Research Article

Importance of Complete Blood Count and it's Derived Parameters in Type II Diabetic Patients (with and without foot ulcer).

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Abstract

Background: Diabetes mellitus (DM) is a chronic condition affecting 1 out of every 11 people worldwide. Diabetic foot ulcer is one of the major consequences of diabetes, causing considerable socioeconomic and health problems. The influence of diabetes on hematological markers has been evaluated with challenged results. However, Hematological parameters may not be regularly used as laboratory diagnostic biomarkers to monitor diabetes and its consequences. **Objective**: to investigate the effect of DM on the hematological parameters and the diagnostic capability of discriminating such medical conditions. **Method**: Prospective case-control study of 49 healthy control and 98 diabetic patients (49 without diabetic foot ulcer- 49 with diabetic foot ulcer) were included. Age and sex were matched among the three studied groups. Blood sample was collected from each subject and used for complete blood count and its analysis. **Result**: The ratio of Male to Female was (88/59=1.49). Most of the patients were in the 5th and 6th decades among three studied groups. A significant difference in the mean level of Hemoglobin (HB), Red Blood Cell (RBC), Total White Blood Cell (WBC), Neutrophils, Lymphocytes, Platelets, Neutrophils Lymphocyte Ratio (NLR), Platelets Lymphocyte Ratio (PLR), and Platelets Neutrophils Ratio (PNR) among the three studied groups was observed. The Receiver Operating Characteristic (ROC) curve analysis documented that PLR had good discrimination power between DFU and Healthy subjects (AUC=0.814) in addition to NLR (AUC=0.802). **Conclusions**: PLR is simple, inexpensive, and is a useful marker for discrimination between DFU and healthy control.

Introduction

Diabetes mellitus (DM) is a chronic disease that affects one in every eleven individuals, nearly 422 million people worldwide,[1] and approximately 11.5% of annual global deaths are attributed to diabetes.[2] The prevalence of type 2 diabetes mellitus is predicted to increase globally by 2030, with 7,079 cases per 100,000 people. [3]

Diabetic foot ulcer (DFU) is one of the most common consequences of diabetes mellitus, which has serious health and socioeconomic implications that negatively impact the patient's quality of life [4] and It is a significant cause of morbidity, often including hospitalization and lower extremity amputation and mortality.[5] 19% to 34% of diabetic individuals will acquire a DFU in their lifetime, and around 20% of persons who develop a DFU will require lower-extremity amputation.[6] Ulceration and infection are frequently occurring foot complications in people with diabetes and peripheral neuropathy.[7] patients with DFU encounter a markedly elevated risk of general infection.[8]

Complete blood count (CBC) is a simple, routinely requested test in all healthcare settings. Analysis of the hematological parameters (CBC) such as white blood cells (WBC), red blood cells (RBC), and platelets (PLT) could contribute

Study design

A prospective case-control study of 49 healthy control groups and 98 diabetic patients (49 without DFU- 49 with DFU) visited AL-Kafeel Hospital at Karbala and Imam Al-Hassan Center for Endocrinology and Diabetes. This study was conducted between October 2023 to January 2024. Demographic data of participants was collected including age, sex, and BMI (body mass index which was calculated by using the formula weight/height^2 (kg/m^2)).

to the following-up of the development of degenerative complications in DM.[9]

CBC-derived parameters, such as Platelets/Lymphocyte Ratio (PLR), Platelet/Neutrophil Ratio (PNR), and Neutrophil/Lymphocyte Ratio (NLR) are regarded as creative indicators for predicting the inflammatory process. [10]
The influence of diabetes mellitus on hematological markers has been reviewed with controversial results. However, Hematological parameters may not be regularly assessed as laboratory diagnostic biomarkers for monitoring diabetes and diabetes-related comorbidities.[11] Also, it is still useful to investigate whether CBC indicators possess sufficient diagnostic ability to discriminate such medical disorders. Thus, the current study aimed to assess the effect of DM on the hematological parameters including CBC and CBC-derived parameters (involved (NLR), (PLR) and (PNR)).

Material and Method
Ethics

The study protocol was approved by the Ethical Committee of the College of Applied Medical Science/University of Kerbala. All patients engaged in this study were informed, and each participant verbally approved before the sample collection.

Sample Selection

Patients with confirmed T2DM, with age more than 18 years old from both sexes (with or without diabetic foot ulcer were included in this study. Age and sex-matched healthy participant was included. Individuals with ages less than 18 years, other types of diabetes mellitus, non-diabetic patients with foot ulcers, T2DM patients with infection or inflammation other than DFU, T2DM patients with any hematological disorders or

any other disease that can affects on CBC test, pregnant or lactating women, and participant with steroids or any hormone therapy supply were excluded from the study.

Blood sample collection and laboratory analysis

three milliliters of venous blood were withdrawn from each participant using tubes containing ethylene diamine tetra acetic acid (EDTA) and used for CBC analysis. The CBC was performed using Cell-Dyn Ruby (Abbott- Germany), and the CBC-derived indicators were determined as follows: PLR acquired by dividing platelet /lymphocyte count, NLR obtained by dividing neutrophil /lymphocyte count, and PNR generated by dividing platelet by neutrophil count.[12]

Statistical analysis

The data was analyzed using the Statistical Package for the Social Sciences (SPSS), version 22 software (IBM Corp., NY, and USA). Data from the normal distribution were represented as means ± standard deviation (SD), and one-way ANOVA was used for group comparisons. Count data were presented as frequency and percentage (%), and the chi-square test was performed to compare groups. To determine the area under the curve, a Receiver Operating Characteristic (ROC) analysis was performed. The statistical significance level was set at P < 0.05.

Results

One hundred and forty-seven subjects (49 participants for each group, DFU, T2DM, and Healthy) were included in this study from October 2023 to January 2024 in Karbala Governorate, Iraq. Both age and sex were matched among the three studied groups. The participants' ages were 32-76 years old, of them, 88 (59.9%) were male and 59 (40.1%) were female. Nineteen (38.8%), 21(42.9%), and 19 (38.8%) were female in DFU, T2DM and control groups, respectively, as shown in Table 1. Concerning age, about two-thirds of the participants 88 (59.9%) with an age range from 51-69 years among the three studied groups, as shown in Table 1. The mean age of the three groups were (57.31 ± 10.27), (57.43 ± 10.23), and (55.73 ± 9.36) for DFU, T2DM, and health control, respectively. In both sex and age, there were no statistically significant differences amongst the three studied groups, as shown in Table 1.

Regarding BMI, 18 (36.7%) of DFU, 5 (10.2%) of T2DM and 8 (16.3%) of healthy group had normal weight, whereas, 17 (34.7%), 21(42.9%), and 30 (61.2%) were overweight and 14 (28.6%), 23 (46.9%), and 11 (22.4%) were obese in DFU, T2DM, and Healthy groups, respectively. A statistically significant difference was revealed in the current study according to BMI (*p*=0.001). A higher mean of BMI was found in the T2DM patients which had a highest frequency of obese patients, and a higher frequency of overweight was seen in the control participants while, a higher frequency of normal weight was seen in DFU, as shown in Table 1.

Table 1: Demographic Data of the Patients and Healthy Control.

Variables	Control	T2DM	DFU	Total	Chi-Square\ANOVA *P-value*
Number	**49**	**49**	**49**	**147**	
Sex N (%)					
Male	30 (61.2)	28 (57.1)	30 (61.2)	88	0.893
Female	19 (38.8)	21(42.9)	19 (38.8)	59	
Age					
Mean ± SD	55.73 ± 9.36	57.43 ± 10.23	57.31 ± 10.27	147	0.645
Age Groups N (%)					
≤ 50 years	14 (28.6)	12 (24.5)	11 (22.4)	37 (25.2)	0.590
51-69 years	31(63.3)	28 (57.1)	29 (59.2)	88 (59.9)	
>69 years	4 (8.2)	9 (18.4)	9 (18.4)	22 (15.0)	
BMI kg/m2					
(Mean ± SD)	27.87 ± 3.89	30.03 ± 4.60	27.96 ± 5.88	147	0.049[*]
BMI groups N (%)					
Normal	8 (16.3)	5 (10.2)	18 (36.7)	31	0.001[*]
Overweight	30 (61.2)	21 (42.9)	17 (34.7)	68	
Obese	11 (22.4)	23 (46.9)	14 (28.6)	48	

Hematological Characteristics of the Three Studied Groups

Some hematological markers were investigated in the current study. The results showed that the mean level of HB, RBC, Total WBC, Neutrophils, Lymphocytes, Platelets, NLR, PLR, and PNR was significant differences among the three studied groups, as shown in Table 2.

Also, the mean of lymphocyte was significantly lower and neutrophils were signifecantly hgher in DFU patients compared to T2DM and control groups.

Multiple comparisons shown that significant differences were observed in all studied hematological parameters between DFU and control / T2DM. In DFU patients, the mean of Platelets, PLR, and NLR was significantly higher in comparison to T2DM and control groups whereas, the mean of PNR was significantly lower in T2DM in comparison to DFU and control groups. Also, the mean of RBC in DFU patients was significantly lower in comparison to other groups, as shown in Table 2.

Table 2: Hematological Markers Among Studied Groups.

Parameter	Control (N=49)	T2DM (N=49)	DFU (N=49)	*P-*value	Multiple Comparison Mean Difference (P- value)		
					DFU vs Control	DFU vs T2DM	T2DM vs Control
HB Mean ± SD	14.31 ± 1.65	14.23 ± 1.64	12.03 ± 1.95	0.000*	-2.28* (0.000*)	-2.19* (0.000*)	-0.08 (0.807)
RBC Mean ± SD	4.89 ± 0.60	4.89 ± 0.54	4.23 ± 0.64	0.000*	-0.66* (0.000*)	-0.66* (0.000*)	-0.00 (0.997)
Total WBC Mean ± SD	7.91 ± 8.74	7.80 ± 1.50	11.33 ± 9.14	0.028*	3.42* (0.023*)	3.53* (0.019*)	-0.11 (0.941)
Neutrophiles Mean ± SD	52.09 ± 10.68	57.62 ± 7.80	65.65 ± 10.08	0.000*	13.55* (0.000*)	8.02* (0.000*)	5.53* (0.005*)
Lymphocytes Mean ± SD	35.89 ± 10.89	31.63 ± 7.11	24.68 ± 9.07	0.000*	-11.21* (0.000*)	-6.95* (0.000*)	-4.26* (0.023*)
Platelets Mean ± SD	233.92 ± 60.18	216.37 ± 58.29	296.59 ± 96.42	0.000*	62.67 (0.000*)	80.22 (0.000*)	-17.55 (0.241)
NLR Mean ± SD	1.66 ± 0.77	2.00 ± 1.00	3.46 ± 2.52	0.000*	1.80 (0.000*)	1.46 (0.000*)	0.33 (0.305)
PLR Mean ± SD	7.39 ± 5.30	7.37 ± 3.26	15.58 ± 12.51	0.000*	8.19 (0.000*)	8.21 (0.000*)	-0.02 (0.988)
PNR Mean ± SD	4.89 ± 2.54	3.78 ± 1.03	4.52 ±1.31	0.008*	-0.37 (0.298)	0.73 (0.040*)	-1.10 (0.002*)

Receiver Operative Characteristic Curve (ROC) for the studied variables

In the present study, the ROC curve was analyzed to determine the performance of the NLR, PLR, and PNR in discrimination of DFU from T2DM patients and DFU patients from healthy subjects and between T2DM patients and Healthy subjects. The results found a statistically significant difference was present in NLR, PLR, and PNR between DFU and T2DM patients (Area under curve (AUC) for NLR= 0.707, *p-value* 0.000), (AUC for PLR= 0.789, *p-value* 0.000) and (AUC for PNR= 0.681, *p-value* 0.002), as shown in Figure 1.

ROC curve for NLR, PLR, and PNR between T2DM and control participants, (AUC for NLR= 0.651, *p* value 0.010), (AUC for PLR= 0.699, *p* value 0.000) and (AUC for PNR= 0.335, *p* value 0.005), as shown in Figure 2. ROC curve for NLR, PLR, and PNR between DFU and T2DM patients was (AUC for NLR= 0.802, *p-value* 0.000), (AUC for PLR= 0.814, *p-value* 0.000) and (AUC for PNR= 0.503, *p-value* 0.958), as shown in Figure 3.
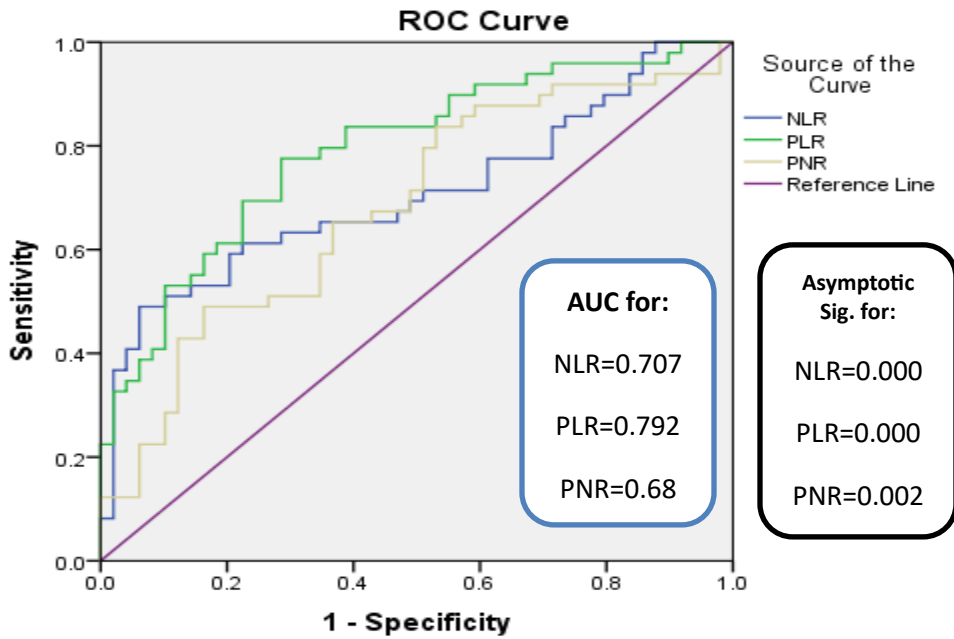
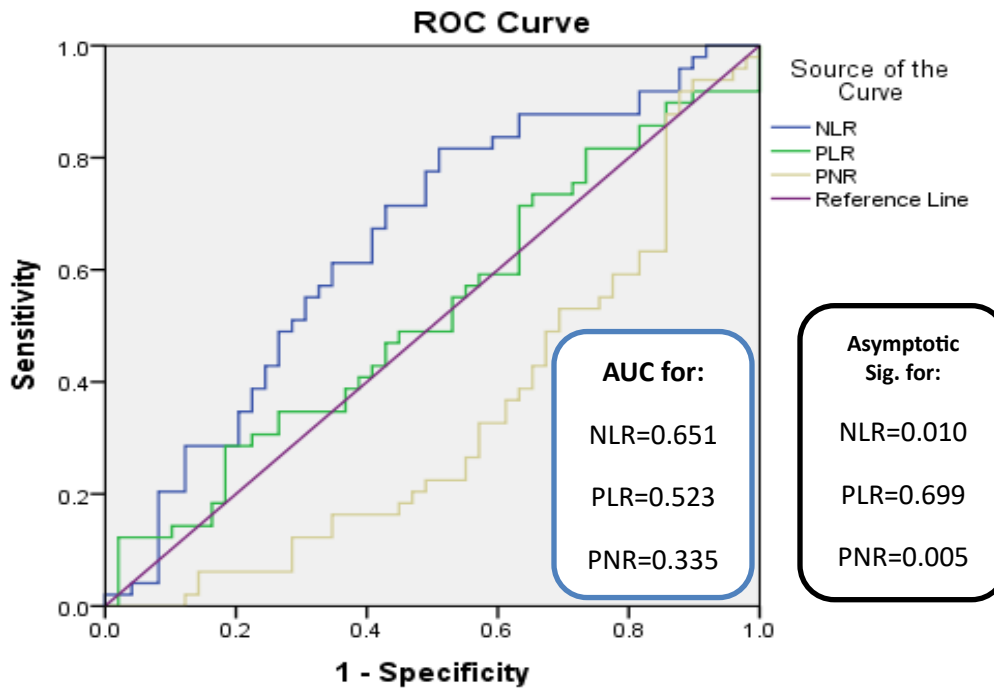**Figure 1: ROC Analysis for DFU vs T2DM.**

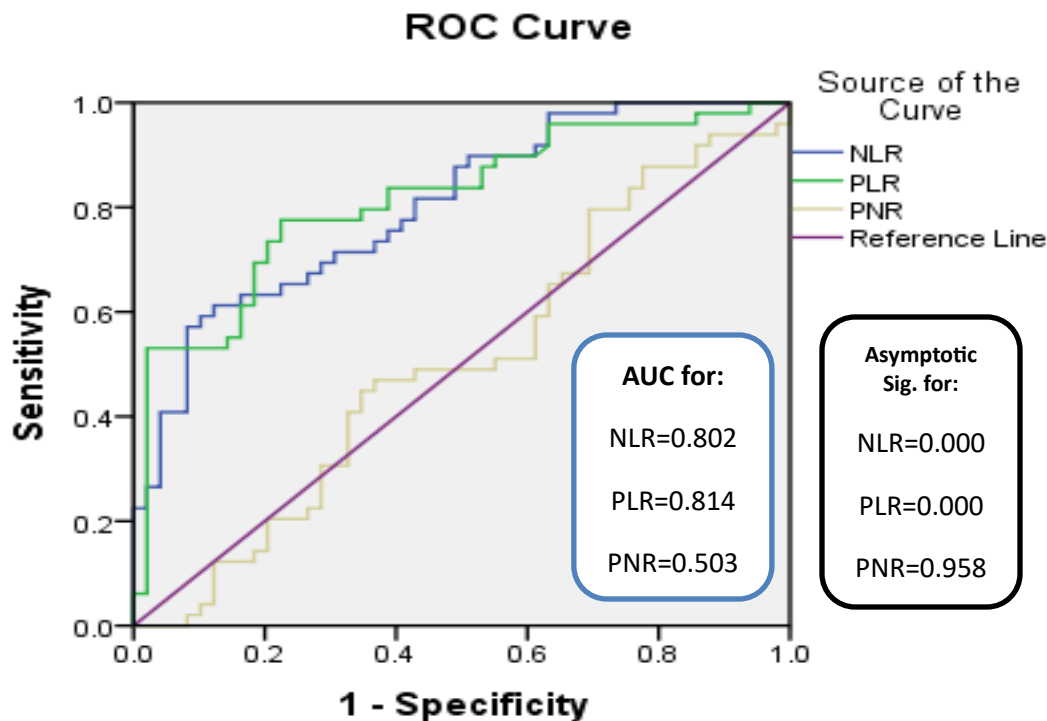**Figure 2: ROC Analysis for T2DM vs Control.**

Figure 3: ROC Analysis for DFU vs Control.

Discussion

Chronic hyperglycemia causes several pathological and physiologic processes, involving oxidative stress, [13] and increase oxidative stress causes hematological changes that impact the structure, function, and metabolism of white blood cells, red blood cells, and platelets.[14] Thus, the aim of the current study is to evaluate the influence of diabetes mellitus on the hematological indicators including CBC and CBC-derived parameters (involving PLR, NLR, and PNR).

This study showed that the Male/ Female ratio was (88/59=1.49), Table1. Similar findings were reported by Tola *et al.*[15] Anderson *et al.,* [16] reported a higher frequency of DFU among males (55.7%) and Mineoka *et al.,* [17] reported a higher frequency of males in DFU (56.2%) and T2DM (61.2%). This may be due to the fact that the male population is more likely than female to be subjected to heavier work and a higher risk of trauma in work zone. [18] Inversely, Younis *et al.,* [19] documented higher frequency in DFU among females (63%).

Most of the patients were in the 5th and 6th decades among the three studied groups, Table 1. Similarly, Singh *et al.,* [20] reported that the highest age range of DFU patients was in 5th and 6th decade of life. This might be due to the fact that today's numerous factors, such as professional activities and lifestyle, lead the foot to withstand higher pressure, which is more typically found in middle-aged and older persons [21] or due to the fact that elderly persons pose the risk of

diminished capacity to self-care due to poor vision and limited mobility. [22]

In this study, the three studied groups were shown Statistically significant differences regarding to BMI (*p*=0.001), as shown in Table 1. Tola *et al.,* [15] found a significant difference in BMI between T2DM and DFU groups and between T2DM and control groups. The higher mean BMI which was seen in T2DM patients included in this study might possibly predict the possible development of DFU in the future in those patients because obese diabetic individuals may develop DFU due to decreased normal blood circulation to their lower extremities and increased foot pressure compared to DM patients with normal body weight. [15]

The current study found significant differences in the mean levels of HB, RBC, Total WBC, Neutrophils, Lymphocytes, Platelets, NLR, PLR, and PNR among the three studied groups, the highest mean of Platelets, NLR, and PLR was seen in DFU patients whereas the lowest mean of NLR was seen in T2DM, whereas, significant lower mean of PNR was seen in T2DM, Table 2. Similarly, Zhao *et al.,* found significant differences in Total WBC, Neutrophiles, Lymphocytes, Platelets, PLR, and NLR among DFU, T2DM, and normal groups. [23] A previous study reported that the platelets were significantly differ between DFU and T2DM/or control groups while the total WBC, lymphocyte, and PLR in DFU significantly differed among DFU, T2DM, and control groups. [24]

Also, the DFU patients showed significantly highest in the mean of neutrophils and significantly lowest of lymphocyte in Comparison to T2DM and control groups. A similar finding hypothesized by KALELİ *et al.* [25] Also, Previous studies showed that PLR and Lymphocytes were significantly differ between T2DM and DFU groups. [17]

Additionally, previous study documented statistically significant differences in PNR

for the T2DM patients in comparison to the healthy control while platelets (290 ± 72 vs 286 ± 67) and PLR were comparable among groups. [14] However, Essawi *et al.,* reported the absence of significant difference in the mean of total WBC between T2DM (6.8±2) and control groups (6.6±1.8). [14] The raised PLT count may suggest a high inflammatory state and thrombotic risk, whereas the reduced lymphocyte count reflects the relative deficiency of immune control. [24]

Platelets are assumed to be the pathogenetic mechanism of atherosclerosis because they interact with a range of various types of cells that cause inflammatory processes in the arterial wall. [17] Other studies indicated the importance of activated platelets, which may play a major role in enhanced atherogenesis, whereas lymphocytes suggest the protective element of inflammation. [26]

A significant Lower mean of RBC seen in DFU patients could be attributable to the consequences of persistent hyperglycemia. Hyperglycemic condition promotes reactive oxygen species (ROS) generation, affecting RBC deformability and lifespan. RBC alterations in the diabetes mellitus (DM) influence microcirculation and thereby contribute to microangiopathy. [14]

The ROC curve of the current study revealed that PLR has the highest discriminatory ability (which is fair) in differentiation between DFU and T2DM patients, (AUC=0.792), as shown in Figure 1, and had good discrimination power between DFU and Healthy subjects (AUC=0.814) in addition to NLR (AUC=0.802), as shown in Figure 3. Whereas, NLR had the highest discriminitory power (poor) in differentiation between T2DM patients and healthy subjects, (AUC=0.651), as shown in Figure 2. This means that PLR has the best discriminatory ability among CBC-derived parameters that could be used in

differentiation between DFU and healthy subjects.

Eren *et al.,* was concluded that because the PLR and NLR are computed from a conventional complete blood count, they may be assessed using a low-cost, easily available technique. Furthermore, they are novel inflammatory indicators that can be used to predict the grade of ulcers in

Conclusion

A higher frequency of male sex was affected T2DM and DFU. Most of the patients were in the 5th and 6th decade of life. A significant higher mean of BMI was seen in T2DM patients. Significant differences in the mean level of HB, RBC, Total WBC, Neutrophils, Lymphocytes, Platelets, NLR, PLR, and PNR were found among the three studied groups. PLR is simple, inexpensive, and had

hospitalized patients with infected DFUs. [27] A previous study documented a similar finding for PLR in which the author documented that the PLR could be used as a simple clinical indicator for the clinical diagnosis of DFU. [24]

the highest discriminatory power in differentiation between DFU and healthy subjects.

Reccommendations

This article recommended to:

1-Undergoing future study with larger numbers

2- Study the relationship of these parameters to severity of diabetic foot

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