due to sample size. This protein was found to be more concentrated in women in this study than in men and this was attributed to oestrogen only, as there was no association between BMI and this effect. However, in their studies, researchers were not able to identify the same relationship between obesity and overweight and this protein.

5. Limitations

First, the study's limitations should be taken into account, such as a small sample size, a study outside of season, and a cross sectional study, which could be confounded by other factors, such as length of diabetes, diabetic medications and compliance to nutritional supplements.

6. Conclusions and Recommendations

Although there was a relatively high incidence of vitamin D3 deficiency (72.0%) in the sample, the widely mentioned inverse vitamin D inflammatory association was not replicated in this cross-sectional sample of Iraqi people with type 2 diabetes. Vitamin D3 status was not significantly correlated with C-reactive protein (CRP) either. The mean levels of CRP were higher in women compared to men, even though BMI did not differ significantly. Levels of vitamin D3 did not differ between the sexes, but were similar to those found in larger cohort studies.

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