

Manuscript ID ZUMJ-2308-2861 (R2)

DOI 10.21608/ZUMJ.2023.231856.2861

ORIGINAL ARTICLE

Relation between 25-hydroxy Vitamin D Levels and Neutrophil-to-Lymphocyte Ratio in Chronic Renal Disease Patients Under Hemodialysis.

Eman Ahmed Elsayed Abo-Elela¹, Mabrouk Ibrahim Ismail², Ibrahim Mohammed Ibrahim Salem², Amany Mohyeldin Sediq³

¹ Resident at Hehia central Hospital-Nephrology unit

² Internal Medicine Department, Faculty of Medicine, Zagazig university

³ Clinical Pathology Department, Faculty of Medicine, Zagazig university

Correspondence to:

Eman Ahmed Elsayed Abo-Elela

Email:

emyahmedaboalola@gmail.com

Submit Date 2023-08-26

Revise Date 2023-09-12

Accept Date 2023-09-16



ABSTRACT

Background and Aim: The progression of chronic kidney disease (CKD) toward the need for hemodialysis can be predicted by the neutrophil-to-lymphocyte ratio (NLR), NLR in hemodialysis patients may be predicted by their serum 25-hydroxy vitamin D levels. This study aimed to diagnose inflammation as early as possible among hemodialysis patients.

Patients and Methods: This cross-sectional study was done on 80 selected CKD patients from the nephrology units at Hehia Central Hospital and internal medicine department at Zagazig University Hospital. Subgroup analyses comparing the mean 25-hydroxy vitamin D (VD) levels and NLR between men and women, patients with and without the hypertensive disease (HTN), and patients with and without diabetes mellitus (DM).

Results: Mean level of Vitamin D was significantly higher in males than females with p value=0.001, however, a significantly higher NLR was found in females than in males with p value=0.001, Vit D level was significantly correlated with inflammatory markers (neutrophils, lymphocytes, as well as NLR with p value=0.001 for each), we found clinical and statistically significant prediction of NLR by Vit D with p value=0.001.

Conclusion: We showed a significant inverse correlation between serum vitamin D levels and the NLR values, which are inexpensive and broadly available inflammation markers. Our regression analyses indicate a significant relationship between these inflammatory markers and vit D levels level.

Keywords: 25-hydroxy Vitamin D, Neutrophil-to-Lymphocyte Ratio, Chronic Renal Disease Hemodialysis.

INTRODUCTION:

One of the biggest causes of death and morbidity around the world is chronic kidney disease (CKD). Increased rates of cardiovascular disease, atherosclerosis, and mortality are all results of the micro-inflammatory condition brought on by CKD. ^[1]

Chronic kidney disease (CKD) frequently has a co-morbid component known as persistent subclinical inflammation, especially in patients on chronic dialysis. ^[2]

Inflammatory markers, such as neutrophil-to-

lymphocyte ratio (NLR), have emerged as a useful diagnostic tool in recent years for a variety of chronic, subclinical disorders, including CKD ^[3].

Some evidence shows that this ratio can be used as a marker of inflammation. One of these is that neutrophils emerge with an inflammatory effect. ^[4], which in turn is a measure of inflammation ^[5], whereas lymphocyte apoptosis is elevated by inflammation ^[6]. NLR is also useful for predicting whether or not a patient would require hemodialysis when their CKD worsens. ^[7]. Patients on hemodialysis are more likely to die if

their neutrophil count is high and their lymphocyte count is low.^[8]

Serum vitamin D levels are also important in chronic kidney disease. Patients with chronic kidney disease and those undergoing dialysis have been found to have low levels of vitamin D, as evaluated by blood 25-hydroxy vitamin D and 1,25(OH)₂D. Secondary hyperparathyroidism, and the health problems connected with it, are caused by insufficient levels of vitamin D.^[9]

In hemodialysis patients, the levels of 25-hydroxy vitamin D and NLR were found to be inversely related in a study conducted in 2021. As a result, hemodialysis patients' 25-hydroxy vitamin D blood levels may be utilized as a predictor of NLR.^[10]

Vitamin D's impact on inflammation and inflammation's effect on vitamin D levels are both hotly debated topics. Nonetheless, vitamin D has been shown in multiple trials to have anti-inflammatory properties.^[11]

The aim of this work is early diagnosis of inflammation among hemodialysis patients.

METHODS:

This cross-sectional study was performed on 80 Selected CKD patients from the nephrology unit at Hehia Central Hospital in collaboration with the Nephrology unit of the internal medicine department at Zagazig University Hospital.

The Internal Medicine Division of Zagazig University Hospitals gave its approval to the study after it was accepted by the hospital's Institutional Review Board (IRB) (#10001/26-10-2022). Written informed consent was obtained from all participants. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All cases with age \geq 18 years from both sexes female and male, having end-stage renal disease (ESRD), who have been on hemodialysis for at least three months were included. Any cases with the following criteria were excluded; immunodeficiency, any active inflammatory diseases, malignancy, or having an active infection.

Methods:

Complete medical histories were obtained from all patients, with an emphasis on preexisting conditions such as diabetes mellitus (DM), hypertension (HTN) disease, as well as polycystic kidney disease. The clinical examination included vital signs, weight, height, and local abdominal examination. Blood samples were obtained from

the patients to measure the following laboratory parameters: (a) EDTA blood sample to measure CBC on Celltac- MEK-6510K (Nihon Kohden, Japan), and (b) Whole blood samples on the plain tube for separation of serum to measure serum creatinine, blood urea nitrogen (BUN), sodium, potassium, serum calcium, serum phosphorus, iron profile, lipid profile. All were measured on Beckman Coulter/au480 chemistry analyzer (Beckman Coulter Diagnostics, USA). Meanwhile, Intact PTH and the serum level of 25(OH)D were measured by TOSOH AIA-360 immunoanalyzer using a dedicated reagent (Tosoh Bioscience, Inc., USA).

A normal NLR is roughly 1-3, the values higher than 3.0 and below 0.7 in adults are pathological, An NLR of 6-9 suggests mild stress (e.g. a patient with uncomplicated appendicitis). Critically ill patients will often have an NLR of \sim 9 or higher (occasionally reaching values close to 100).

Normal range of vitamin D is considered when it is above 30 ng/ml, vitamin D deficiency is considered when (serum 25-OH-D levels < 20 ng/mL) and insufficiency (serum 25-OH-D levels of 21 to 30 ng/mL).

ECG, Echocardiography, and pelvic and Abdominal Ultrasound were also done.

STATISTICAL ANALYSIS:

Microsoft Excel was used to code, process, and analyze data gathered from a patient's medical history, physical examination, laboratory tests, and outcome measurements. Statistics for the Social Sciences (SPSS) was then used to import the data (SPSS version 27.0). IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp. According to the type of data qualitative data is presented as number and percentage, parametric quantitative data is presented as mean \pm SD, non-parametric quantitative data is presented as range and median, and the Pearson correlation test was used to find the correlation between the studied parameters. Linear regression is done to detect the prediction of NLR. P value was set at <0.05 for significant results and <0.001 for high significant results.

RESULTS:

In the present study, most of the cases were males 46(57.5%), and the age ranged from 22 to 75 years with a mean age of 52.2 years, other demographics and comorbidities are shown in (Table 1).

Table 1: Demographic and clinical data of studied group

		N	%
Gender	Female	34	42.5
	Male	46	57.5
		Mean	SD
Age (Years)		52.2	13.6
Weight (Kg)		77.5	16.6
		N	%
Co-morbidity	None	23	28.7
	HTN	41	51.2
	HTN/DM	6	7.5
	Hypotension	7	8.8
	DM	3	3.8
		Median	Range (min-max)
Duration of HD (months)		48	3-132

HTN: Hypertension, DM: Diabetes Mellitus, HD: Hemodialysis.

Males had a significantly higher mean vitamin D level compared to females with p value=0.001, (Table 2),

Table 2: Laboratory data of studied group

	Mean	SD
Hb (g/dl)	9.8	1.3
TLC (10³)	7.4	2.2
Neutrophile (%)	71.5	8.4
Lymphocyte (%)	22.6	7.5
	Median	Range
NLR	3.2	1.2-8.7
	Mean	SD
Vitamin D (ng/ml)	17.8	4.21
Ca (mg/dl)	9.3	0.65
Phosphorus (mg/dl)	4.0	0.88
Creatinine (mg/dl)	7.4	1.96
BUN (mg/dl)	134.4	19.47
Na (mmol/L)	139.3	2.50
K (mmol/L)	4.6	0.76
	Median	Range
Fe (mcg/dl)	316	10-1920
PTH (pg/dl)	191	11-1807

Hb: Hemoglobin, TLC: Total leucocyte count, NLR: Neutrophil lymphocyte ratio, Ca: calcium, BUN: Blood urea nitrogen, Na: Sodium, K: Potassium, Fe: Ferritin, PTH: Parathyroid hormone.

we also found a significantly higher NLR in females than males with p value=0.002, while Patients with and without hypertension and diabetes showed no statistically significant differences in NLR. We studied the correlation

between NLR, and participants' factors such as age, gender, and duration of disease, NLR was significantly correlated with age only with p value=0.005 (Table 3).

Table 3: Comparison of Vit D level, and NLR across gender, HTN and DM

	Vit D (ng/ml)			NLR		
	Mean	SD	P value	Mean	SD	P value
Female	15.36	2.71	0.001*	4.40	2.01	0.002*
Male	19.60	4.23		3.18	1.40	
With HTN	17.11	3.36	0.22	3.92	1.83	0.36
Without HTN	18.28	4.70		3.54	1.75	
With DM	18.00	4.09	0.22	3.57	1.68	0.07
Without DM	16.17	5.05		4.70	2.37	

Independent t-test for testing significance difference in means

* Statistical significance at <0.05

HTN: Hypertension, DM: Diabetes Mellitus NLR: Neutrophil lymphocyte ratio

With liner analysis of the correlation between vitamin D level and inflammatory markers, we found an insignificant negative correlation between Vit D level and TLC with p=0.8, a significant positive correlation between Vit D level and Lymphocyte count with p=0.001, There

is a significant inverse relationship between vitamin D and neutrophil count, and an inverse relationship between vitamin D and neutrophil-lymphocyte ratios with p values=0.001 for each (Table 4).

Table 4: Correlation between Vit D and inflammatory markers

	r	P value
TLC	-0.027	0.8
Neutrophile	-0.56	0.001*
Lymphocyte	0.64	0.001*
NLR	-0.65	0.001*

r= spearman correlation; * Statistical significance at <0.05

TLC: total leucocyte count, NLR: Neutrophil lymphocyte ratio

From linear regression analysis of different effector factors, we found clinical and statistically

significant predictions of NLR by Vit D with p value=0.001 (Table 5).

Table 5: Linear regression to predict the NLR

	Standardized Coefficients		P value
	Std. Error	Beta	
Age	0.015	-0.131	0.261
Gender	0.393	-0.291	0.010*
Duration	0.006	0.011	0.916
Vit D	0.036	-0.654	0.001*
HTN	0.398	-0.127	0.252
DM	0.629	0.214	0.060

* Statistical significance at <0.05

HTN: Hypertension, DM: Diabetes Mellitus

DISCUSSION

The neutrophil-to-lymphocyte ratio (NLR) is used as an inflammatory marker for several chronic, subclinical inflammatory illnesses, including CKD [3].

Vitamin D's impact on inflammation and inflammation's effect on vitamin D levels are both hotly debated topics. Nonetheless, vitamin D has been shown in multiple trials to have anti-inflammatory properties. [11].

As with other chronic diseases, such as CKD, NLR have recently been recognized as readily available and affordable markers that can be utilized to quantify and predict bad outcomes and survival rates. [12].

The low levels of 25-hydroxyvitamin D in ESRD patients are likely the result of a combination of

factors, including inactivity while on dialysis, a lack of sun exposure, a diet low in vitamin D-rich foods, and perhaps altered skin synthesis of vitamin D due to uremia. [13].

The current study aimed to determine if hemodialysis patients' 25-hydroxy vitamin D levels were correlated with their neutrophil-to-lymphocyte ratio. The goal of this study was to determine whether or not serum 25-hydroxy vitamin D levels were a good predictor of NLR in hemodialysis patients.

In the present study, most of the cases were males 46(57.5%), and the age ranged from 22 to 75 years with a mean age of 52.2 year, in contrast to our finding of the high frequency of male patients, Satirapoj et al., [14] in their study

reported that the male sex was less frequent. The difference between sexes probably reflects the cultural and religious practices leading to less skin exposure in women than in men.

In the present study, NLR ranged from 1.2 to 8.7 with a mean of 3.7, this was supported by Ahbap et al.,^[12], NLR in patients with ESRD was 3.7 ± 0.2 . There is a worldwide epidemic of vitamin D deficiency, but hemodialysis patients have an even greater prevalence of both deficiency (serum 25-OH-D values 20 ng/mL) and insufficiency (serum 25-OH-D levels 21 to 30 ng/mL).^[9]

In the present study, vitamin D levels ranged from 10.5 to 28.2 ng/ml with a mean of 17.8 ng/ml . 65(81.3%) with Vit D deficiency and 13(16.3%) Vit D insufficiency.

Comparing our results with those of Annibale, who found vitamin D deficiency in 73% and insufficiency in 23% of HD patients, we find that they are both consistent with each other.^[15] In another study, vitamin D deficiency and insufficiency were observed in 40 and 20 %, respectively.^[16]

Wolf et al.,^[17] found 22% of the United States hemodialysis patients to be vitamin D sufficient but only 18% were classified as severely deficient ($< 10 \text{ ng/dL}$). The differences may be due to the use of fortified food with vitamin D in the United States population.

The variant prevalence of vitamin D levels in different studies could be due to different races, nutritional behavior, sun exposure, different cultural, and religious customs, and different cut-off point for Vit D level category.

In the present study, we found that Vit D was significantly higher in males than females with $p \text{ value} = 0.001$. Previous research contrasting non-hemodialysis people with hemodialysis populations is consistent with our results.^[18]

One theory puts this discrepancy down to the fact that women are less likely to get enough vitamin D from the sun because of the customary religious attire they wear (complete body covering). An increase in vitamin D sequestration in adipose tissue likely contributes to women's poor vitamin D status.^[18] Our results were comparable to Mirchi et al.,^[19], as regards to correlation between Vit D levels and different parameters; Vitamin D levels were significantly correlated with body mass index, serum calcium, parathyroid hormone, diabetes, hypertension, and dialysis duration.

In the present work, we studied the correlation between NLR, and participants' factors such as age, gender, and duration of disease, NLR was

significantly correlated with age only with $p \text{ value} = 0.005$. however, NLR has been linked in certain research to people with diabetes or hypertension. NLR, for example, is much higher in type 2 diabetes patients.^[20] The NLR was also significantly associated with the incidence of HTN in a large cohort study^[21]. This difference could be attributed to different sample groups and participants selection.

Researchers have found a link between vitamin D and inflammation, although the direction of the correlation is unclear. A decrease in vitamin D levels has been linked to inflammation, whereas an increase in vitamin D levels has been shown to prevent inflammation in other studies^[18]

Very little is known about vitamin D and its association with inflammatory markers in the HD population. Mirchi et al., showed that the group with 25-OH-D 10 ng/mL had significantly higher levels of NLR and CRP; they also identified a modest negative connection between 25-OH-D and CRP^[18].

New inflammatory markers NLR and PLR were shown to have a negative correlation with 25-OH-D levels, as discovered by Villafuerte-Ledesma et al.^[16]. The research adds credibility to our investigation.

With liner analysis of the correlation between vitamin D level and inflammatory markers, we found an insignificant negative correlation between Vit D level and TLC with $p = 0.8$, a significant positive correlation between Vit D level and Lymphocyte count with $p = 0.001$, There is a significant inverse relationship between vitamin D and neutrophil count, and an inverse relationship between vitamin D and neutrophil-lymphocyte ratios with $p \text{ values} = 0.001$ for each. This negative correlation may be consistent with reports about the anti-inflammatory effects of 25(OH)D levels^[22].

Unlike our finding, Yildirim et al.,^[23] studied the association of 25-OH-D with CRP, ESR, and TLC in a population with and without CKD, and did not find any association between inflammatory markers and 25-OH-D. This could be explained by, high levels of CRP, ESR and TLC in vitamin D deficient patients might be related to other factors such as infectious, inflammatory status, malnutrition, cachexia, or multivitamin deficiency. These factors and others may affect high morbidity and mortality in patients with vitamin D3 deficiency. Therefore, replacement of vitamin D alone could be corrected only in patients with vitamin D deficient patients in the foreground.

From linear regression analysis of different effector factors, we found clinical and statistically significant predictions of NLR by Vit D and gender. Supported by Kara and Soylu,^[18], in their regression analysis they found a significant relationship between 25(OH)D levels and CRP, and NLR values.

Study of Forget et al.,^[24] indicate more complications were associated with higher levels of NLR, they suggested the inflammatory status of a patient could be assessed easily with the NLR which could be considered as an independent marker of poor prognosis.

Our work has limitations:

A cross-sectional approach was taken in this paper. Therefore, we are unable to determine whether NLR has a role in regulating vitamin D levels in the blood. The biochemical measurements were only performed once, which does not take time-dependent changes into account. Enough patients were included despite the short sample size to demonstrate statistically significant associations.

Conclusion:

We found that the NLR values were significantly higher in the vitamin D deficiency patients. We showed a significant inverse correlation between serum vitamin D levels and the NLR values, which are inexpensive and broadly available inflammation markers. Our regression analyses indicate a significant relationship between these inflammatory markers and vit D levels level. Our study showed a statistically significant negative correlation between 25-hydroxy vitamin D levels and NLR in hemodialysis patients. Therefore, serum 25-hydroxy vitamin D levels may be considered a predictor for NLR in hemodialysis patients.

Reference

1. Sterling KA, Eftekhari P, Girndt M, Kimmel PL, Raj DS. The immunoregulatory function of vitamin D: implications in chronic kidney disease. *Nat Rev Nephrol.* 2012;8(7):403-12.
2. Nowak KL, Chonchol M. Does inflammation affect outcomes in dialysis patients. *Semin Dial.* 2018;31(4):388-97.
3. Yuan Q, Wang J, Peng Z, Zhou Q, Xiao X, Xie Y et al. Neutrophil-to-lymphocyte ratio and incident end-stage renal disease in Chinese patients with chronic kidney disease: results from the Chinese Cohort Study of Chronic Kidney Disease (C-STRIDE). *J Transl Med.* 2019;17(1):86.
4. Soehnlein O, Steffens S, Hidalgo A, Weber C. Neutrophils as protagonists and targets in chronic

- inflammation. *Nat Rev Immunol.* 2017;17(4):248-61.
5. Solak Y, Yilmaz MI, Sonmez A, Saglam M, Cakir E, Unal HU et al. Neutrophil to lymphocyte ratio independently predicts cardiovascular events in patients with chronic kidney disease. *Clin Exp Nephrol.* 2013;17(4):532-540.
6. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med.* 2003;348(2):138-50.
7. Yoshitomi R, Nakayama M, Sakoh T, Fukui A, Katafuchi E, Seki M, et al. High neutrophil/lymphocyte ratio is associated with poor renal outcomes in Japanese patients with chronic kidney disease. *Ren Fail.* 2019;41(1):238-43.
8. Uludag K, Arikan T. Is White Blood Cell Count Associated With Mortality in Peritoneal Dialysis Patients: A Retrospective Single-Center Analysis. *Cureus.* 2021;13(11):e19728.
9. Jean G, Souberbielle JC, Chazot C. Vitamin D in Chronic Kidney Disease and Dialysis Patients. *Nutrients.* 2017;9(4):328.
10. 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. *Journal of Preventive Epidemiology,* 2021 6(2), e26–e26.
11. Haddad Kashani H, Seyed Hosseini E, Nikzad H, Soleimani A, Soleimani M, Tamadon MR, et al. The Effects of Vitamin D Supplementation on Signaling Pathway of Inflammation and Oxidative Stress in Diabetic Hemodialysis: A Randomized, Double-Blind, Placebo-Controlled Trial. *Front Pharmacol.* 2018;9:50.
12. Ahbap E, Sakaci T, Kara E, Sahutoglu T, Koc Y, Basturk T, Sevinc M, et al. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in evaluation of inflammation in end-stage renal disease. *Clin Nephrol.* 2016;85(4):199-08.
13. Ali M, Ejaz A, Solangi SA, Junejo AM, Yaseen M, Iram H, Solangi SA. Vitamin D Deficiency in End Stage Renal Disease Patients with Diabetes Mellitus Undergoing Hemodialysis. *Cureus.* 2020;12(11):e11668.
14. Satirapoj B, Limwannata P, Chairprasert A, Supasynhd O, Choovichian P. Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. *BMC Nephrol.* 2013; 14:206.
15. Marinelli A, Pistolesi V, Rossi V, Battista M, Buono A, Della Grotta F, et al. Deficit severo di 25-OH Vitamina D in emodialisi cronica [Severe 25-OH vitamin D deficiency in patients on

chronic hemodialysis]. *G Ital Nefrol.* 2014;31(5):gin/31.5.7.

16.Villafuerte-Ledesma HM, Moragrega B, Castellón E, Luzón-Alonso M, García-Mena M. Association between vitamin D serum levels and inflammatory markers in patients on hemodialysis. Asociación entre los niveles séricos de vitamina D y marcadores inflamatorios en pacientes en hemodiálisis. *Gac Med Mex.* 2020;156(6):509-15.

17.Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, et al. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int.* 2007;72(8):1004-13.

18.Kara AV, Soylu YE. The relationship between vitamin D and inflammatory markers in maintenance hemodialysis patients. *Int Urol Nephrol.* 2019;51(9):1659-65.

19.Mirchi E, Saghafi H, Gharehbeblou M, Aghaali M, Rezaian Z, Ghaviahd M. Association Between 25-Hydroxyvitamin D Level and Inflammatory and Nutritional Factors in Hemodialysis and Peritoneal dialysis Patients in Qom, Iran. *Iran J Kidney Dis.* 2016;10(4):205-12.

20.Duman TT, Aktas G, Atak BM, Kocak MZ, Erkus E, Savli H. Neutrophil to lymphocyte ratio

as an indicative of diabetic control level in type 2 diabetes mellitus. *Afr Health Sci.* 2019;19(1):1602-6.

21.Jhuang YH, Kao TW, Peng TC, Chen WL, Li YW, Chang PK, et al. Neutrophil to lymphocyte ratio as predictor for incident hypertension: a 9-year cohort study in Taiwan. *Hypertens Res.* 2019;42(8):1209-14.

22.Wang X, Zhang G, Jiang X, Zhu H, Lu Z, Xu L. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: a meta-analysis of observational studies. *Atherosclerosis.* 2014;234(1):206-13.

23.Yildirim I, Hur E, Kokturk F. Inflammatory Markers: C-Reactive Protein, Erythrocyte Sedimentation Rate, and Leukocyte Count in Vitamin D Deficient Patients with and without Chronic Kidney Disease. *Int J Endocrinol.* 2013; 802165.

24.Forget P, Dinant V, De Kock M. Is the Neutrophil-to-Lymphocyte Ratio more correlated than C-reactive protein with postoperative complications after major abdominal surgery?. *PeerJ.* 2015;3: e713.

To Cite:

Abo-Elela, E., Ismail, M., salem, I., Sediq, A. Relation between 25-hydroxy Vitamin D Levels and Neutrophil-to-Lymphocyte Ratio in Chronic Renal Disease Patients Under Hemodialysis. *Zagazig University Medical Journal*, 2024; (1039-1045): -. doi: 10.21608/zumj.2023.231856.2861