IMPACT OF VITAMIN D DEFICIENCY ON HEMATOLOGICAL PARAMETERS

Luzana SHABANI[1], Jehona ALI[2,3], Sheqibe BEADINI[2], Mimoza BAFQARI-BAKIJI[2,3], Arita SABRIU HAXHIJAHA[4]

[1]Faculty of Mathematics and Natural Science, Department of Biology – Biochemistry University of Tetova,,
[2] Faculty of Medical Sciences, University of Tetova,
[3]Clinical Hospital of Tetovo
[4]Faculty of Technological Sciences, Mother Theresa University, Skopje
E-mail of corresponding author: luzana.shabani@unite.edu.mk

Abstract

Vitamin D is a steroid hormone with an important role in bone metabolism and immune function, while its deficiency represents a global health problem. This study aims to evaluate the relationship between vitamin D levels and hematological parameters, including the neutrophil-to-lymphocyte ratio (N/L), as an indicator of inflammation. This retrospective study included 201 outpatient individuals of different ages, analyzed during the period September 2025 – March 2026 at the Clinical Hospital of Tetovo. Patients were divided into three groups according to vitamin D levels: >30 ng/ml, 20–30 ng/ml, and <20 ng/ml. The results showed that 47.8% of patients had vitamin D deficiency. Analysis of hematological parameters indicated no statistically significant differences in erythrocyte and leukocyte counts among the groups. However, for hemoglobin, a statistically significant difference was observed between the group with levels of 20–30 ng/ml and the group with <20 ng/ml ($p=0.017$), suggesting a possible association between vitamin D deficiency and decreased hemoglobin levels. Additionally, the N/L ratio was significantly higher in patients with vitamin D deficiency, with statistically significant differences between groups (<20 ng/ml compared to the other groups), indicating a potential link with inflammatory processes. In conclusion, vitamin D deficiency is associated with partial changes in hematological parameters, particularly with decreased hemoglobin and increased N/L ratio. These findings suggest a potential role of vitamin D in modulating inflammation and hematological status, highlighting the importance of its monitoring in clinical practice.

Keywords: vitamin D, hemogram, hemoglobin, neutrophil-to-lymphocyte ratio

Introduction

Vitamin D is a steroid hormone that is very important for human health and belongs to the group of fat-soluble vitamins. Biologically inactive vitamin D acts by being transformed into its active form, 1,25-dihydroxyvitamin D [1,25$(OH)_2$] (Ergenç et al., 2023). Vitamin D is essential for the formation and maintenance of strong and healthy bones.

Vitamin D deficiency may occur due to insufficient exposure to sunlight, inadequate dietary intake, reduced absorption, abnormal metabolism, or resistance to vitamin D (Shabani et al., 2021). Vitamin D and its metabolites show a strong binding affinity to the vitamin D-binding protein (VDBP) in plasma, and the plasma half-life of VDBP is approximately 2.5–3.0 days (Cavalier et al., 2024).

There has been an ongoing debate regarding which concentrations of 25(OH)D define vitamin D deficiency. The Institute of Medicine (IOM, U.S. National Academy of Sciences) considers a minimum concentration of 25(OH)D of 20 ng/mL (50 nmol/L) as physiologically sufficient for at least 97.5% of the population. The Endocrine Society, in 2011, recommended a level of >30 ng/mL (>75 nmol/L) (Ekmekcioglu & Poteser, 2025).

Furthermore, inflammatory markers derived from the hemogram, particularly the neutrophil-to-lymphocyte ratio (NLR), have attracted considerable attention for study. It has been shown that vitamin D deficiency may influence changes in hematological parameters (Książek et al.,

2021). Evidence is increasing that $1,25(OH)_2D$ may stimulate erythropoiesis in red blood cells by increasing sensitivity to erythropoietin. Moreover, $1,25(OH)_2D$ may enhance the proliferation of hematopoietic precursor cells (Ernst et al., 2016)

Aim of the Study

This study aims to evaluate the relationship between serum vitamin D levels and hemogram parameters by analyzing the impact of vitamin D status on hematological changes. The study was designed to determine whether variations in vitamin D concentration are associated with significant changes in blood parameters and to further elucidate its potential role in hematological function and overall patient health.

Materials and Methods

This study is of a retrospective type and was conducted during the period from September 2025 to March 2026 at the Biochemical Diagnostic Laboratory of the Clinical Hospital of Tetovo.
A total of 201 outpatient patients of different ages were included in the study. The mean age of the patients was 51.24 ± 18.40 years, with a minimum age of 1 year and a maximum age of 89 years. They were categorized into three groups based on vitamin D levels: >30 ng/mL, 20–30 ng/mL, and <20 ng/mL.
Biological material for vitamin D analysis was collected in tubes with gel for serum separation, while for the measurement of hematological parameters, blood was collected in tubes containing the anticoagulant K_2EDTA
The determination of serum vitamin D concentration was performed using a chemiluminescent immunoassay method on the Siemens Advia Centaur analyzer. This method is based on the antigen–antibody reaction, where vitamin D binds to specific antibodies, forming an immune complex.The hemogram was determined using the Medonic M32 hematology analyzer with a flow cytometric method. The parameters included in the study were hemoglobin, erythrocytes, leukocytes, and the leukocyte differential, namely neutrophils, lymphocytes, and monocytes. The principle of this method is based on the separation of blood cells, which pass one by one through a small aperture between two electrodes.
The Medonic analyzer provides a three-part differentiation of white blood cells (lymphocytes, monocytes, and granulocytes) based on their volume after treatment with a specific reagent. For the measurement of hemoglobin and erythrocytes, a photometric method is used, where after the lysis of red blood cells, hemoglobin is released and light absorbance is measured at a wavelength of approximately 540 nm.
In the statistical analysis of the data, minimum and maximum values, mean, and standard deviation were determined using the statistical software IBM SPSS version 20. Values with $p < 0.05$ were considered statistically significant.

Results

In this study, patients of both genders were included, with a higher percentage of females (69.7%) compared to males (30.3%). This gender distribution reflects the actual composition of the studied population and represents an important factor in the interpretation of laboratory and clinical data.

Table 1. Vitamin D levels and hematological parameters according to vitamin D status groups

	Median							
>30 ng/ml		**Mean**	**SD**	**Min**	**Max**	**25**	**50**	**75**
	Vit.D	38.97	9.91	30.28	77.90	32.12	36.44	40.05
	Hb	8.25	1.06	6.39	10.42	7.78	8.41	8.69
	Er	4.53	0.52	3.57	5.96	4.16	4.40	4.83
	Le	7.72	2.49	3.79	13.50	6.01	7.30	9.68
	Neutro	4.42	1.77	1.92	8.39	2.75	4.03	5.08
	Limfo	2.78	1.52	0.88	8.72	1.77	2.49	3.58
	Mono	0.65	0.22	0.29	1.23	0.46	0.62	0.79
20-30 ng/ml	Vit.D	24.41	2.89	20.02	29.83	2.83	23.86	26.65
	Hb	8.57	1.08	4.03	10.67	8.00	8.63	9.30
	Er	4.71	0.59	2.32	6.08	4.40	6.72	5.09
	Le	7.70	2.15	4.16	16.09	6.20	7.30	8.75
	Neutro	4.31	1.69	1.65	10.30	3.20	3.94	5.07
	Limfo	2.64	0.91	0.85	5.65	1.99	2.45	3.12
	Mono	0.81	1.18	0.15	8.30	0.48	0.60	0.73
<20 ng/ml	Vit.D	14.85	3.36	6.10	19.94	12.54	14.85	17.73
	Hb	8.16	1.06	4.16	9.92	7.50	8.32	8.92
	Er	4.62	0.57	3.08	6.0	4.22	4.69	5.02
	Le	7.90	3.01	2.50	19.60	5.88	7.30	9.20
	Neutro	4.53	2.09	0.80	11.80	3.13	3.87	5.71
	Limfo	2.58	1.53	0.62	14.97	1.80	2.47	2.92
	Mono	0.63	0.25	0.25	1.43	0.46	0.58	0.73

The analysis shows that vitamin D levels vary noticeably between the groups, decreasing gradually from a mean of 38.97 ng/mL in patients with vitamin D levels >30 ng/mL, to 24.41 ng/mL in the insufficiency group (20–30 ng/mL), and to 14.85 ng/mL in the deficiency group (<20 ng/mL), reflecting a considerable proportion of patients with vitamin D deficiency.

Hematological parameters, including hemoglobin, erythrocytes, leukocytes, and their components (neutrophils, lymphocytes, and monocytes), remain relatively stable across the groups, with minimal variations.

Table 2. Distribution of patients according to vitamin D levels

	N	**%**
>30 ng/ml	38	18.90
20-30 ng/ml	67	33.33
< 20 ng/ml	96	47.76

The table shows that of the total patients included in the study, 18.9% had vitamin D levels above 30 ng/mL (normal level), 33.3% had levels in the range of 20–30 ng/mL (insufficiency), while 47.8% had levels below 20 ng/mL, indicating vitamin D deficiency. These results highlight that nearly half of the patients have vitamin D deficiency, making monitoring and potential interventions necessary for correcting this deficiency.

Table 3. Intergroup comparison of vitamin D with hematological parameters

	p value		
	>30 ng/ml vs. 20-30 ng/ml	>30 ng/ml vs. < 20 ng/ml	20-30 ng/ml vs. < 20 ng/ml
Hb	0.145	0.650	0.017
Er	0.120	0.400	0.329
Le	0.965	0.744	0.641

The statistical analysis of hematological parameters shows that for hemoglobin (Hb), a statistically significant difference was observed only between the group with levels of 20–30 ng/mL and the group with levels <20 ng/mL ($p = 0.017$), suggesting that vitamin D deficiency may be associated with a slight decrease in hemoglobin levels. Other comparisons for Hb between >30 ng/mL and 20–30 ng/mL ($p = 0.145$), and between >30 ng/mL and <20 ng/mL ($p = 0.650$), were not statistically significant.

For erythrocytes (Er) and leukocytes (Le), no significant differences were observed between the groups, with all p-values above 0.05.

Table 4. Distribution of vitamin D levels according to gender and vitamin D status groups

	>30 ng/ml	**20-30 ng/ml**	**< 20 ng/ml**						
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
Male	39.73	12.48	13	24.79	2.11	24	15.54	3.04	24
Female	36.83	11.40	25	24.20	3.24	43	14.66	3.46	72
p value	0.47	0.42	0.26						

Table 4 shows the mean vitamin D levels in males and females across three groups: >30 ng/mL, 20–30 ng/mL, and <20 ng/mL. In the >30 ng/mL group, males had a mean of 39.73 ng/mL, while females had 36.83 ng/mL. In the 20–30 ng/mL group, the mean was 24.79 ng/mL for males and 24.20 ng/mL for females, and in the <20 ng/mL group, the mean was 15.54 ng/mL for males and 14.66 ng/mL for females.

The p-values (0.47, 0.42, and 0.26) indicate that there are no statistically significant differences between males and females in any of the vitamin D groups, suggesting that the distribution of vitamin D is similar across both genders.

Table 5. Neutrophil-to-lymphocyte ratio (N/L) according to vitamin D level groups

	>30 ng/ml	**20-30 ng/ml**	**< 20 ng/ml**
Mean	1.91	1.80	2.43
SD	1.50	0.89	1.27
Min	0.31	0.55	0.73
Max	9.53	4.82	7.85
N	38	67	96

The above table shows the mean neutrophil-to-lymphocyte ratio (N/L) in three groups of patients according to vitamin D levels. In patients with vitamin D >30 ng/mL, the mean ratio was 1.91 ± 1.50, while in the group with levels of 20–30 ng/mL, this ratio was slightly lower at 1.80 ± 0.89.

The group with vitamin D deficiency (<20 ng/mL) had a higher mean value of 2.43 ± 1.27, indicating a tendency toward a higher N/L ratio in patients with low vitamin D levels. The minimum and maximum values show considerable variability across all groups, while the number of patients is higher in the deficiency group (<20 ng/mL), reflecting the distribution of

the studied population. This suggests that vitamin D deficiency may be associated with a tendency toward a higher neutrophil-to-lymphocyte ratio, a potential indicator of inflammatory response.

Table 6. Statistical comparison of the neutrophil-to-lymphocyte ratio (N/L) between vitamin D groups

	p value
>30 ng/ml vs. 20-30 ng/ml	0.63
20-30 ng/ml vs. < 20 ng/ml	0.0006
>30 ng/ml vs. < 20 ng/ml	0.04

The analysis shows that patients with severe vitamin D deficiency (<20 ng/mL) have a significantly higher N/L ratio compared to the group with levels of 20–30 ng/mL ($p = 0.0006$) and the group with >30 ng/mL ($p = 0.04$), indicating a possible association between vitamin D deficiency and an increased inflammatory response. In contrast, the difference between the >30 ng/mL group and the 20–30 ng/mL group is not statistically significant ($p = 0.63$), suggesting that the N/L ratio remains similar in these two groups with higher vitamin D levels.

Discussion

Low levels of vitamin D represent a global problem affecting a considerable proportion of the population. Although some studies suggest a relationship between vitamin D deficiency and chronic inflammatory diseases, the association with inflammatory markers remains unclear and requires further investigation (Akbas et al., 2016). Our results are consistent with the findings of the aforementioned study, showing that vitamin D_3 deficiency does not have a significant effect on erythrocyte count, erythrocyte indices, and leukocyte differential count. This agreement suggests that, despite the well-known role of vitamin D in various physiological and immune processes, its impact on basic hemogram parameters may be limited or not direct (Tabatabaei et al., 2023).

Similar results, consistent with these studies, indicating no statistically significant differences in hematological parameters—particularly in erythrocyte and leukocyte counts—depending on vitamin D status, were also reported in the study by Mobarki et al. in 2022 (Mobarki et al., 2022). Similarly, the study by Erkus et al. reported no significant differences in these parameters, suggesting that vitamin D may not have a direct or pronounced effect on the basic components of the hemogram. Although some correlations between vitamin D and certain hematological parameters have been observed, they were not statistically significant, which further reinforces our findings and supports the idea that the relationship between vitamin D and these parameters remains limited (Erkus et al., 2018). Although the literature reports that anemia is more frequent among individuals with vitamin D deficiency and that mean hemoglobin values are lower in these groups, our results partially support this finding. In our study, a statistically significant difference in hemoglobin levels between groups was observed, while no significant change was found in erythrocyte count. This suggests that vitamin D

deficiency may have a greater impact on hemoglobin content rather than on the total number of erythrocytes (Alpakra et al., 2025).
In a study conducted by Basaran & Aktas (2024), which included 240 patients, of whom 170 had vitamin D deficiency, a statistically significant difference in the N/L ratio among patients was observed ($p = 0.025$) (Basaran & Aktas, 2024). These findings are consistent with the results of our study, where a statistically significant difference ($p < 0.05$) was also found. Studies by Akbas et al. (2016) show that low levels of vitamin D are associated with a higher neutrophil-to-lymphocyte ratio (NLR), suggesting a link between vitamin D deficiency and inflammation. This highlights the value of NLR as a simple and practical indicator for monitoring inflammatory status. However, they suggest that larger studies are needed to better clarify this relationship (Akbas et al., 2016).

Conclusion

From the results of this study, it can be concluded that vitamin D deficiency (<20 ng/mL) is associated with a slight decrease in hemoglobin levels and a noticeable increase in the neutrophil-to-lymphocyte ratio (N/L), suggesting a potential impact of vitamin D deficiency on inflammatory response and hematological status. Vitamin D levels are low in nearly half of the patients, while gender distribution does not significantly affect the measured parameters. These findings highlight the need for monitoring and targeted interventions to correct vitamin D deficiency in the studied population.

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