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Evaluation of the relationship between neutrophil-to-lymphocyte ratio and 25-hydroxy vitamin D levels in hemodialysis patients, Isfahan, Iran

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Received: 11 Jan. 2021

Accepted: 4 June 2021

ePublished: 3 July 2021

Keywords: Neutrophil-to-lymphocyte ratio, Vitamin D, End-stage renal disease, Hemodialysis, Inflammation, Inflammatory markers

Abstract

Introduction: Chronic low-grade inflammation is a comorbid factor in Chronic kidney disease (CKD), and especially in chronic dialysis patients. Recently, neutrophil-to-lymphocyte ratio (NLR), which is very available and affordable, has emerged as an inflammatory index in many disorders such as CKD. Several studies also have shown the anti-inflammatory effects of vitamin D, and scientists believe that we must prevent vitamin D deficiency in CKD patients. Vitamin D supplementation may decrease mortality and morbidity in end-stage renal disease (ESRD) patients.

Objectives: There are few studies on the association between serum 25-hydroxy vitamin D levels and NLR in ESRD patients. Therefore, we tried to evaluate this correlation, hoping to be a key for more researches.

Patients and Methods: We enrolled 140 ESRD hemodialysis patients in a cross-sectional study and evaluated the correlation of NLR with serum 25-hydroxy vitamin D levels and some demographic factors in these patients.

Results: Our results showed a statistically significant negative correlation between NLR and serum 25-hydroxy vitamin D levels ($P=0.010$, $r=-0.216$). However, other variables such as disease duration and age had no correlation with NLR ($P=0.649$, $r=-0.039$ and $P=0.781$, $r=-0.024$, respectively). We also used a linear regression test to check any confounder associated with NLR, and the regression was only significant for serum 25-hydroxy vitamin D levels ($P=0.011$, $B=-0.009$).

Conclusion: Based on the results, serum 25-hydroxy vitamin D levels can be considered a predictor for NLR in hemodialysis patients.

Introduction

The role of low-grade inflammation in the pathogenesis of many chronic diseases is an agreed issue, and the inflammatory base of many chronic diseases revealed in the last decades (1). Chronic kidney disease (CKD) is one of these diseases. It is a progressive and non-reversible disorder that is a health problem worldwide. The micro-inflammatory state of CKD leads to atherosclerosis and cardiovascular disease and an increased rate of morbidity and mortality (2,3). In other words, chronic subclinical inflammation is regarded as a comorbid factor in CKD, and especially in chronic dialysis patients (4). Recently, neutrophil-to-lymphocyte ratio (NLR) has emerged as an inflammatory marker for several chronic subclinical inflammatory diseases such as CKD (5,6). Some facts explain how calculating this ratio can be an indicator of inflammation. One of these

Key point

In a study on 140 hemodialysis patients, we found a statistically significant negative correlation between NLR and serum 25-hydroxy vitamin D levels ($P=0.010$, $r=-0.216$). We concluded that 25-hydroxy vitamin D levels might be considered a predictor for NLR in hemodialysis patients.

facts is that neutrophil has inflammatory effects (7), and its count represents the inflammation (6), while inflammation itself increases lymphocyte apoptosis (8). Besides, lymphocytes mostly have a regulatory function in the immune system (9), and their count shows the general stress and nutritional state, not inflammation (6). The increased neutrophil count with the decreased lymphocyte count predicts mortality in hemodialysis patients (10). In addition, NLR can determine the CKD progression toward the hemodialysis state (11).

Citation: Baradaran A, Hoseini Z, Hedayati P, Shirvani R. Evaluation of the relationship between neutrophil-to-lymphocyte ratio and 25-hydroxy vitamin D levels in hemodialysis patients, Isfahan, Iran. J Prev Epidemiol. 2021;6(2):e26. doi: 10.34172/jpe.2021.26.



Another factor that is noteworthy in CKD is the serum level of vitamin D. Vitamin D deficiency, including the low level of serum 25-hydroxy vitamin D and 1,25(OH)₂D, has been reported in CKD and dialysis patients generally.

Vitamin D deficiency prompts secondary hyperparathyroidism and its complications (12). An optimal level of serum 25-hydroxy vitamin D has been considered more than 30 ng/dL (13). However, studies on the optimal vitamin D level and the safety and effectiveness of vitamin D administration in these patients are continuing (14).

Vitamin D supplementation may decrease mortality and morbidity in end-stage renal disease (ESRD) patients (3). However, metastatic calcification is the dramatic result of the high level of calcium and phosphorous and can occur by using supplements (3, 15). Therefore, the general recommendation of taking vitamin D supplementation needs more researches.

A study in 2016 showed the correlation of 25(OH) D serum levels and inflammatory markers such as NLR in hemodialysis patients (16). Another cross-sectional study in 2019 on 129 maintenance hemodialysis patients revealed that serum 25-hydroxy vitamin D levels has a significant but weak inverse correlation with NLR (17).

The effect of vitamin D on inflammation and the impact of inflammation on vitamin D level are controversial issues. However, several studies show the anti-inflammatory effects of vitamin D (18-20).

Objectives

Since few studies have been carried out on the association between vitamin D and NLR in hemodialysis patients, we evaluated the correlation between NLR and serum 25-hydroxy vitamin D levels, hoping to be a key for more researches.

Patients and Methods

We conducted this cross-sectional study in 2021 in hemodialysis departments of Amin and Fatemiyeh hospitals, Isfahan, Iran. Blood sampling of hemodialysis patients routinely is conducted to check some parameters such as CBC and 25-hydroxy vitamin D levels. We were present in the hemodialysis departments on the days of blood sampling. After taking consent from patients and considering some inclusion and exclusion criteria, finally, 140 patients were selected. The inclusion criteria were; the ESRD patients who were at least 18 years old, had been on hemodialysis for at least three months, and had no special

conditions such as drug or alcohol addiction (based on patient self-expression). The exclusion criteria based on their medical history and physical examination were; having an active infection, having malignancy or any active inflammatory disease, and taking any immunosuppressive drugs. We write information including age, gender, duration of being on hemodialysis, and having a medical history of hypertension (HTN) disease, diabetes mellitus (DM), or polycystic kidney disease. Then we followed up their blood test results to obtain NLR and the serum level of 25(OH)D.

Statistical analysis

We analyzed data by using the SPSS software version 25. All quantitative variables followed the normal distribution. We also used the Pearson correlation coefficient, independent t-test, and linear regression test. The significance level in the present study was less than 0.05.

Results

One hundred forty patients participated in this study, 89 of whom were male, and 51 were female. Ninety-five patients had DM, 79 patients had HTN disease, and no one had polycystic kidney disease. The mean age of patients was 61.9 ± 12.9 years. They have been on hemodialysis for an average of 2.94 ± 2.43 years. The mean of NLR was 2.39 ± 0.94 , and the average serum levels of vitamin D was 36.03 ± 23.30 ng/mL. The maximum and minimum amount of these variables are in Table 1.

The mean level of 25-hydroxy vitamin D levels was compared in subgroups including men or women, patients with or without HTN disease, and patients with or without DM. There were no statistically significant differences between men or women ($P=0.900$) and between patients with or without HTN disease ($P=0.368$). However, the 25-hydroxy vitamin D serum levels were significantly different between diabetic and non-diabetic patients ($P=0.043$). The mean of NLR also was evaluated in these subgroups, and there was no statistically significant difference (gender; $P=0.298$, HTN \pm ; $P=0.191$, DM \pm ; $P=0.463$; Table 2).

We evaluated the correlation of serum levels of 25(OH)D and NLR by Pearson's correlation coefficient test. We found a statistically significant negative correlation between these two variables ($P=0.010$, $r=-0.216$). However, other variables such as disease duration and age did not correlate with NLR ($P=0.649$, $r=-0.039$ and $P=0.781$, $r=-0.024$, respectively). We also used a linear regression test to check

Table 1. Mean and standard deviation of the basic information

Variable	Minimum	Maximum	Mean	Standard Deviation
Age (y)	28.00	85.00	61.9	12.9
NLR	0.28	5.94	2.39	0.94
25-hydroxy vitamin D levels (ng/mL)	8.00	106.00	36.03	23.30
Disease duration	0.30	11.00	2.94	2.43

NLR: Neutrophil-to-lymphocyte ratio.

any confounder associated with NLR, and the regression was only observed for vitamin D ($P=0.011$, $B=-0.009$) (Tables 3 and 4).

Discussion

Two cross-sectional studies in 2016 (16) and 2019 (17) showed the negative correlation of 25(OH)D serum levels and inflammatory markers including NLR in hemodialysis patients ($P<0.001$, $r=-0.408$, $P=0.013$, $r = -0.219$, respectively). Similar to these studies, we showed the existence of this correlation. This result means that higher levels of serum 25(OH)D are associated with lower ratio of NLR in hemodialysis patients. Since NLR is a subclinical inflammatory marker (21), this negative correlation may be consistent with reports about the anti-inflammatory effects of 25(OH)D levels. In our study, DM and HTN that are the two main underlying causes of ESRD (22) were not the confounders of NLR. Only 25-hydroxy vitamin D levels were meaningful in association with NLR. However, some studies have shown the association of NLR with some factors in diabetic or hypertensive patients. For instance, in DM type 2 patients NLR median has been significantly ($P<0.001$) elevated comparing to healthy controls [2.44 (1.9) versus 1.5 (0.9), respectively] (23). In addition, in type 2 DM patients, increased NLR levels were associated with elevated HbA1c and poor glycemic control (24). Furthermore, patients with DM who were vitamin D-deficient had the highest neutrophil count ($P =$

0.001) (25). Besides, a 9-year cohort study on 6278 patients revealed a significant association between the NLR and incident HTN (26). Hence, it seems that more studies are needed, especially for evaluating underlying diseases, to accurately determine the value of NLR and its relationship with 25(OH)D in nephrology.

Conclusion

Our study showed a statistically significant negative correlation between 25-hydroxy vitamin D levels and NLR in hemodialysis patients. Thereby, serum 25-hydroxy vitamin D levels may be considered a predictor for NLR in hemodialysis patients. However, more studies are needed.

Limitations of the study

We did this study in only two departments of hemodialysis. More studies with more sample sizes are needed, especially for evaluating the effect of underlying causes of ESRD. Besides, lots of ESRD patients take vitamin D supplements. Neutrophils have a circulating half-life of 6–8 h, and they are produced at a rate of 5×10^{10} – 10×10^{10} cells/day (27). Therefore, we checked the serum level of 25(OH)D in association with NLR regardless of the source of vitamin D or time course of taking vitamin D supplementation. However, maybe more studies with controlling these factors may also be required.

Authors' contribution

The principal investigators of the study were AB and ZH. They were included in preparing the concept and design of study and writing the manuscript and critically evaluated the intellectual contents. PH and ZH participated in designing the method of study, and data collection. RS participated in data collection and drafted the manuscript. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare no conflict of interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Isfahan University of Medical Sciences approved this study. The institutional ethical committee at Isfahan University of Medical Sciences approved the study protocols (IR.MUI.MED.REC.1399.1073). Accordingly, informed consent was

Table 2. Comparison of the serum 25-hydroxy vitamin D levels and NLR across gender, DM and HTN

Variable		Mean	SD	P value
Vitamin D levels	Male	36.22	23.85	0.900
	Female	35.70	22.54	
NLR	Male	2.44	1.01	0.298
	Female	2.28	0.79	
Vitamin D levels	With hypertension	37.59	23.95	0.368
	Without hypertension	34.00	22.47	
NLR	With hypertension	2.48	1.014	0.191
	Without hypertension	2.27	0.83	
Vitamin D levels	With diabetes mellitus	33.29	21.49	0.043
	Without diabetes mellitus	41.81	26.05	
NLR	With diabetes mellitus	2.43	0.94	0.463
	Without diabetes mellitus	2.30	0.93	

NLR: Neutrophil-to-lymphocyte ratio.

Table 3. Pearson's correlation between NLR and other effective factors

Variable		Disease duration	Vit D	Gender	Age	NLR
Disease duration	Pearson (r)	1	0.242	0.043	0.124	-0.039
	P value	-	0.004	0.618	0.144	0.649
Vitamin D levels	Pearson (r)	0.242	1	-0.011	-0.038	-0.216
	P value	0.004	-	0.900	0.654	0.010
Gender	Pearson (r)	0.043	-0.011	1	0.001	-0.083
	P value	0.618	0.900	-	0.993	0.330
Age	Pearson (r)	0.124	-0.038	0.001	1	-0.024
	P value	0.144	0.654	0.993	-	0.781

NLR: Neutrophil-to-lymphocyte ratio.

Table 4. Linear regression to predict the neutrophil-to-lymphocyte ratio

Variable	Unstandardized coefficients		P value
	Beta coefficient	Standard error	
Diabetes mellitus	0.213	0.186	0.255
Hypertension	0.323	0.168	0.057
Disease duration	0.017	0.034	0.614
Vitamin D levels	-0.009	0.004	0.011
Gender	-0.232	0.166	0.166
Age	-0.004	0.006	0.565

taken from all participants. This study was extracted from the M.D., thesis of Zahra Hoseini at this university (Thesis# 399971). Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

None.

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