CLINICAL PRACTICE ARTICLE

Haemogram Parameters in Vitamin D Deficiency

Edip Erkus, Gulali Aktas, Burcin Meryem Atak, Mehmet Zahid Kocak, Tuba T. Duman and Haluk Savli

ABSTRACT

Objective: To study haemogram derived inflammatory indices, mean platelet volume (MPV), red cell distribution width (RDW), and neutrophil to lymphocyte ratio (NLR) in vitamin D deficient patients and to compare to those subjects with normal vitamin D levels.

Study Design: Descriptive study.

Place and Duration of Study: Abant Izzet Baysal University Hospital, Bolu, Turkey, from March to May 2017.

Methodology: Patients' data visiting the outpatient clinics of the institution was retrospectively obtained from patients' files and computerised database. Subjects were grouped into normal vitamin D and vitamin D deficiency groups, according to the serum Vitamin D levels. Seasonal threshold value for vitamin D in Bolu region was set as 10 ng/ml. General characteristics and laboratory data of the study population were recorded and compared.

Results: Vitamin D deficiency was more common in subjects working inside as compared to those working outside (p=0.02), and in subjects with comorbidities compared to those without comorbidities (p=0.31). Body mass index (BMI), MPV, NLR were significantly higher in vitamin D deficient group as compared to subjects with normal vitamin D levels. A MPV greater than 6.22 has 89% sensitivity and 55% specifity for vitamin D deficiency, and NLR greater than 1.69 has 76% sensitivity and 55% specifity for vitamin D deficiency.

Conclusion: Elevated MPV and NLR may be the indicator of underlying serious vitamin D deficiency. Physicians should be alert and order a vitamin D assay in patients with elevated MPV or NLR, especially in endemic areas for vitamin D deficiency.

Key Words: *Mean platelet volume, Neutrophil to lymphocyte ratio, Inflammation, Vitamin D.*

INTRODUCTION

Vitamin D is considered as a steroid hormone, which regulates calcium metabolism and the structure of bone skeleton. Vitamin D deficiency may affect whole population and cause osteoporosis in adulthood. Besides the well-known effects on bone mineralization, vitamin D deficiency may also have a role in development of hypertension, malignancy, and type 2 diabetes mellitus.[1] Moreover, low vitamin D levels have been found to be associated with inflammation, arterial stiffness, and endothelial dysfunction.[2-4] Because vitamin D has immunomodulatory effect in human,[5] vitamin D deficiency can lead to immunological diseases and low grade continuous inflammation.[6] Authors have reported elevated serum levels of inflammatory markers in vitamin D deficiency.[5,7]

Haemogram derived inflammatory markers, such as mean platelet volume (MPV), red cell distribution width (RDW), and neutrophil to lymphocyte ratio (NLR) attracted great interest of researchers, recently. MPV refers the size of platelets which tends to be larger after infectious or inflammatory stimulus. Inflammatory conditions were reported to be related with increased MPV values.[8] Not only the high grade, but also low grade inflammatory diseases were associated with elevated MPV.[9] Moreover, MPV, NLR and RDW were also reported to be associated with inflammatory conditions.[10-12] It has been demonstrated that vitamin D deficiency may cause an elevation in mean platelet volume even in healthy subjects.[6] Therefore, we hypothesised that deficiency of vitamin D could have some effects on haemogram parameters.

The aim of this study was to compare MPV, NLR and RDW values of subjects with vitamin D deficiency to those with normal serum vitamin D levels.

METHODOLOGY

Data of patients visiting outpatient clinics of the Abant Izzet Baysal University Hospital, Bolu, Turkey, from March to May 2017, was retrospectively collected from patients' files and computerised database. Inclusion criteria were age >18 years, not using vitamin D products, and non-pregnant women. Subjects were grouped into normal vitamin D and vitamin D deficiency groups, according to the serum vitamin D levels. Seasonal threshold value for vitamin D in Bolu region was set as 10 ng/ml. Patients with active inflammation, infection or chronic kidney disease were excluded from the study. Age, gender, and other general characteristics, such as height, weight, accompanying comorbidities (*e.g.* hypertension, diabetes mellitus, coronary heart

Department of Internal Medicine, Abant Izzet Baysal (AIB) University Hospital, Bolu, Turkey.

Correspondence: Dr. Gulali Aktas, Department of Internal Medicine, Abant Izzet Baysal University Hospital, Golkoy, 14280, Bolu, Turkey.
E-mail: draliaktas@yahoo.com

Received: December 06, 2017; Accepted: July 12, 2018.

disease), and characteristics of the living environment (rural or urban), working place conditions (outside or inside,) marital status (single or married) were also recorded. BMI was calculated by dividing the weight in kilograms to the square of height in meters.

Venous blood samples obtained into sterile standard tubes containing constant amount of anticoagulant. Laboratory tests have been held within several minutes after blood samples obtained. Vitamin D levels and c-reactive protein (CRP) levels were obtained from database and recorded. Haemogram parameters; white blood cell (WBC), neutrophil (neu), lymphocyte (lym) and platelet (PLT) counts, hemoglobin (Hb), Hematocrit (Htc), mean corpuscular volume (MCV), RDW, and MPV values were also recorded. NLR was calculated by simply dividing of neu to lym. The complete blood count analysis were performed in automatic analyser of LH 780 model of Beckman Coulter device (Beckman Coulter In.; Bre CA). Original kits of the manufacturer were used in haemogram assays. Vitamin D levels were detected by measuring serum 25-hydroxyvitamin D.

Data were analysed by SPSS software. (SPSS 15.0; IBM Inc., Chicago, IL, USA). Distribution of variables among study groups were analysed with Kolmogorov-Smirnov test. Results expressed as either mean ±SD or median (interquartile range). Variables were conducted with independent samples t-test or Mann-Whitney U-test. Chi square test used in comparison of categorical variables between study groups. A ROC analysis was performed to determine thresholds of haemogram parameters in detecting vitamin D deficiency. A p-value of <0.05 was considered as statistically significant.

RESULTS

A total of 85 subjects (45 in vitamin D deficiency group and 40 in normal vitamin D group) were enrolled to the study. Median age of the vitamin D deficient group (38 years) was not significantly different than that of the control subjects with normal vitamin D levels (39 years, p=0.96). Six (13%) of 45 in vitamin D deficient group and 13 (32%) of 40 in control group were men. Women were more prevalent in vitamin D deficient group compared to control group (p=0.034). Body weight (74 ±17 kg in study group versus 71 ±15 kg in control group, p=0.35), CRP [1 (3.75) mg/L in study group versus 0.95 (2.4) mg/L in control group, p=0.30], WBC (6581 ±1695 cells/mm^3 in study group versus 6707 ±1800 cells/mm^3 in control group, p=0.74), Hb (13.5 ±1.5 gr/dl in study group versus 13.6 ±1.3 gr/dl in control group, p=0.73), Htc (39.8 ±4 in study group versus 40.5 ±4 in control group, p=0.41), MCV [88.4 (5.3) fL in study group versus 87.5 (6.4) fL in control group, p=0.69], RDW [15.9 (2.1) in study group versus 16.3 (1.7) in control group, p=0.80] and PLT [259089 ±71504 cells/mm^3 in study group versus 235825 ±70382 cells/mm^3 in control group, p=0.14] were not significantly different in vitamin D deficiency group compared to controls. General characteristics of the study population were summarised in Table I and laboratory data of the study population were summarised in Table II.

Vitamin D deficiency was more common in subjects working inside as compared to those working outside (p=0.023), and in subjects with comorbidities compared to those without comorbidities (p=0.031).

Table I: General characteristics of the study groups.

	Vitamin D deficient n (%)	Vitamin D normal n (%)	p
	χ^2 test		
Gender:			
Men	6 (13)	13 (32.5)	0.034
Women	39 (87)	27 (67.5)	
Working environment:			
Outside	17 (38)	25 (62)	0.023
Inside	28 (62)	15 (38)	
Marital status:			
Married	31 (69)	22 (55)	0.19
Single	14 (31)	18 (45)	
Living environment:			
Urban	38 (84)	32 (80)	0.59
Rural	7 (16)	8 (20)	
Comorbidity:			
Absent	29 (64)	34 (85)	0.03
Present	16 (36)	6 (15)	
	Mean ±SD		
Height (m)	1.62 ±0.07	1.67 ±0.09	0.11
Weight (kg)	74.3 ±17	71 ±15.3	0.35
BMI (kg/m^2)	28.4 ±6.6	25.7 ±5.7	0.04
	Median (IQR)		
Age (years)	38 (24)	39 (27.8)	0.96

BMI = Body mass index

Table II: Comparison of laboratory data of the study population.

	Vitamin D deficient n (%)	Vitamin D normal n (%)	p
	Mean ±SD		
WBC (cells/mm^3)	6581 ±1695	6707 ±1800	0.74
Hb (g/dL)	13.5 ±1.5	13.6 ±1.3	0.73
Htc (%)	40 ±4	40 ±3.8	0.41
Plt (cells/mm3)	259089 ±71504	235825 ±70382	0.14
MPV (fL)	7.2 ±0.9	6.3 ±0.7	<0.001
	Median (IQR)		
Vitamin D (ng/ml)	7 (3)	15.9 (10.5)	<0.001
cRP (mg/L)	1 (3.75)	0.95 (2.4)	0.30
NLR (%)	2.3 (1.2)	1.6 (0.8)	0.001
MCV (fL)	88 (5)	87.5 (6)	0.69
RDW (%)	15.9 (2.1)	16.3 (1.7)	0.80

cRP: c-reactive protein.

Table III: Sensitivity and specifity of MPV and NLR in predicting vitamin D deficiency.

	Sensitivity (%)	Specifity (%)	AUC
MPV (at >6.22 fL level)	89	55	0,77
NLR (at >1.69 level)	76	55	0.72

Subjects in control group (1.67 ±0.09 m) were taller than vitamin D deficient patients (1.62 ±0.07 m). The difference was statistically significant (p=0.011). Body mass index of the vitamin D deficient and control groups were 28.4 ±6.5 Kg/m^2 and 25.7 ±5.7 Kg/m^2, respectively (p=0.048). Both MPV (7.2 ±0.9 fL in vitamin D deficients and 6.3 ±0.7 fL in controls) and NLR [2.3 (0.6-5.6) in vitamin D deficients and 1.6 (1-5.8) in controls] were significantly higher in vitamin D deficiency group compared to control subjects (p<0.001 for MPV and p=0.001 for NLR).

ROC analysis indicated that a MPV greater than 6.22 has 89% sensitivity and 55% specifity for Vitamin D deficiency (AUC: 0.77). Another ROC analysis for NLR showed that a NLR greater than 1.69 has 76% sensitivity and 55% specifity for Vitamin D deficiency (AUC: 0.72, Table III).

DISCUSSION

Present study showed that both NLR and MPV could be the markers of the inflammatory burden in vitamin D deficiency.

Association between vitamin D deficiency and inflammatory status has been well established. Nand *et al*. reported that vitamin D has an important role in lowering inflammatory burden.[13] On the other hand, authors found that the deficiency of vitamin D had caused more enormous inflammation in immuno-compromised subjects.[14] Interleukin, a marker of inflammation, is inversely correlated with serum vitamin D levels.[15] These evidences show the relation between vitamin D deficiency and inflammation.

Low grade inflammatory diseases are characterised with increased MPV values.[16] Elevated MPV has also been found in ulcerative colitis.[17] Elevated MPV and NLR in vitamin D deficient subjects in present study is not surprising because both these haemogram derived indices were supposed to be markers of inflammation. Increased MPV in vitamin D deficient subjects in our study was a result that was compatible with literature data. Moreover, we showed that a MPV greater than 6.22 fL has a 89% sensitivity and 55% specifity for vitamin D insufficiency.

Neutrophil to lymphocyte ratio, as a haemogram derived inflammatory marker, have been mostly studied in cancer and acute coronary diseases.[18] Systemic inflammation, even in low grade, produce neutrophilia and lymphopenia, together result in increased NLR.[19] Because vitamin D deficiency induces inflammation, increased NLR in the present study in subjects with insufficient vitamin D levels is an inevitable result.

Present study did not show an association between RDW and vitamin D deficiency. Although RDW has been introduced as an inflammatory marker in certain conditions. Accompanied diseases may affect the RDW indice in haemogram test, such as iron deficiency anemia, which causes elevation in RDW.[11,20] On the other hand, similar to our results, a study reported that there was no association between vitamin D and red blood cell count and indices in vitamin D deficient subjects.[21]

Measurement of 25-hydroxyvitamin D is widely used for detecting vitamin D stores,[22] because half-life of serum 25-hydroxyvitamin D is about three weeks,[23] and longer than vitamin D and 1,25(OH)2D. However, there are challenges in measuring serum 25-hydroxyvitamin D by different assays. The most important challenge may be the need for well-trained and motivated operators for valid test results.[24] Thus, elevation in MPV and/or NLR in otherwise healthy subjects may indicate underlying vitamin D deficiency in the regions exposing less sunlight.

Continuous low grade inflammatory environment in vitamin D deficiency may lead to interaction between inflammatory cytokines and megakaryopoiesis in bone marrow and cause production of larger platelets, which increase the measured MPV value.[25]

Limitations of present study are relatively small cohort and retrospective design. Therefore, prospective larger studies are needed to confirm the results we have presented.

CONCLUSION

Elevated MPV and NLR may be the indicator of underlying serious vitamin D deficiency. Physicians should be alert and order a vitamin D assay in patients with elevated MPV or NLR, especially in endemic areas for vitamin D deficiency.

REFERENCES

1. Yousef FM, Jacobs ET, Kang PT, Hakim IA, Going S, Yousef JM, *et al*. Vitamin D status and breast cancer in Saudi Arabian women: case-control study. *Am J Clin Nutr* 2013; **98**:105-10.
2. Syal SK, Kapoor A, Bhatia E, Sinha A, Kumar S, Tewari S, *et al*. Vitamin D deficiency, coronary artery disease, and endothelial dysfunction: observations from a coronary angiographic study in Indian patients. *J Invasive Cardiol* 2012; **24**:385-9.
3. Shor R, Tirosh A, Shemesh L, Krakover R, Chaim AB, Mor A, *et al*. 25 hydroxyvitamin D levels in patients undergoing coronary artery catheterization. *Eur J Int Med* 2012; **23**:470-3.
4. Kunadian V, Ford GA, Bawamia B, Qiu W, Manson JE. Vitamin D deficiency and coronary artery disease: a review of the evidence. *Am Heart J* 2014; **167**:283-91.
5. Beilfuss J, Berg V, Sneve M, Jorde R, Kamycheva E. Effects of a 1-year supplementation with cholecalciferol on interleukin-6, tumor necrosis factor-alpha and insulin resistance in overweight and obese subjects. *Cytokine* 2012; **60**:870-4.
6. Cumhur Cure M, Cure E, Yuce S, Yazici T, Karakoyun I, Efe H. Mean platelet volume and vitamin D level. *Ann Lab Med* 2014; **34**:98-103.

7. Di Rosa M, Malaguarnera G, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immuno-modulatory effects of vitamin D3 in human monocyte and macrophages. *Cellular Immunol* 2012; **280**:36-43.
8. Polinska B, Matowicka-Karna J, Kemona H. Assessment of the influence of the inflammatory process on the activation of blood platelets and morphological parameters in patients with ulcerative colitis (colitis ulcerosa). *Prostaglandins* 2011; **12**:16.
9. Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HKM, *et al*. Mean platelet volume in type 2 diabetes mellitus. *J Lab physicians* 2012; **4**:5.
10. Ahsen A, Ulu MS, Yuksel S, Demir K, Uysal M, Erdogan M, *et al*. As a new inflammatory marker for familial Mediterranean fever: neutrophil-to-lymphocyte ratio. *Inflammation* 2013; **36**:1357-62.
11. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* 2009; **133**:628-32.
12. Song CS, Park DI, Yoon MY, Seok HS, Park JH, Kim HJ, *et al*. Association between red cell distribution width and disease activity in patients with inflammatory bowel disease. *Dig Dis Sci* 2012; **57**:1033-8.
13. Nand N, Mittal R. Evaluation of effect of vitamin D deficiency on anemia and erythropoietin hyporesponsiveness in patients of chronic kidney disease. *J Assoc Physicians India* 2017; **65**:38.
14. Manion M, Hullsiek KH, Wilson EM, Rhame F, Kojic E, Gibson D, *et al*. Vitamin D deficiency is associated with IL-6 levels and monocyte activation in HIV-infected persons. *PLoS One* 2017; **12**:e0175517.
15. Toujani S, Kaabachi W, Mjid M, Hamzaoui K, Cherif J, Beji M. Vitamin D deficiency and interleukin-17 relationship in severe obstructive sleep apnea-hypopnea syndrome. *Ann Thoracic Med* 2017; **12**:107.
16. Yuri Gasparyan A, Ayvazyan L, Mikhailidis D, Kitas G. Mean platelet volume: a link between thrombosis and inflammation? *Curr Pharmaceutic Design* 2011; **17**:47-58.
17. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, *et al*. Mean platelet volume: a useful marker of inflammatory bowel disease activity. *Am J Gastroenterol* 2001; **96**:776-81.
18. Tamhane UU, Aneja S, Montgomery D, Rogers E-K, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. *Am J Cardiol* 2008; **102**:653-7.
19. Zahorec R. Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. *Brat Lek listy* 2001; **102**:5-14.
20. Aktas G, Sit M, Dikbas O, Tekce B, Savli H, Tekce H, *et al*. Could red cell distribution width be a marker in Hashimoto's Thyroiditis? *Exp Clin Endocrinol Diab* 2014; **122**:572-4.
21. Soliman AT, Eldabbagh M, Elawwa A, Ashour R, Saleem W. The effect of vitamin D therapy on hematological indices in children with vitamin D deficiency. *J Tropic Pediatr* 2012; **58**: 523-4.
22. Zerwekh JE. Blood biomarkers of vitamin D status. *Am J Clin Nutr* 2008; **87**:1087S-91S.
23. Clemens TL, Zhouf X-Y, Myles M, Endres D, Lindsay R. Serum vitamin D2 and vitamin D3 metabolite concentrations and absorption of vitamin D2 in elderly subjects. *J Clin Endocrinol Metab* 1986; **63**:656-60.
24. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. *Am J Clin Nutr* 2008; **88**: 507S-10S.
25. Bath P, Butterworth R. Platelet size: measurement, physiology and vascular disease. *Blood Coagul Fibrinol* 1996; **7**:157-61.