Research Article

Association of Inflammatory Markers with Vitamin D Deficiency in Adult Population

Maryam Sadaqat [1], Feroza Hamid Wattoo [1], Asma Nafisa[2], Rizwan Ahmed Kiyani[1]

[1] University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan;
[2] Rawalpindi Medical University, Rawalpindi, Pakistan;
* Correspondence: maryamsadaqat602@gmail.com (+923125477400)

Abstract: Background: Vitamin D deficiency has emerged as a significant global health concern, extending beyond its fundamental role in bone regulation to affecting immune metabolism.

Aim: This study aimed to assess the association of vitamin D with inflammatory markers in a healthy adult population of twin cities Islamabad and Rawalpindi, Pakistan.

Methodology: This cross-sectional study included 330 healthy adults of both genders. Blood samples were drawn to measure the serum concentrations of vitamin D and inflammatory markers including C-reactive protein, interleukin-6, ferritin, and plasma levels of white blood cells.

Results: The majority of the study participants were female 75.2% compared with male 24.8%. The mean concentration of vitamin D in the study population was 22.4 ng/ml ± 13.9. Among the participants, 53.7% were vitamin D deficient, 27% were insufficient whereas 18.4% had sufficient levels. Logistic regression analysis showed significant association between Vitamin D and CRP, IL-6, and WBCs whereas ferritin showed no association in adjusted model. **Conclusion: A** negative correlation of vitamin D with inflammatory markers including CRP, IL-6, and WBCs however no significant association was observed between serum vitamin D and ferritin levels. These findings propose that sufficient vitamin D may reduce the immune disruptions in adult individuals.

Keywords: Inflammation; Inflammatory markers; Vitamin D deficiency

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1. Introduction

Vitamin D is essential for proper growth and development. It is mainly obtained by diet and sunshine exposure. Serum 25(OH) D, the main circulating form of vitamin D, is produced by the liver after being absorbed from the diet and sunlight [1]. Its functions extend beyond bone homeostasis to encompass anti-inflammatory and immune modulation. It exerts pleiotropic effect on physiological activities of keratinocytes, endothelial cells, lymphocytes, and osteoblasts through binding to vitamin D receptors (VDR) [2]. VDR is a nuclear transcription factor that impacts the gene expression in wide array of cells. Due to universal presence of VDR in almost all tissues vitamin D deficiency is one of the major worldwide health issues nowadays. Insufficient levels of Vitamin D have been implicated in several chronic inflammatory conditions, including rheumatoid arthritis, liver cirrhosis, colon cancer, breast cancer, asthma, and cardiovascular disease [3], inflammatory bowel disease, preeclampsia, multiple sclerosis, muscle weakness, fatigue, and impaired immune systems.

Despite abundant sunlight, Vitamin. D deficiency is highly prevalent in Pakistan. Over 20% of the populations of India, Pakistan, and Afghanistan have vitamin D insufficient levels below 30 nmol/l [4]. The frequency of vitamin D insufficiency in Pakistan has recently raised affecting 60-80% of the population [5]. In the Islamabad and Rawalpindi, 56% of subjects were vitamin D deficient, 18% insufficient and 25% had sufficient vitamin D levels [6].

Research studies emphasizing the association between vitamin D and inflammatory markers suggest that insufficient vitamin D may lead to systemic inflammatory disruption. Moreover, its deficiency has been linked to elevated white blood cell (WBC) count, C-reactive protein (CRP), interleukin-6 (IL-6), and ferritin levels causing immune activation and inflammation markers. IL-6 serves as a pro-inflammatory cytokine produced during viral or immune responses [7]. Similarly, CRP and ferritin levels reflect chronic low-grade inflammation and oxidative stress. Both of which are intensified by low vitamin D [8]. Moreover, the balanced iron uptake and vitamin D status further support the link between its deficiency and inflammatory dysregulation, as observed in female athletes having low vitamin D and altered iron status [9]. These studies indicate that maintaining vitamin D levels may help regulate immune responses and inflammation with balanced levels of cytokines, acute-phase proteins, and hematologic markers. This study investigates the association between vitamin D and inflammatory markers including white blood cells, interleukin-6, ferritin, and C-reactive protein where high levels of inflammatory markers could be observed due to insufficient vitamin D in the adult age group residing in the twin cities, Rawalpindi and Islamabad of Pakistan.

2. Materials and Methods

A cross-sectional, observational study was conducted on 330 healthy adults (male and female) aged 18-70 years residing in Rawalpindi and Islamabad. Subjects who had no diagnosed chronic inflammatory diseases were, enrolled through a non-probability purposive sampling technique. Blood samples were taken to measure the inflammatory markers CRP, IL-6, ferritin, and white blood cells (WBCs). Demographic Data was collected and Vitamin D status was determined by measuring serum 25(OH)D concentration, and participants were categorized into deficient, insufficient, and sufficient groups

The ethical committee of Pir Mehar Ali Shah Arid Agriculture University, Rawalpindi, under the reference no. PMAS-AAUR/IEC/687 approved the research work.

Participants with chronic inflammatory diseases such as cardiovascular disease, diabetes, rheumatoid arthritis, asthma, cancer, gastrointestinal disease, or those taking any treatment for these diseases were excluded. A nutritional dietary questionnaire [10] was designed, including participants' weight, height, and body mass index, dietary history, and vitamin D history.

The blood samples of healthy subjects were collected by using a 5ml disposable syringe from the antecubital vein of the subjects and drawn into KEDTA and gel tube. The tubes containing serum were kept stored at -20^{o}C until analysis.

Serum Vitamin D was measured with an Enzyme-linked immunoassay (Arigobio). The vitamin D standard cut-off value was determined according to the Journal of Pakistan Medical Association. As in Pakistan ng/mol unit is used for the measurement of 25(OH) D. The cut-off value was considered as <20 ng/mol for vitamin D deficiency, <30 ng/mol for insufficiency, and >30-100 ng/mol for sufficient concentration.

Serum ferritin and C-reactive protein were measured by a chemistry analyzer (AU680- Beckman Coulter) reference range<15-150 ng/ml for females, <15-200ng/ml for males [11] and <5 mg/l [12] respectively. The cut-off range of CRP was considered as per the normal or low-grade inflammation status in clinical and research settings, whereas the ferritin cut-off value was considered according to the WHO-defined inflammatory status of apparently healthy males and females with ferritin [11]. WBC count was measured in the range of 4.5-11.5x10^9cells/l by a (Mindray BC) hematology analyzer. The serum interleukin-6 was measured by enzyme chemiluminescent assay in a Cobas 6000 analyzer with a cut-off value < 7pg/ml to express the inflammation level, as reported with a normal reference range of IL-6 in healthy adults [13].

Data analysis was done by SPSS software (version 24). The normality of the data was verified with the Kolmogorov-Smirnov test. Means of different variables in various age groups were compared with the Kruskal-Wallis test. The Mann-Whitney U test was used to compare data in both genders. Logistic regression analysis was used to understand the relation between serum Vitamin D and inflammatory markers. Normal levels of WBCs of below median, i.e., 7.7×10^9cells/l, interleukin-6 as <7pg/ml, CRP as <5 mg/l, and Ferritin as <15ng/ml. The serum vitamin D, considered as an independent variable, was divided into four quartiles: the lowest quartile <13.89ng/ml, the second quartile (13.90 until 19.77ng/ml), the third quartile (19.78 until 29.99ng/ml), and the fourth quartile ranged from (30.0 until > 64ng/ml). The last quartile was considered a reference category. As ferritin acts as a positive acute-phase protein reported to have elevated levels during inflammation [14Therefore was considered a complementary marker in this study to define inflammatory status with vitamin D rather than an individual inflammatory marker such as CRP and IL-6. From the analyzed unadjusted group 1, 4 adjusted groups were derived for the relationship of vitamin D with inflammatory markers, as Group 2: age and gender; Group 3: BMI, physiological measurement; Group 4: smoking, sun exposure, sunscreen usage; Group 5: daily food intake.

3. Results

3.1. Anthropometric Analysis

The majority of the study participants were female (75.2%) compared with males 24.8%. (The mean age of the participants was 37.87± 13.02 year. The mean BMI of the participants was 23.45

± 2.62 with no significant difference between genders. The variables assessed in the questionnaire analysis are shown in Table 1.

Table 1. Analysis of characteristics of study individuals.

Gender N(%age)		**Age Group N(%age)**		**BMI N(%age)**		**Blood Pressure N(%age)**		**Sunlight exposure N(%age)**	
Male	82(24.8)	0-20	18(5.5)	Underweight	13(3.9)	SBP100-	243(73.6)	<30min	133(40.3)
Female	248(75.2)	21-40	198(60)	Normal	232(70.3)	120	87(26.3)	>30min	197(59.7)
		41-60	95(28.8)	Overweight	81(24.5)	130-140	269(81.5)		
		61-100	19(5.8)	Obese	4(1.2)	DBP 70-	61(18.5)	**Sunscreen usage N(%age)**	
						8090			
								No	210(63.5)
								Yes	120(36.5)
RDA milk uptake N(%age)		**Diarrheic condition N(%age)**		**Fish oil intake N(%age)**		**Daily recommended egg uptake N(%age)**		**Smokers N(%age)**	
No	**279(84.4)**	**No**	**302(91.5)**	**No**	**268(81.2)**	**No**	**216(65.5)**	**No**	**278(84.2)**
Yes	**51(15.5)**	**Yes**	**28(8.5)**	**Yes**	**62(18.8)**	**Yes**	**114(34.5)**	**Yes**	**52(15.8)**

3.2. Vitamin D and Inflammatory Markers Measurement

The mean vitamin D concentration of the study population was 22.4 ng/ml ± 13.9. Among the participants, 53.7% had vitamin D deficiency, 27% showed insufficiency, whereas 18.5% had sufficient vitamin D levels. The distribution of inflammatory markers is shown in Figure 1. Vitamin D Deficiency and insufficiency were most common in participants aged 21-60 years (37.7 ± 13.0). The mean concentration of WBCs was as 8046.3x10^9 cells/l, interleukin-6 11.66 pg/ml ± 12.7 CRP 14.7 mg/l ± 15.6, and the mean ferritin was 96.77ng/ml ± 37.6. The mean of inflammatory markers in females and males is shown in Table 2. Figure 2.

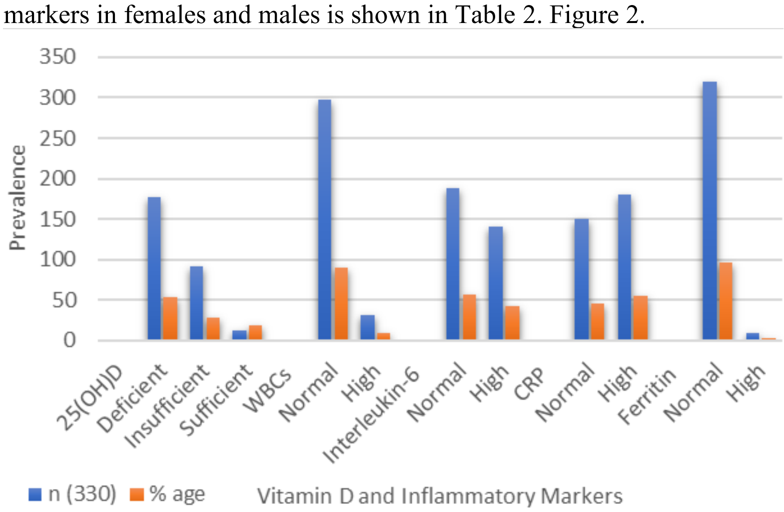

Figure 1. Prevalence of Vitamin D and Inflammatory Markers

Table 2. Vitamin D and inflammatory markers measurement

Variables	**Male (mean)**	**Female (mean)**	**p-value**
25(OH)D	22.3± 16.407	22.5± 13.088	0.418

WBCs	$8.13x10^9 \pm 1784.996$	$8.03x10^9 \pm 1688.261$	0.562
Interleukin-6	11.65± 12.62903	11.67± 12.78221	0.844
CRP	16.7± 15.54366	14.0± 15.70202	0.083
Ferritin	94.4± 39.10430	97.5± 37.14478	0.628

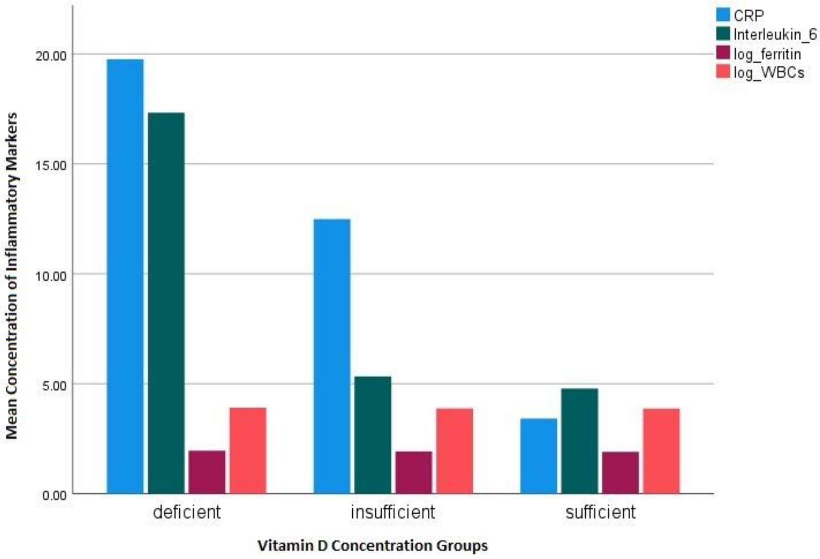

Figure 2. Mean concentration graph of vitamin D versus the mean concentration of inflammatory markers

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3.3 Regression Analysis for Vitamin D and Inflammatory Markers

Both Univariate and Multivariate Multinomial regression analysis were done to assess the relationship between Vitamin D quartiles and inflammatory markers (WBC, CRP, IL-6, Log- ferritin). As shown in Table 4 and 5, no significant association was observed between vitamin D levels and total WBC count across the quartiles, with odds ratios (OR) close to 1.0 and confidence intervals (CI) overlapping unity ($p > 0.05$). In contrast, a significant inverse association was noted between vitamin D and CRP levels. Participants in the lower vitamin D quartiles (Q1–Q3) exhibited markedly higher odds of elevated CRP compared to those in the highest quartile (Q4), with OR values ranging from 1.25 to 1.29 and all p-values < 0.001. Similarly, IL-6 demonstrated a strong and consistent association with vitamin D status, where individuals in the lowest quartile of 25(OH)D had approximately 1.8-fold higher odds of increased IL-6 levels ($p < 0.001$), indicating enhanced pro-inflammatory activity with declining vitamin D concentration. Conversely, no significant relationship was found between vitamin D and log-transformed ferritin levels ($p > 0.05$). Overall, these findings suggest that lower vitamin D levels are independently associated with elevated inflammatory markers, particularly CRP and IL-6, supporting the potential role of vitamin D in modulating inflammatory responses in apparently healthy adults.

Table 4. Multinomial logistic regression unadjusted analysis for the correlation of vitamin D and inflammatory markers

	Unadjusted Analysis (overall)			
	WBCs	**CRP**	**IL-6**	**Log-ferritin**

Group 1	**OR**				
	25(OH)D				
	Higher Q4	1	1	1	1
	Third Q3	1.0	1.27	1.28	1.00
	Second Q2	1.0	1.29	1.72	1.00
	Lower Q1	1.0	1.28	1.73	1.00
	95%CI				
	25(OH)D				
	Higher Q4	-	-	-	-
	Third Q3	1.00,1.00	1.11,1.46	1.01,1.63	0.98,1.01
	Second Q2	0.98,1.00	1.12,1.49	1.36,2.19	0.98,1.02
	Lower Q1	0.97,1.01	1.12,1.48	1.36,2.20	0.98,1.02
	p-value				
	25(OH)D				
	Higher Q4	-	-	-	-
	Third Q3	0.572	<0.001	0.019	0.841
	Second Q2	0.507	<0.001	<0.001	0.976
	Lower Q1	0.003	<0.001	<0.001	0.940

Table 5. Multinomial logistic regression adjusted analysis for the correlation of vitamin D and inflammatory markers

	Adjusted Analysis											
	Group 2			**Group 3**			**Group 4**			**Group 5**		
	OR	**95%CI**	**Sig.**	**OR**	**95%CI**	**Sig.**	**OR**	**95%CI**	**Sig.**	**OR**	**95%CI**	**Sig.**
WBCs												
25(OH)D												
Higher Q4	1	-		1	-		1	-		1	-	
Third Q3	1.000	0.99,1.00	0.189	0.98	1.00,1.02	0.256	1.00	0.99,1.00	0.756	1.00	1.00,1.02	0.362
Second Q2	1.000	0.98,1.00	0.405	1.00	1.01,1.00	0.397	1.00	0.99,1.01	0.119	1.00	1.01,1.00	0.365
Lower Q1	1.001	1.02,0.98	<0.001	1.00	1.01,1.02	<0.001	1.00	1.01,1.00	<0.001	1.00	1.01,1.02	0.001
CRP												
25(OH)D												
Higher Q4	1	-		1	-		1	-		1	-	
Third Q3	1.25	1.11,1.41	<0.001	1.26	1.11,1.43	<0.001	1.275	1.13,1.45	<0.001	1.25	1.10,1.41	<0.001
Second Q2	1.26	1.12,1.41	<0.001	1.28	1.13,1.45	<0.001	1.285	1.12,1.45	<0.001	1.26	1.11,1.42	<0.001
Lower Q1	1.26	1.10,1.42	<0.001	1.27	1.12,1.44	<0.001	1.295	1.12,1.44	<0.001	1.26	1.11,1.43	<0.001
IL-6												
25(OH)D												
Higher Q4	1	-		1	-		1	-		1	-	
Third Q3	1.357	1.08,1.70	0.008	1.37	1.09,1.71	0.006	1.327	1.06,1.65	0.013	1.38	1.10,1.74	0.013
Second Q2	1.814	1.42,2.28	<0.001	1.83	1.45,2.31	<0.001	1.757	1.40,2.19	<0.001	1.84	1.46,2.32	<0.001
Lower Q1	1.818	1.43,2.29	<0.001	1.84	1.45,2.32	<0.001	1.758	1.40,2.20	<0.001	1.84	1.46,2.33	<0.001
Log-ferritin												
25(OH)D												
Higher Q4	1	-		1	-		1	-		1	-	
Third Q3	1.002	0.99,1.01	0.607	0.99	0.99,1.01	0.868	1.001	0.99,1.01	0.814	1.00	0.99,1.01	0.702
Second Q2	1.003	0.91,1.00	0.750	1.00	0.98,1.01	0.886	1.003	0.99,1.02	0.796	1.00	0.99,1.02	0.740
Lower Q1	1.003	0.99,1.01	0.786	1.00	0.98,1.01	0.997	1.002	0.98,1.02	0.910	1.00	0.98,1.02	0.832

When comparing the unadjusted and adjusted multinomial logistic regression analyses, the overall trend of association between vitamin D levels and inflammatory markers remained consistent. Both models demonstrated a significant inverse relationship between serum 25(OH)D and the inflammatory markers CRP and IL-6, whereas no association was observed with WBC count or log-ferritin. In the unadjusted model, lower vitamin D quartiles (Q1–Q3) were associated with markedly higher odds of elevated CRP (OR 1.27–1.29, $p < 0.001$) and IL-6 (OR 1.72–1.73, $p < 0.001$) compared to the highest quartile. After adjustment for potential confounding factors, these associations remained statistically significant confirming the independent inverse relationship of vitamin D with CRP and IL-6 levels. The lack of association between vitamin D and WBC or ferritin persisted in both models. Thus, adjustment for demographic variables did not alter the strength of associations, reinforcing that vitamin D deficiency is independently linked to elevated systemic inflammation, particularly through increased CRP and IL-6 concentrations

4. Discussion

The present study assessed the relationship between serum vitamin D levels and inflammatory markers (WBCs, CRP, IL-6, and log-ferritin) among apparently healthy adults residing in the twin cities Islamabad and Rawalpindi. Despite abundant sunlight throughout the year, a high prevalence of vitamin D deficiency was observed, consistent with previous national reports highlighting widespread hypovitaminosis D in Pakistan. In this study, more than half of the participants (53.7%) were vitamin D deficient, while only 18.5% had sufficient levels. This trend highlights the inadequate dietary intake of vitamin D and poor awareness regarding its importance in maintaining optimal health. Younger participants (21-40 years) showed deficient vitamin D levels as compared older age groups. Another study showed similar findings indicating that vitamin D-deficient subjects were younger compared to non-deficient participants [18] suggesting that younger individuals of the Pakistani population are not well aware of the significance of a healthy diet and vitamin D uptake. A review of studies conducted in Pakistan [5] showed that 64% of the population was vitamin D deficient. Regional variations reported in Karachi, the percentage varied up to 84 %. In Islamabad, 11% of the participants were of the normal range, whereas the present study showed 53.7% of deficient individuals with 18.4 in % normal range compared to another study [19] with deficiency up to 53.5%, 31.2% in the insufficient category, and 15.3% with normal vitamin D levels in a random population.

. Participants showed normal BMI on average. The previous study reported the mean value of the BMI of participants in the range of 23.1-28.8 [20]. Another study conducted observed the mean BMI of participants as 23.43.

Most of the participants had normal blood pressure levels with mean systolic blood pressure of 117.9 ± 10.1 and diastolic pressure of 79.1± 6.7 [21]. Our findings are closer to another study which reported mean systolic and diastolic blood pressure of 109.8 and 68.7, respectively [22]. The mean 25 (OH) D concentration in non-smokers and smokers was not significantly different indicating that smoking status did not appear to influence the Vitamin D level. Similarly, a study by Aldaham, et al. [23] showed no significant association between gender, smoking status and Vitamin D levels, however, smoker had raised concentration of Interleukin 6 suggesting a possible link between smoking and inflammation. The current study found the mean vitamin D level 22.4 ng/ml which is significantly lower compared to the findings of [16] where the vitamin D mean value was 43.9, 45.8 in males, and 41.3 in females, Raftery et al. reported the mean of vitamin D in their study as 59.8. In research [21], vitamin D levels were given a mean value of 16.26±6.8. Out of these, 16% of participants were deficient, 58% were insufficient, and 24.6% were sufficient. The study reported the concentration of WBCs varied from 1.22 to 32.32 x10^ (9) cells/l. The mean concentration was observed as 6.51x10^ (9) cells/l [15]. Another study reported elevated levels of WBCs in their study [24]. The above-mentioned studies showed that vitamin D has been related to several inflammatory markers. A few studies have been conducted to understand this association. It was also studied with the pandemic disease COVID-19, representing higher levels of interleukin 6 and CRP in infected patients with lower vitamin D levels. It showed higher levels of interleukin 6 and CRP in lung disease, liver damage, peripheral artery disease, and cancer [25,26,27,28].

Our study showed a significant negative association for sufficient and deficient groups of vitamin D with ferritin levels compared to the insufficient group; however, the association did not remain significant after the adjustment for other variables, which suggests that it might be influenced by factors of inflammation and physiological parameters. Some studies report a positive association of ferritin with vitamin D [18]. Moreover, studies reported the mean value of serum ferritin in males and females separately as 116.23±75.49 and 32.47±31.21, respectively [29]. In previous research, vitamin D insufficiency and low ferritin levels in athletes have been observed in a positive correlation [9]. In the present study, Vitamin D showed a significant inverse relationship with inflammatory markers in apparently healthy participants. In another study, a significant decrease in the concentration of CRP and IL-6 was observed against vitamin D sufficient levels [16]. Moreover, another study reported an inverse relationship of serum ferritin levels in their study conducted based on gender [29]. Similarly, a significant association was observed in this study between vitamin D and inflammatory markers based on gender, where females showed an association between vitamin D levels and WBCs, interleukin-6 only but no association for CRP and ferritin. The male participants showed no association with any inflammatory parameter, representing females more closely related to inflammatory responses. Thus, this inverse relationship of vitamin D with inflammatory markers in apparently healthy participants suggests that to reduce inflammatory actions, the normal vitamin D concentration in the body has fundamental importance. Further study should be carried out on the diseased and healthy populations in order to relate inflammatory markers with vitamin D levels on a large scale in Pakistan.

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