Research Article

High-dose supplementation of vitamin D affects measures of systemic inflammation: reductions in High-Sensitivity C-Reactive Protein level and Neutrophil to lymphocyte ratio (NLR) distribution†

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Abstract

Background: The prevalence of Vitamin D deficiency is increasing worldwide, which has be shown to be associated with increased risk of cardiovascular disease (CVD), autoimmune disease and metabolic syndrome. These conditions are also associated with a heightened state of inflammation. The aim of the current study was to evaluate the effect of vitamin D supplementation on serum C - reactive protein (CRP) level and Neutrophil‐to‐lymphocyte ratio (NLR) distribution in a large cohort of adolescent girls.

Methods: 580 adolescent girls were recruited follow by evaluation of CRP and hematological parameters before and after supplementation with vitamin D supplements as 9 of 50000 IU cholecalciferol capsules for 3 months taken at weekly intervals.

Results: At baseline, serum hs-CRP level was 0.9 (95%CI: 0.5-1.8), while this value after intervention was reduced to 0.8 (95%CI: 0.3-1.6; P = 0.007). Similar results were also detected for NLR (e.g., NLP level was 1.66±0.72 and 1.53±0.67, P = 0.002, before and after therapy with compliance rate of >95.2%). Moreover we found an association between hs-CRP and BMI, triglyceride, white blood cell count and lymphocytes. Interestingly we observed a significant reduction in neutrophil count and CRP level after high dose vitamin D supplementation.

Conclusion: Our findings showed that the high-dose supplementation of vitamin D affects measures of systemic inflammation: reductions in High-Sensitivity C-Reactive Protein level and Neutrophil‐to‐lymphocyte ratio (NLR) distribution.

Key words: vitamin D; supplementation; NLR; CRP.

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Introduction

Vitamin D deficiency defined as a serum 25(OH)D (25- hydroxy vitamin D) <20 ng/mL or <50 nmol/L (1) is a worldwide problem with a high prevalence, reaching 75% in some populations (2). This prevalence is also high in the Iranian population (3) . In recent studies the prevalence of vitamin D deficiency in Iranian adolescents was reported to be 79- 81.3 %(4). Though, a normal serum level of 25-hydroxy vitamin D is important for the skeletal system, Vitamin D deficiency is also associated with cardiovascular risk factors, and there is recent evidence for an association between vitamin D deficiency and cardiovascular disease (CVD). But there are few randomized control trials (RCT) to determine the effects of vitamin D supplementation on CVD (5). Previous studies have shown that vitamin D deficiency can be associated with autoimmune disease and metabolic syndrome and that these conditions are also associated with a heightened state of inflammation (6). Some studies have shown that C - reactive protein (CRP) can predict CVD (7-9). The Centers for Disease Control and Prevention and the American Heart Association (AHA) has suggested that serum hs-CRP can be used as part of a general assessment of cardiovascular risk(10). The Canadian Cardiovascular Society also recommended serum hs-CRP level in the assessment of patients with "intermediate risk," which defined as the expectancy risk of a cardiovascular event over the 10 years 10%- 20% (11). American Heart Association–AHA has recommended that assessment of hs-CRP levels is cost effective for all patients with intermediate risk (that mentioned above) (12). So, it is possible to restrain CVD with reducing CRP. Neutrophil-to-lymphocyte ratio (NLR) is an inexpensive and easily measured marker for determination of inflammation. Previous studies have shown that neutrophil-to-lymphocyte ratio associated with diseases that have inflammatory pathogenesis like, cardiovascular disease, chronic disease and malignancy (13-15). There is evidence for reducing inflammation by using

of vitamin D (16, 17) and it is possible that by decreasing CRP and NLR using vitamin D supplements it may be possible to reduce CVD risk. Recently, some clinical trials have investigated the effects of vitamin D supplementation on serum CRP level but to the best of our knowledge not on NLR. Furthermore, the studies on the effects of supplementation on CRP were undertaken in adult populations. Therefore, as CVD prevention should be started early in a population, we have now conducted this RCT to evaluate the effect of vitamin D supplementation on serum C - reactive protein level and NLR in adolescent girls.

Methods

Participants. This randomized, clinical study was performed in Mashhad, Iran, during January 2015 to April 2015. The total population in our RCT was 580 healthy girls aged between 12 to 18 years. We did not include subjects with any past medical history of hypertension, diabetes or any diagnosed chronic diseases like cardiovascular diseases or gastrointestinal diseases, or that were taking any drugs, or supplements. We also excluded those with any history of hypersensitivity to vitamin D or any facture of the formulation and pregnant or lactating girls. The Ethical Committee of Mashhad University of Medical Sciences approved the study, and informed written consent was received for all participants.

Study design. At study baseline, blood samples were taken and data on blood samples, height, weight, any diseases and medications were recorded. All participants received vitamin D supplements as 9 of 50000 IU cholecalciferol capsules for 3 months taken at weekly intervals. After 3 months blood samples were taken and data on height, weight, any diseases and medications were recorded. Vitamin D supplements were provided by Zahravi Pharmaceutical Company (Tabriz, Iran). We have verified vitamin D pearls by an outside laboratory and the pearls contain 50000 IU of cholecalciferol. We asked participants not to change their usual

physical activity or routine regimen and diets during the study and not to take any supplements other than the supplement that provided to them by the research fellow. We assessed and monitored participants' compliance with the consumption of vitamin D supplements every 10 days through phone or direct interviews.

Assessment of variables. General demographic data including age, any drug or supplement use, chronic diseases were collected as previously described (18). Waist circumference (WC) and systolic and diastolic blood pressure were measured as previously described (19). Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. A full fasted lipid profile was obtained for each subject. Cell blood counts, fasting blood glucose (FBG) concentrations, liver enzymes, serum lipids and 25(OH) vitamin D were measured by methods that have been previously described(20-22)

Statistical analysis. We used SPSS software version 15.0 (SPSS Inc, Chicago, IL, USA) for all statistical analyses. We categorized individuals by quartiles of hs-CRP. We used one-way analysis of variance to examine the significant differences in continuous variables across quartile categories of hs-CRP. Pearson correlation was done for all variables. Multinomial logistic regression was used to investigate the association between hs-CRP and the other variables and we adjusted all variables for age. A Wilcoxon test was used for comparing serum hs-CRP before and after intervention because of hs-CRP was non-normally distributed. For normally distributed variable paired sample t-test was used. We also use bivariate correlation for assessment of correlation of our measured factors with hs-CRP. A P-value < 0.05 was considered statistically significant.

Results:

The mean (±SD) age, weight and BMI of the participants were 14.5±1.5 y, 52.8±12.0 Kg, and 21.2±4.3 Kg/m^2, respectively (Table 1). The mean (±SD) of serum vitamin D level at baseline was 9.4±8.8 nanograms per milliliter (ng/mL) and after high dose vitamin D supplementation it increases to 36.4±15.4 (ng/mL) (P< 0.001). At baseline, serum hs-CRP, Median (IQ) was 0.9 (0.5-1.8) in adolescent girls and after the high-dose supplementation it fell to 0.8 (0.3-1.6) (P = 0.007) (Table 4). NLR at baseline has a mean ± SD of 1.66±0.72 and after intervention it reduces to 1.53±0.67 (P = 0.002) (Table 4).

On average, the compliance rate was high in our study, and such that >95.2% of vitamin D capsules were taken by subjects. After 3 months of intervention we have seen a significant decrease in serum hs-CRP (P-interaction=0.007). Also, there was significant association between weight (Kg) and hs-CRP quartiles (P< 0.001) (Table 1) at baseline. We have seen this association after calculating BMI with hs-CRP quartiles (P< 0.001). Furthermore, there were significant associations between biochemical factors like triglyceride (P< 0.001), white blood cell count (P=0.001) and lymphocytes (P=0.001) with hs-CRP (Table 1). After high dose vitamin D supplementation we observed a significant reduction in neutrophil count (P< 0.001) (Table 3). A significant Pearson's correlation was seen between hs-CRP and weight, BMI, triglyceride, white blood cells, platelets and lymphocytes.

In multinomial logistic regression (after adjusted variables for age), there were significant association between weight and first hs-CRP quartile with 3^{th} and 4^{th} hs-CRP quartile, respectively (OR=1.04, P< 0.001/ OR=1.06, P< 0.001), BMI and first hs-CRP quartile with 3^{th} and 4^{th} hs-CRP quartile, respectively (OR=1.14, P= P< 0.001/ OR=1.21, P= P< 0.001), WBC

and first hs-CRP quartile with 2th, 3th and 4th hs-CRP quartile, respectively (OR=1.18, P=0.014/ OR=1.17, P=0.021/ OR =1.39, P< 0.001), respectively (OR=1.06, P=0.002/ OR=1.08, P< 0.001) and triglyceride and first hs-CRP quartile with 2th, 3th and 4th hs-CRP quartile, respectively (OR=1.00, P=0.022/ OR=1.01, P< 0.001/ OR =1.01, P< 0.001) (Table 2).

Discussion

To the best of our knowledge this is the first study showing that high-dose supplementation of vitamin D affects measures of systemic inflammation: reductions in High-Sensitivity C-Reactive Protein level and Neutrophil‑to‑lymphocyte ratio (NLR) distribution in a large population of adolescent girls. This can be explained at least in part by its biological pathways via inflammatory and stress responses which is activated through nuclear factor kappa B pathway (NF-κB). There are evidences about the effect of activation of nuclear factor kappa B pathway (NF-κB) in induction of endogenous CRP (23). Studies have shown that in passively sensitized smooth muscle cells in human airways and murine macrophage cells, active form of vitamin D (1,25-dihydroxyvitamin D_3), had inhibition effects on NF-κB. The mechanism of inhibition is by reduction of IkB-α phosphorylation and also up regulation of NF-κB inhibitors (24, 25). Therefore, mega dose supplementation of vitamin D can lead to increasing in active form of vitamin D (1,25-dihydroxyvitamin D_3) and it can decrease CRP by inhibition effects on NF-κB.

Our finding are similar to the results of study in colorectal adenoma patients (26). In another study, Year-Long showed that vitamin D supplementation in overweight and obese subjects lead to significantly increase in hs-CRP concentration but decreased in serum interleukin-6 (27). There are also other studies that have shown the effect of vitamin D supplementation on inflammation. Eleftheriadis et al. (28) showed that participants with higher serum 25(OH) D

have lower serum hs-CRP concentration. Also, there was similar association of 25(OH) D and hs-CRP in patients with type 2 diabetes that undergoing coronary angiography (29). Similar results have shown in asymptomatic adults (30). Vitamin D supplementation can lead to less production of parathyroid hormone (31), as we know reduction in parathyroid hormone can lead to suppression of inflammatory factors production. Yombi et al. (32) showed that Neutrophil-to-lymphocyte ratio (NLR) distribution can be comparable to hs-CRP for showing early inflammation. Also, Shin et al. have investigated the association of hs-CRP and Neutrophil-to-lymphocyte ratio (NLR) with mortality in patients with acute myocardial infarction and have seen that high NLR and high CRP are independent predictors for all cause death in this patients (33). Both hs-CRP and NLR are biomarkers that associated with inflammatory processes. In this study we have seen that mega dose vitamin D supplementation significantly decreases NLR and hs-CRP that is similar to other studies that have shown lessen inflammatory process with vitamin D supplementation. NLR was used to assess inflammation in different patients like patients with cardiovascular disease, chronic diseases disease and malignancy (13-15). However, to the best of our knowledge, there is no study to assess the effect of vitamin D supplementation in adolescent on NLR level. Previous studies had shown that there is an association between vitamin D deficiency and endothelial dysfunction that was because inflammatory processes (34, 35). Also, the relation between NLR and atherosclerosis demonstrated previously (36). According to these studies, NLR might be an inexpensive marker for revealing inflammatory processes for assessing its relation with vitamin D supplementation.

In our study, we found that there was positive correlation between BMI, waist circumference, triglyceride, with hs-CRP. As we know, hypertriglyceridemia and obesity associated with cardiovascular diseases (CVD) and in this situation there is an inflammatory process (37)(38).

This association may attribute to inflammatory processes and maybe it's association with elevated hs-CRP level.

Conclusion

In conclusion, hs-CRP and NLR fell significantly after high dose vitamin D supplementation. Our study showed that vitamin D supplementation can decreases inexpensive inflammatory markers like hs-CRP and NLR. Further RCTs are required for confirmation of these findings, and better understanding the relationship of vitamin D and inflammatory markers.

Conflict of interest

The authors have no conflict of interest to disclose.

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Table 1 Baseline demographic, anthropometric, clinical and laboratory characteristics of the participants according to the serum high sensitvity C-reactive protein quartiles

Variables	Q1	Q2	Q3	Q4	P value*
Age (Years)	14.4±1.5	14.8±1.5	14.5±1.5	14.6±1.6	0.100
Weight (Kg)	49.0±8.8	50.9±10.3	54.3±11.2[a, b]	57.6±15.6[a, b, c]	< 0.001
Height (Cm)	157.2±6.3	157.5±6.3	158.1±6.0	157.9±7.0	0.537
Body Mass Index (Kg/m^2)	19.8±3.1	20.4±3.6	21.6±3.8[a, b]	22.9±5.6[a, b, c]	< 0.001
Waist Circumference (Cm)	67.5±6.7	68.7±7.6	72.3±8.5[a, b]	74.2±11.8[a, b]	0.000
Systolic Blood Pressure (mmHg)	96.9±14.6	96.3±14.3	97.3±13.6	96.7±14.4	0.919
Diastolic Blood Pressure (mmHg)	61.9±13.2	63.1±12.9	63.7±13.2	62.1±13.1	0.504
Cholesterol (mg/dL)	170.8±64.2	185.1±84.6	178.6±64.0	164.5±56.6[b]	0.027
Triglyceride (mg/dL)	76.0±30.3	79.9±33.3	92.7±40.4[a, b]	96.6±53.1[a, b]	< 0.001
White Blood Cell Count (billion cell/L)	5.8±1.5	6.5±3.4[a]	6.3±1.8	6.8±1.9[a]	0.001
Red Blood Cell Count (trillion cell/L)	4.8±0.6	4.9±0.7	5.0±0.8	5.0±0.9	0.282
Hemoglobin (grams/dL)	13.8±2.1	13.9±1.9	14.2±2.8	14.0±2.3	0.551
Hematocrit (percent)	42.9±5.7	43.8±6.1	44.2±6.9	43.6±7.4	0.340
Mean Corpuscular Volume (fL/red cell)	87.9±6.8	88.4±6.7	88.0±7.1	87.2±6.4	0.424
Mean Corpuscular Hemoglobin (pg/cell)	28.4±2.3	28.3±2.4	28.0±2.4	28.2±2.3	0.642
Mean Corpuscular Hemoglobin Concentration (g/dL)	32.1±1.6	31.9±1.8	31.9±2.1	32.2±1.6	0.407
Platelets (billion/L)	244.1±65.4	244.4±56.6	266.0±67.2	270.5±76.0[a, b]	0.001
Lymphocytes (percent)	38.5±8.1	37.6±9.0	36.8±8.6	34.4±9.2[a, b]	0.001
Neutrophils (percent)	53.4±9.1	53.4±11.1	55.2±9.6	56.1±11.1	0.081
Platelet Distribution Width (percent)	12.9±1.8	12.8±1.8	12.6±1.9	12.9±2.1	0.617
Red Cell Distribution Width (percent)	12.8±1.2	12.9±1.3	12.8±1.2	13.0±1.4	0.571
Mean Platelet Volume (fL)	10.5±3.7	10.7±3.8	10.0±0.9	10.3±2.2	0.299
Platelet Larger Cell Ratio	27.2±6.6	27.4±8.7	25.8±7.2	26.9±7.6	0.379

Data are expressed as mean ± SD.
a: Q1 with Q2, Q3, Q4/ b: Q2 with Q3, Q4/ c: Q3 with Q4 significance are according to post hoc analysis.
*Comparison of variables with hs-CRP quartiles.

Table 2 multinomial logistic regression: Odds ratio of variables according to the serum high sensitvity C-reactive protein quartiles

	Reference group* and hs-CRP quartile 2		Reference group and hs-CRP quartile 3		Reference group and hs-CRP quartile 4	
variables	**Odds Ratio**	**P- Value**	**Odds Ratio**	**P- Value**	**Odds Ratio**	**P- Value**
BMI (Kg/m^2)	1.03 (0.97-1.10)	0.250	1.14 (1.07-1.20)	< 0.001	1.21 (1.14-1.28)	< 0.001
Waist Circumference (Cm)	1.01 (0.98-1.04)	0.270	1.07 (1.04-1.10)	0.000	1.09 (1.06-1.12)	< 0.001
WBC (billion cells/L)	1.18 (1.03-1.35)	0.014	1.17 (1.02-1.33)	0.021	1.39 (1.22-1.59)	< 0.001
Lymphocytes (percent)	0.98 (0.96-1.01)	0.455	0.97 (0.94-1.00)	0.121	0.95 (0.92-0.97)	< 0.001
Platelet (billion/L)	1.00 (0.99-1.00)	0.734	1.00 (1.00-1.00)	0.031	1.00 (1.00-1.01)	0.001
Triglyceride (mg/dL)	1.00 (1.00-1.01)	0.022	1.01 (1.00-1.02)	< 0.001	1.01 (1.01-1.02)	< 0.001

All variables adjusted for age.

*hs-CRP quartile 1 is the Reference group.

Table 3 CBC at baseline and 12 wk after intervention in healthy adolescent girls who received mega dose vitamin D supplements

	Before Supplementation	After Supplementation	P- Value
White Blood Cell Count (billion cells/L)	6.37±2.34	6.31±1.66	0.557
Red Blood Cell Count (trillion cells/L)	4.99±0.81	4.93±0.65	0.144
Hemoglobin (grams/dL)	14.03±2.24	14.00±1.83	0.807
Hematocrit (percent)	43.64±6.51	43.36±5.51	0.399
Mean Corpuscular Volume (fL/red cell)	87.42±6.80	88.13±5.76	< 0.001
Mean Corpuscular Hemoglobin (pg/cell)	28.17±2.37	28.53±2.29	< 0.001
Mean Corpuscular Hemoglobin Concentration (g/dL)	32.17±1.78	32.35±1.29	0.004
Platelets (billion/L)	260.90±69.10	249.09±60.02	< 0.001
Lymphocytes (percent)	36.57±8.49	37.51±8.50	0.037
Neutrophils (percent)	55.17±9.68	52.38±9.43	< 0.001
Platelet Distribution Width (percent)	12.76±1.97	12.70±1.96	0.473
Red Cell Distribution Width (percent)	12.91±1.18	12.75±1.02	< 0.001
Mean Platelet Volume (fL)	10.25±1.78	10.07±0.90	0.032
Platelet Larger Cell Ratio	26.60±7.61	26.00±7.18	0.041

Data are expressed as mean ± SD.

Table 4 Concentrations of serum hs-CRP and Neutrophil-to-lymphocyte ratio (NLR) at baseline and 12 wk after intervention in healthy adolescent girls who received mega dose vitamin D supplements

	Before Supplementation	After Supplementation	P- Value
hs-CRP*	0.975 (0.500-1.850)	0.860 (0.387-1.61)	0.007
NLR†	1.66±0.72	1.53±0.67	0.002

*Data is expressed as Median (IQR).

† Data is expressed as mean ± SD.