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RESEARCH ARTICLE

IMPACT OF VITAMIN D3 CORRECTION ON NEW CHRONIC INFLAMMATION MARKERS : NEUTROPHIL - TO - LYMPHOCYTE RATIO, PLATELET - TO - LYMPHOCYTE RATIOIN PATIENT WITH VITAMIN D3 DEFICIENCY AND DIABETES TYPE 2/DIABETIC NEPHROPATHY

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Abstract

Introduction: Chronic inflammation plays an important role in the development and progression of diabetes and diabetic nephropathy as one of its major complications. Vitamin D3 regulates two separate, but interacting, types of immunity: innate and adaptive. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are markers of inflammation.The aim of the study was to determine the values of NLR and PLR in patients with diabetic nephropathy and vitamin D3 deficiency, before and after correction of vitamin D3 levels.

Materials and Methods: Participants with diabetes type 2 and diabetic nephropathy were divided into two groups: study and control group of 45 patients each, with vitamin D3 deficiency and comparable characteristics in terms of therapy and laboratory parameters. The study group of patients received cholecalciferol at a dose necessary to achieve the intended optimal vitamin D3 level of 90-100 nmol /L in the blood, while the control group received their previous therapy.

Results: The NLR in the study and control groups together on inclusion was 2.36 ± .98 and for PLR 121.77 ± 42.24. NLR was lower in women (2.18 ± .72) than in men (2.58 ± 1.01) as well as PLR was lower in women (118,59±57,51) than in men (123,15±54,43). A continuous positive effect on NLR, which has statistical significance, was found in group (p = 0.047; and p = 0.011), as well as in men (p= 0.001; and p = 0.006).

Conclusion: Vitamin D3 correction exerts anti-inflammatory activity in patients with diabetic nephropathy that is most pronounced on neutrophil-to-lymphocyte ratio and less on platelet-to-lymphocyte ratio.

Introduction:-

Diabetes mellitus (DM) is a chronic systemic disease in whose development, progression, pathogenesis of its complications, chronic inflammation has a very important role (Mertoglu C.et al.2017, Calle MC.2012). Immune response and metabolic regulation are highly integrated so far that their functionality is interdependent.This interface can be viewed as a central homeostatic mechanism, dysfunction of which can lead to chronic metabolic disorders, like type 2 diabetes (T2DM) (Hotamisligil GS.2006). The existing connection between metabolic

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disorders and inflammation led to a newly defined concept called "metaflammation."Metaflammation is a form of low-grade systemic and chronic inflammation (Zhong J.et al. 2017).There are a cluster of evidence that low-grade inflammation is present in patients with T2DM (Calle M.C et al.2012,Hameed I.et al.2015). Presence of inflammation in diabetic disorder suggest that an innate immune response has a role in its pathogenesis and emerging data indicate that elements of the adaptive immune system could also be emphasized (Zhong J.et al.2017,Vellosos A.L.et al.2013,Zhou T. et al.2017). Vitamin D3 as immunomodulatory agent has signaling role both for innate immune (antimicrobial activity and antigen presentation) and adaptive immune (T and B lymphocyte function) responses. These include coordinated actions of the vitamin D-activating enzyme, 1α-hydroxylase (CYP27B1), and the vitamin D3 receptor (VDR) in mediating intracrine and paracrine actions of vitamin D3 (Zhou T.et al. 2017).Hypovitaminosis D is a common and emerging health problem worldwide especially in patients with T2DM (Mithal A. et al.2009, Rafiq S. et al.2018). A correction of vitamin D deficiency in T2DM patients has multiple significance, including the reduction of inflammation.Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) are indicators of inflammation. NLR and PLR were introduced as easily measured, reproducible, and inexpensive markers to determine inflammation (Akbas EM. et al.2016).

The aim of the study:
The aim of this study was to examine whether the correction of vitamin D3 in patientswith T2DM/DN is accompanied by reduction in the values of NLR and PLR.

Method and Participants:-

This study was performed as nonrandomised controlled clinical examination held in General Hospital in Subotica, Serbia. The 24-week study included patients followed for T2DM with diabetic nephropathy (DN) defined with proteinuria >150 mg/24h, who were treated and controlled at the outpatient Clinics for nephrology and diabetic patients. It included the population of the wider area of Subotica, located at 46 °6 '0"north latitude and 19 ° 40' 0.01" east longitude, with a pronounced pannonian-continental climate. The study was conducted and lasted from May 2018 to November 2019.After the initial screening phase, 90 patients were selected for the study, who were divided into two groups, experimental and control group, each of 45 patients.The experimental group received cholecalciferol, and a control group received their standard therapy.The lower limit of normal vitamin D values for each patient was determined on the basis of seasonally defined limits for required level of vitamin D given by months of the year, as well as their gender structure.For the assessment of vitamin D status in our patients was used a table that was adapted to our climate conditions (Bolland MJ. et al. 2007).(Table 1).For optimal values of vitamin 25- (OH) D, a value of 90-100 nmol/L was determined.

Solution Vigantol (MERCK KGaA) was administered 20,000 IU / ml as oral drops (500 IU of vitamin D in one drop). The number of cholecalciferol drops was determined based on the difference between the patient's serum vitamin D values and the set optimal values.Participants in both experimental and control group were followed for a complete blood count (CBC) every two months and for the value of vitamin D in all patients at baseline, in the experimental group also at the end of the study.

The 25-(OH) D3 is determined by the chemiluminescence method with acridinium ester-CMIA on the Abbott Architect i1000 Immunochemical Analyser of MEDLAB laboratory (accreditation number: 03-008, with accepted requirements prescribed by SRPS ISO 15189: 2014). Abbot tests were used. For blood sampling vacutainers and vacutainer needles Becton Dickinson, ref 367955 were used. Samples were sent the same day to a central laboratory and were analysed within 6 hours.

Results of the simultaneously performed measurements of complete blood count (CBC) (Sysmex XN-550 and Sysmex XT-1800i) were recorded. Methods used for these analyses were as follows: WBC diff. - Fluorescence flow cytometry, WBC - Flow cytometry, RBC/PLT - Hydrodynamic focusing impedance andHgb - SLS cyanide-free method.

Ethical aspects:
Each participant in the study signed a consent form.

Statistical Analysis:
Using the International Business Machines Corporation (IBM) Statistical Package for Social Sciences (IBM SPSS) version 20 and STATISTICA version 11 data was analysed. Qualitative data was presented in the form of numbers

and percentages while quantitative data with parametric distribution in the form of means, SD and ranges.Whole tests were two sided. When the p value was less than 0.05, it was considered statistically significant, when less than 0.001 it was highly statistically significant, and greater than or equal to 0.05: was statistically non-significant. The paper uses a simple linear curvilinear regression analysis with ANOVA tests in regression models where their statistical significance was determined by the F test. Finally, a correlation analysis was made where we have interpreted the obtained Pearson correlation coefficient (r).

Resultes:-

Clinical and biochemical characteristics of patients in the study and control group a presented in (Table 2).

Vitamin D3 in the study group at inclusion was 43.2 ±15.02 nmol /L and at the end it was 90.57± 23.74 nmol / L.

Table 3: presentation of NLR / PLR values in the study and control group during follow-up

Table 4: p –value within the SG group: whole sample, men and women

Based on a simple linear curvilinear regression analysis of the effect of vitamin D3 on NLR in whole study groupit was concluded that NLR rises to the point of maximal function t max (108.66; 2.58) and after that the NLR falls. The regression function by which the NLR value is monitored is y = -0.9652 + 0.0652x-0.0003x2.

The PLR value by regression square function increases to the point t max (102.2; 128.98) at 0 measurements, at the second control at the action of Vitamin D3 PLR increases to t max (131.1; 134.98). In the third control, the PLR rises to the point t max (148.75; 137.57), while in the last control the PLR is increasing to the t max (175.72; 186.86). None of the regression models showed statistical significance. The correlation coefficients werer1 = 0.15 r2 = 0.132 r3 = 0.282 r4 = 0.16, indicating a weak correlation strength.

Graph 1.NLR regression functions in men
Graph 2. NLR regression functions in women
Graph 3. PLR regression functions in men
Graph 4. PLR regression functions in women

Results of correlation analyses between vitamin D and NLR and PLR of all controls in a whole group sample and only in men and women are shown on graph 5, 6 and 7.

Discussion:-

After the study of Akbas EM.and al. (2016) which was the first one evaluating the relationship between vitamin D deficiency and inflammation with the novel inflammatory markers NLR and PLR, the present study is the first one whitch investigated the impact of vitamin D3 correction on NLR and PLR in T2DM patients. This study is also, to our knowledge, the first one which took into account seasonally defined limit values for this vitamin, by months of the year and by gender of patients,when determining vitamin D levels.

NLR and PLR in relation with T2DM:

Diagnostic and prognostic significance of NLR and PLR in patients with T2DM has been studied in several studies to date.Because NLR and PLR are considered as marker of subclinical inflammation Mertoglu C et al. had investigated the association of NLR and PLR with prediabetes and T2DM with an aim to determine whether these indicators are reliable markers for diagnosis. They concluded that NLR significantly increases in prediabetic and diabetic patients. PLR significantly decreases in prediabetes and early stages of diabetes but increases in later stages, so they found that NLR and PLR values may be reliable predictors in prediabetes and diabetes mellitus (Mertoglu C.et al.2017).

NLR and PLR in connection with DN:

DN is one of the major complications of diabetic disease.Therefore, the identification of markers for the detection of its early stage is very important. Huang W. et al.(2015) in their study reported that, in addition to several other predictors, high NLR values may be a reliable predictive marker of early-stage DN. Alsayyad MM. et al.(2019) assessed the prognostic value of lymphocyte to monocyte ratio- LMR, NLR and PLR in DN of T2DM, comparing

them with each other.Their results showed that in predicting the DN risk, NLR came first in regards to specificity followed by LMR and then PLR, but followed by PLR and then LMR in regards to sensitivity.NLR andPLR can be used as factors to determine the prognosis of patients in various clinical situations.

About normal values of NLR and PLR:
Normal values can be specified by analysing these markers in a healthy population, and the mean values in different disease conditions can be determined by analysing the above parameters in certain patient population. However, many differences exist in these markers depending on race, sex, and age (Azab B. et al. 2014). These differences have important clinical implications because the risk stratification in many chronic diseases with inflammatory component is estimated by arbitrary NLR cut off points which were based on the average NLR values of each study population.

The results of other studies showing different means for NLR and PLR in healthy population, DM type 2 patients and patient from hospital database across all ages, for specific age group, total and by gender are presented.(Table 5).

NLR and PLR in a whole group sample:
Our results show that NLR as marker of inflammation in all patients at the beginning of the study was 2.36 ± .98 and for PLR 121.77 ± 42.24, confirming that obtained values for NLR compared to healthy population are elevated and for PLR are within the normal range (Table 5.- Lee JS 2018). This comparison to the above result was shown for a large sample of healthy volunteers (12 160) although in making these comparisons it should be taken into account that the reported normal values (NLR 1.65 ± 0.79 and PLR 132 ± 43.68) refer to healthy younger population (average age = 47 years) in South Korea. Of note is that values of both parameters decrease with age (Lee JS.et al2018). Thus, it is possible that the normal PLR values for the population of South East Europe are lower, as shown by the results in a study also from Serbia and Montenegro, involving 300 healthy volunteers whose average age was 60.32 ± 12.21 years. In that study normal values for PLR was 97.51 ± 31.67 and NLR 1.82 ± 0.83 (Stojkovic Lalosevic M. et al.2019).

In relation to the DN, values in present study were slightly lower than in the study performed in diabetic patients in Egypt, where in the group of patients with DN, NLR was 3.7 (normal value 1.7) and PLR 277.3 (normal value 108,3) in a somewhat younger population of participants (average age=57 years) then in present study. (Alsayyad MM et al. 2019).The most comparable to present study findings are those reported by Akbas EM.et al. (2016) at the University Hospital Ataturk in Anatolia. In contrast to other studies conducted in healthy participants, this one included patients who have been treated for some ill condition. Also, considering the most similar and closest geographical position to us (Erzurum, Turkey 39.90290° N, 41,25284 ° E; Subotica, Serbia 46 ° 6 '0" N 19 ° 40' 0.01" E.), our results can be viewed as the most comparable**.** In their study NLR was 2.3 (1,79-2,96) and PLR 120,1(96,8-157,9).(Akbas EM.et al.2016).(Table 5).

Gender related values of NLR and PLR:
NLR values are different by gender, in women younger than50 years of age theNLR is higher than in men the same age, whereas in age groups older than 51 years of age, it is the reverse.These changes are due to altered hematopoiesis during menopause. Neutrophil recruitment from the bone marrow, as well as delay in apoptosis is influenced by estrogen and progesterone. Decline in the production of these hormones results in higher neutrophil apoptotic rates and increased lymphocyte production. There is also a gender difference in PLR, with higher values in women than in men (Lee JS. et al.2018).The difference may be associated with the higher platelet counts in women, although not all studies confirm this difference (Xianchun M.et al2017). In elderly people a reduction in hematopoietic stem cell reserve would lead to reduction of the platelets formation (Wu L.et al2019).Our results confirm that the NLR values were lower in women (2.18 ± .72) than in men (2.58 ± 1.01) as well as PLR values were lower in women (118,59±57,51) than in men (123,15±54,43).Gender-related differences in sensitivity to vitamin D3 were observed in an experiment on mice that indicating that female mice are more sensitive to changes in serum $1,25(OH)_2$ D levels than males and are more sensitive to the elevated circulating $1,25(OH)_2$ D than male mice (Song Y.2004). The synergistic interplay may exist between estrogen and vitamin D3.Estrogen is able to suppress CYP24A1 transcripts, leading to $1,25\text{-}(OH)_2D_3$ accumulation, it enhances VDR biosynthesis in different tissues and stimulates T cells and macrophages to accumulate $1,25\text{-}(OH)_2D_3$ in immune cells (Correale J.et al.2010).In addition to the gender-related difference in intestinal calcium transport mediated by vitamin D3 (Holick MF.et al.1989) it was also reported that the age-related decline in intestinal calcium absorption which in women is

already evident shortly after age 50, whereas in men it starts only after age 70 (Maggio D.et al.2005). There are observations that inhibition of T cell proliferation by 1,25-$(OH)_2D_3$ is significantly stronger in females compared to males. T cells in males and females synthesize 1,25-$(OH)_2D_3$ at similar rates, but females inactivate it more slowly, favouring accumulation in self-reactive T cells (Correale J.et al. 2010). Gender differences in vitamin D3results in greater protective effects for vitamin D3 in women and it may explain why the thresholdsof normal vitamin D levels is lower in women than in men (Graph 1,2).This was confirmed with the gender difference in the lower normal limit values for this vitamin in each month of the year, which we used as a basis for patient inclusion in the study, adapted from Bolland MJ.et al. (2007).(Table 1).

Interpretation of our results:

Our results indicate constant increase in the PLR breakpoint which is explained by the body's need to increase its vitamin D3 level over time in order to achieve and maintain optimal anti-inflammatory activity. Use of vitamin D3 continuously in men over a long period of time leads to a decline in NLR which has statistical significance while in women its beneficial effect is present, but without statistical significance.

The obtained results show that the intensity of the anti-inflammatory action of vitamin D decreases over time, due to the increase of the activity of counter-regulatory mechanisms, so that its intermittent administration can be recommended instead of continuous. According to our results, the interval of action of vitamin D lasts for about four months, after that time we found a decrease in the values of the monitored indicators. This could be resolved either by increasing the dose of vitamin D (taking into account the potentially toxic effect of high doses), or with a break in therapy until the next cycle of Vitamin D3 application.

When administered constant doses of vitamin D to correct its level in the body, the response of the organism based on monitoring the parameters of inflammation behaves according to the laws of normal schedule (Kolmogorov-Smirnov and Shapiro-Wilk tests for NLR and PLR show the normality of distribution), which is an analogue to the natural movement of the normal values of this vitamin in the body throughout the year.

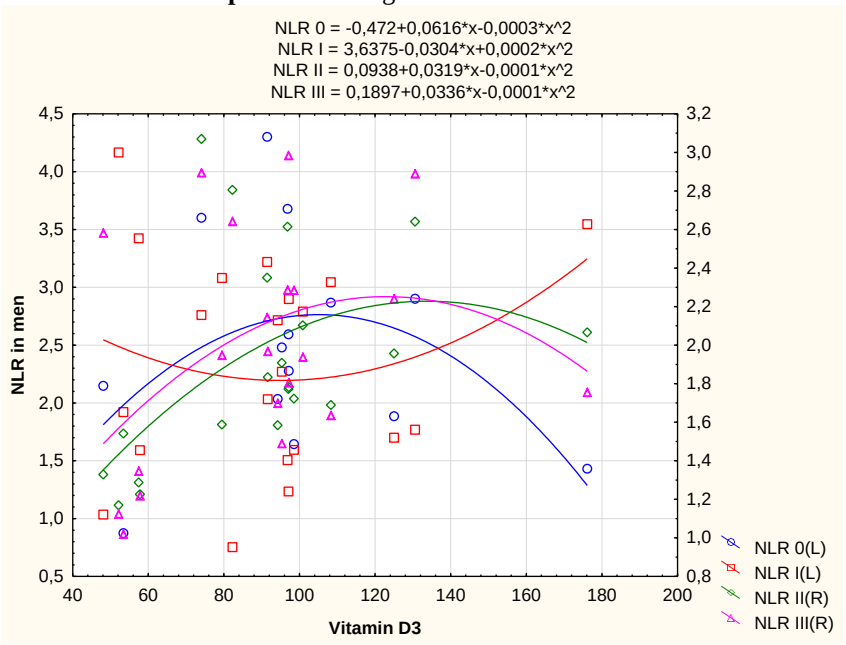
Relationship between vitamin D3 levels and NLR and PLR:

By analysing the relationship between vitamin D3 levels and NLR and PLR a significant association was found, and PLR was found to be an independent predictor of 25-(OH)D levels (Akbas EM.et al.2016). In a group of patients with vitamin D3 level <50 nmol/L, age-comparable group to our study group, NLR was 2.3 (1.86–3.08) and PLR 120,1(96,8-157,9) which presents results that are very similar to ours (NLR 2,36±.98 i PLR 121,77±42,24). It is obvious that there is no difference in levels of NLR and PLR in patients with DN and patients with low vitamin D3, so the question remains whether low 25-(OH) D is a cause of, or a consequence of chronic inflammation.The dilemma between positions that claim that low 25(OH)D causes chronic diseases or that chronic inflammatory process caused by persistent infection with cell wall deficient bacteria which dysregulates vitamin D metabolism, is still unsolved (Holick MF.2008, Mangin M.et al.2014). Perhaps a comparison with the PLR and NLR values in patients with DN and normal vitamin D3 levels could focus properly on the answer.

The weakness of this study is that 25-(OH)D values were not monitored at every two-month control but were determined only at the beginning and end of the study. The study would also be more complete if an additional control group of patients with normal vitamin D3 had been formed. Such a concept would significantly expand the field of our work and increase the cost of the study, however, the guidelines for potential new studies remain that would respond to subsequently identified problems and phenomena.

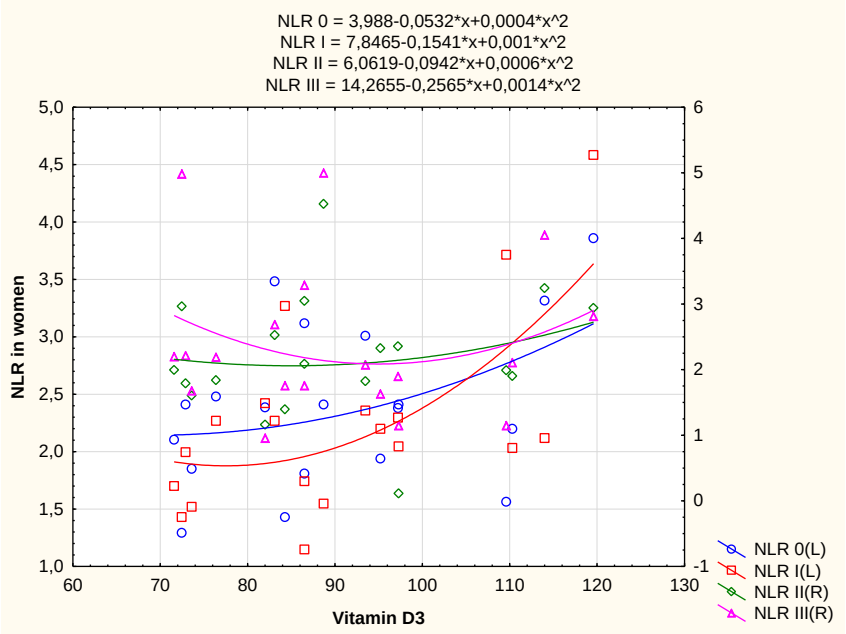
In summary a vitamin D3 correction exhibits anti-inflammatory activity that is most pronounced on NLR, and less on PLR values.The effects of the impact of vitamin D3 are better in women than in men (by regression functions that do not grow). Men need a higher dose of vitamin D than women to achieve the same effect. Due to the decrease of activity during continuous application, intermittent administration of vitamin D or dose escalation is recommended.

Graph 1:- NLR regression functions in men.



In a simple linear curvilinear regression analysis of the effect of vitamin D3 on NLR in men, we concluded that NLR rises to the point of maximal function t max (102,67; 2,69) and after this value NLR falls to the point of minimum t min (76,2; 2,48) and the value at which the NRL will move about is in the interval from 2.48 to 2.69 which is in the interval of the reference value (benchmarks) for a specific age.

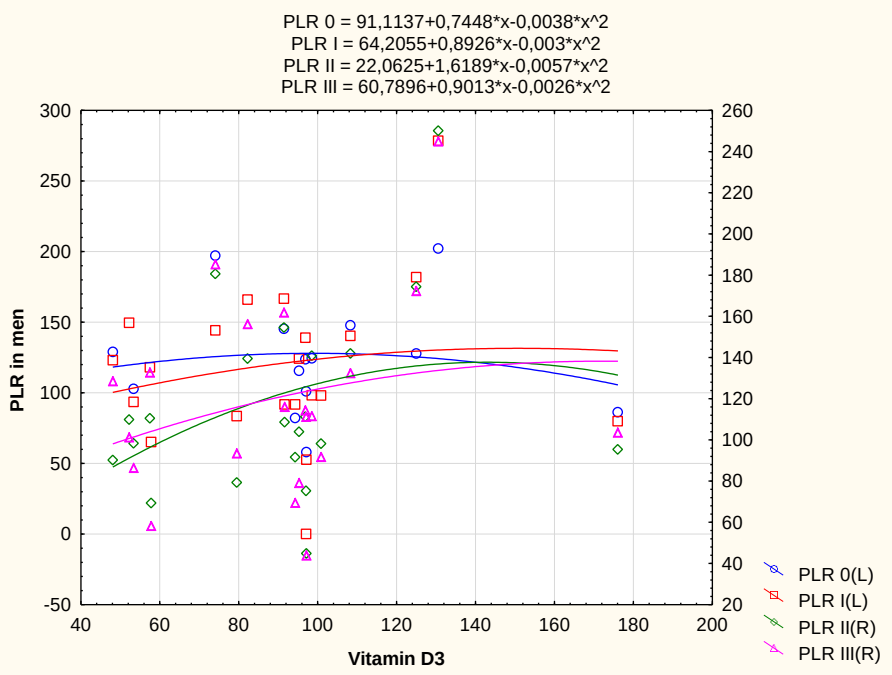
Graph 2:- NLR regression functions in women.



In a simple linear quadratic regression analysis of the effect of vitamin D3 on NLR in women, we conclude that NLR falls to the point of minimum function t min (66.5; 2.22) and will subsequently increase. In the next control, the NLR drops to t min (77.05; 1.91) and then begins to rise. In the next phase, the NLR will drop to the point t min (78.5; 2.36) and then start to rise. In the next control, the NLR will fall to the t min (91.61; 2.51). The regression

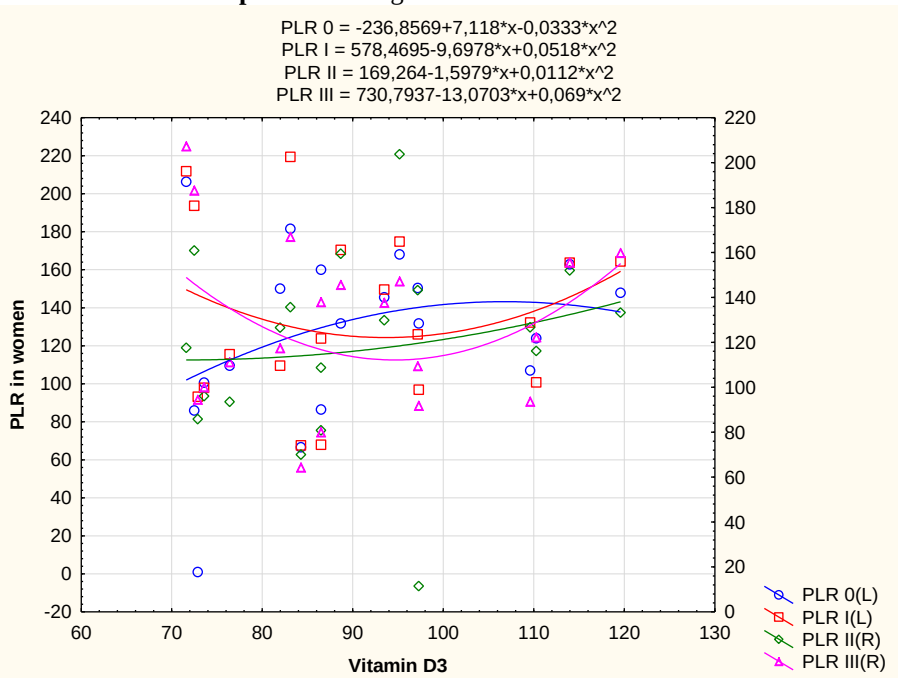
model on the first control shows statistical significance (F = 5,396; p = 0.016) while the remaining 3 regression models have no statistical significance. The correlation coefficient is *r*1 = 0.414, *r*2 = 0.635, *r*3 = 0.2, r4 = 0.239.

Graph 3:- PLR regression functions in men.



The PLR value for men by regression parabolic functions increases to the point t max (98.0; 127.6) at 0 measurements, at the next control at the Vitamin D3 PLR increases to t max 148.77; 130.6. In the next control, the PLR drops to the point t max (142.0; 137) while in the last control the PLR drops to the t max (173.32; 138.89). None of the regression models show statistical significance. The correlation coefficients are *r*1 = 0.154 *r*2 = 0.1431 *r*3 = 0.417 *r*4 = 0.325, which indicates a weak correlation strength.

Graph 4:- PLR regression functions in women.



The quadratic regression functions of vitamin D3 influence on the PLR for women that we defined, do not show statistical significance and show that the PLR will range between t max (106.87; 143.51) and t min (94.71; 111.83).

Graph 5:- Correlation matrix of the entire sample.

Correlations										
		D3II	NLR0	NLR_I	NLR_II	NLR_III	PLR_0	PLR_Ii	PLR_II	PLR_III
D3II	Pearson Correlat									
	Sig. (2-tailed)									
NLR_0	Pearson Correlat	,084								
	Sig. (2-tailed)	,660								
NLR_II0	Pearson Correlat	,193	,271							
	Sig. (2-tailed)	,245	,127							
NLR_III0	Pearson Correlat	,241	,579**	,099						
	Sig. (2-tailed)	,139	,001	,544						
NLR_III	Pearson Correlat	,123	,377*	-,130	,795**					
	Sig. (2-tailed)	,449	,030	,416	,000					
PLR_0	Pearson Correlat	-,018	,615**	,021	,572**	,305				
	Sig. (2-tailed)	,924	,000	,906	,001	,085				
PLR_I	Pearson Correlat	,105	,280	,022	,539**	,387*	,680**			
	Sig. (2-	,517	,109	,890	,000	,010	,000			

	tailed)									
PLR_II	Pearson Correlat	,252	,349	,053	,634**	,407**	,800**	,824**		
	Sig. (2-tailed)	,121	,050	,747	,000	,008	,000	,000		
PLR_III	Pearson Correlat	,153	,213	-,151	,589**	,557**	,634**	,845**	,846**	1
	Sig. (2-tailed)	,346	,227	,340	,000	,000	,000	,000	,000	

p<0,05NLR – neutrophil-to lymphocyte ratio, PLR-platelet –to-lymphocyte ratio.

Correlation analyses between vitamin D3 of all controls during the monitoring of NLR and PLR, show that Pearson's coefficients have no statistically significant association on the total sample.

Graph 6:- Correlation matrix of men only.

Correlations										
		D3_II	NLR_O	NLR_I	NLR_II	NLR_III	PLR_O	PLR_I	PLR_II	PLR_III
D3 II	Pearson Correlat									
	Sig. (2-tailed)									
NLR O	Pearson Correlat	-,080								
	Sig. (2-tailed)	,787								
NLR I	Pearson Correlat	,090	,275							
	Sig. (2-tailed)	,697	,322							
NLRII	Pearson Correlat	,406	,676*	-,224						
	Sig. (2-tailed)	,068	,008	,330						
NLR III	Pearson Correlat	,268	,473	-,517*	,662*					
	Sig. (2-tailed)	,240	,088	,016	,001					
PLR O	Pearson Correlat	-,060	,592*	-,015	,655*	,551*				
	Sig. (2-tailed)	,839	,020	,958	,011	,041				
PLR I	Pearson Correlat	,149	,194	-,071	,474*	,252	,635*			
	Sig. (2-tailed)	,520	,489	,755	,030	,271	,011			
PLR II	Pearson Correlat	,293	,354	-,106	,597*	,469*	,892**	,877**		
	Sig. (2-tailed)	,198	,215	,649	,004	,032	,000	,000		
PLR III	Pearson Correlat	,231	,078	-,245	,605*	,634*	,564*	,790**	,925**	1
	Sig. (2-tailed)	,314	,782	,272	,004	,002	,029	,000	,000	

p<0,05NLR – neutrophil-to lymphocyte ratio, PLR-platelet –to-lymphocyte ratio.

Correlation analyzes between vitamin D3and NLR and PLR, show that Pearson's coefficients have no statistically significant association in men.

Graph 7:- Correlation matrix of women only.

Correlations									
	D3_II	NLR	NLR	NLR	NLR	PLR	PLR	PLR	PLR

			_	_II	_III	_IV	_I	_II	_III	_IV
D3 II	Pearson Correlat									
	Sig. (2-tailed)									
NLR O	Pearson Correlat	,398								
	Sig. (2-tailed)	,091								
NLR I	Pearson Correlat	,584**	,310							
	Sig. (2-tailed)	,009	,150							
NLR II	Pearson Correlat	,158	,374	,013						
	Sig. (2-tailed)	,518	,079	,952						
NLR III	Pearson Correlat	-,036	,221	-,212	,804**					
	Sig. (2-tailed)	,884	,310	,331	,000					
PLR O	Pearson Correlat	,272	,521*	,076	,294	,101				
	Sig. (2-tailed)	,261	,011	,730	,174	,646				
PLR I	Pearson Correlat	,009	,434*	,070	,596**	,520*	,680**			
	Sig. (2-tailed)	,972	,039	,752	,003	,011	,000			
PLR II	Pearson Correlat	,180	,243	,092	,725**	,444*	,477*	,722**		
	Sig. (2-tailed)	,461	,264	,675	,000	,034	,021	,000		
PLR III	Pearson Correlat	-,041	,437*	-,029	,585**	,598**	,669**	,931**	,665**	1
	Sig. (2-tailed)	,869	,037	,894	,003	,003	,000	,000	,001	

p<0,05NLR – neutrophil-to lymphocyte ratio, PLR-platelet –to-lymphocyte ratio

In the female sample, Pearson's rank correlation coefficient showed (r = 0.009) a statistically significant association between vitamin D3 and the first NLR control, while the other variables showed no statistically significant association.

Table 1:- Seasonally defined limits for the required level of Vitamin D

Male		**Female**	
minimum level			
25-(OH)D3	**>50** нмол/Л> 80 нмол/Л	**>50** нмол/Л> 80 нмол/Л	
month			
july	**81**	131	**65**	105
august	**87**	137	**69**	109
september	**87**	137	**71**	111
october	**79**	129	**67**	107
november	**69**	119	**62**	102
december	**59**	109	**57**	97
january	**52**	102	**52**	92
february	**50**	100	**50**	90
marth	**50**	100	**50**	90
april	**53**	103	**51**	91
may	**61**	111	**54**	94
june	**71**	121	**60**	100

adapted from Bolland MJ.et al. (2007)

Table 2:- Clinical and biochemical characteristics of patients in the study and control group.

Variables	**STUDY GROUP (n=45)**	**CONTROL GROUP (n=45)**	***p*value**
Age (years) m/f	63/65	66,5/63	.512

BMI (kg/m^2)	29,936±4.392	29,192±4.278	.418
Duration of diabetes (yr)	8,46±4.679	8,52±4.095	.94
Systolic pressure mmHG	130,9±11.324	128,51±10.224	.296
Diastolic pressure mmHg	79,77±5,999	79,88±5.486	.921
Antihypertensive therapy yes / no	43/2	40/5	
monotherapy	9	8	
dual therapy	20	18	
triple therapy	14	14	
ACEI/ATB yes/no	40/5	37/8	
Orai antidiabetics yes/no	42/3	43/2	
monotherapy	27	20	
dual therapy	15	25	
Hypolipemics yes / no	18/27	23/22	
statins yes / no 15/30	18/27		
fibrates yes / no 3/42	5/40		
Sedimentation mm/h	18±14.69	12,9±10.376	.0433
CRP mg/l	6,19±7.856	4,11±5.099	.668
fibrinogen g/L	4,048±.979	3,71±.755	.88
albumin g/l	43,8±4.145	44,84±5.143	.306
Calcium (s) mmol/l	2,42±.135	2,426±.109	.837
Phosphorus (s) mmol/l	1,097±.164	1,041±.216	.132
Alkaline phosphatase U/L	76,59±72.09	72.09±21.01	.733
HbA1c mmol/mol	47,8.57±5.076	47.592±5.486	.331
Cholesterol mmol/l	5,352±1.164	5,48±1.119	.565
Triglycerides mmol/l	2,091±1.401	1,830±.825	.296
HDL mmol/l	1,171±.297	1,23±.267	.201
LDL mmol/l	3,42±.905	3,443±.960	.806
FACRIZ	4,74±1.125	4,547±1.032	.186
INDART	2.962±.811	2,834±.852	.190
GFR ml/min	100.782±33.76	105.31±46.452	.500
24h proteinuria g/du	0,683±1.446	0,680±1.161	.335
Calcium (U)	3,069±1.496	5,342±3.151	.00 **
Vitamin D3nmol/L	43,2±15.082	47,02±16.069	.198
No. drops of cholecaciferol/i.j.	4,72±1,448/237	-	

Notes:* p<0.05

BMI-body mass index, ACEI-angiotensin converting enzyme inhibitors, ATB- angiotensin receptor blockers, CRP-C-reactive protein, HDL- high density lipoprotein, LDL-low density lipoprotein, FACRIZ- risk factor for atherosclerosis, INDART- index atherosclerosis, GFR-glomerular filtration

Table 3:- Presentation of NLR / PLR values in the study and control group during follow-up.

		NLR				PLR			
	control group	0	I	II	III	0	I	II	III
Whole group	SG	2,42±.87	2,42±.87	2,42±.87	2,42±.87	126,87±39,33	121,8±51,63	114,77±43,8	117,31±45.76
	CG	2,42±.87	2,42±.87	2,42±.87	1,95±.89	116,73±51,15	108,93±48,64	108,78±45,27	114,61±52.79
	p	,409	.011*	,388	.257	,513	.233	,569	.697
men	SG	2.54±.83	2,0±.67	1,97±.78	2,0±.95	136,72±59,57	120,46±63,46	122,80±55,18	116,71±58,72
	CG	2,63±1,2	2,0±.67	1,97±.78	2,0±.95	109,59±49,3	97,17±31,85	96,98±33,34	102,26±50.73
	p	,623	.177	,897	.890	,169	.138	,055	.415
women	SG	2.29±.	2,17±.	2,07±.	2,31±.1,	121,01±4	127,7±45,7	112,49±	121,14±

		68	86	89	1	4,93	8	40,8	38.52
	CG	2,08±.76	1.7±.87	1,83±.65	1.88±.85	116.18±70,1	121,17±60,53	124,98±55,15	128,16±52.15
	p	,118	.103	,001*	.133	,614	.909	,382	,382

P < .05* Statistical sign. SG-study group CG-control group NLR – neutrophil-to lymphocyte ratio, PLR-platelet – to-lymphocyte ratio.

We found a statistically significant difference between the study and control group for NLR I (p =, 011).In relation to men there was no statistically significant difference between the study group and the control group.In a group of women we found a statistically significant difference between the study and control group for NLR II (p=,001).

Table 4:- p - value within the SG group:whole sample, men and women.

	control	NLR I	NLR II	NLR III		PLR I	PLR II	PLR III
Whole group	NLR 0	.391	.011*	.209	PLR 0	.697	.541	.301
	NLR I		.047*	.358	PLR I		.155	.288
	NLR II			.093	PLR II			.388
men	NLR 0	.476	.001*	.006*	PLR 0	.163	.092	.069
	NLR I		.083	.212	PLR I		.721	.622
	NLR II			.350	PLR II			.369
women	NLR 0	.540	.252	.953	PLR 0	.389	.359	.996
	NLR I		.696	.683	PLR I		.035	.078
	NLR II			.105	PLR II			.216

P < .05* Statistical sign.NLR – neutrophil-to lymphocyte ratio, PLR-platelet –to-lymphocyte ratio.

In a study group of whole sample subjects was registered a continuous positive effect on NLR, which is a statistically significant difference between control I and II, and control I and III (i.e., p = 0.047; and p = 0.011) Statistically significant result in terms of continuous positive effect of vitamin D3 on NLR was also obtained in men between 0 and II, and 0 and III control (i.e., p = 0.001; and p = 0.006). With respect to PLR, an effect of action not showing statistical significance was found in whole sample as in men and women, which also applies to NLR in women.

Table 5:- Mean NLR and PLR in healthy and diseased population in total and by age and gender.

		Median age of group	**Mean value for all**	**Mean value for men**	**Mean value for women**	**participants**
LeeJS. 2018.	NLR PLR	47	1.65 (0.79) 132.40 (43.68),	1.66 (0.82)	1.63 (0.76)	across all ages 12,160 healthy adults in South Korea
	NLR PLR	60-69 years		1,7 118	1,4 125	
Wu L. 2019.	NLR PLR			1.59 ± 0.59 92.88 ± 28.70	1.62 ± 0.64 108.02 ± 32.99	across all ages 5000 healthy adults, 2500 men and 2500 women South China
	NLR PLR	60-69 years		1.71± 0,68 91.67 ± 30,38	1,51 ± 0,64 100,53± 31,11	
Alsayyad MM. 2019.	NLR	I/IIA/II	1.8/2.9/3.7/1.2			across all ages 100 DM type 2 patients and 25 healthy controls

	PLR	B/III	175,8/ 249,2/ 277,3/ 108,3				Egypt
Agbas ME.2016.	NLR PLR	45–64 years	2.3 (1.79–2,96) 120,1 (96,8-157,9)				across all ages 4120 patients Ataturk University Hospital database Turkey
			Vitamin D < 20 ng/ml	Vitamin D ≥ 20 ng/ml			across all ages
	NLR PLR		2.38 (1.86–3.08) 124,77 (99.62 – 162,00)	2.25 (1.77–2.95) 117,75 (93,33 – 148,00)			

Table shows an overview of NLR and PLR values by cited authors, with the number of participants who were included in the study and according to their state of health, the country in which the study was conducted, the average age of the subjects for the whole study group, or the population of participants that matches our participants by the average age and gender.

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Data Availability:

The data presented in this paper are a part of the doctoral dissertation project.

Conflicts of Interest:

The author has no conflict of interest to declare. There was no outside funding for the study.