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RESEARCH ARTICLE

Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio in Well Controlled and Uncontrolled Children and Adolescents with Type 1 Diabetes Mellitus.

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ABSTRACT

The platelet lymphocyte ratio (PLR) and neutrophil lymphocyte ratio (NLR) are an inexpensive index useful in predicting the development and control levels of type 2 diabetes mellitus. There are limited research in this regard among patients with type 1 diabetes. This study was aimed to evaluate NLR, PLR and investigate the relationship NLR, PLR and elevated HbA1c in children with T1DM. This was a case-control study which was conducted on 101 children and adolescents with type1DM and59 subjects as controls. Complete medical history, clinical examination, and laboratory assessment of CBC, HbA1C, NLR and PLR were done. No gender differences were seen for HbA1C%, Neutrophil, Lymphocyte, platelet, PLR & NLR. In the present study, Mean PLR & NLR of T1DM patients was significantly higher than those of healthy controls (p=0.000). There was no significant difference in NLR and PLR between patients with HbA1c >= 7.0 (group I) which included 73 subjects and the others with HbA1c < 7.0(group II). There was positive correlation between NLR & PLR (r= .614), Neutrophil r (=.774), and negative correlation between NLR, Lymphocyte, and HbA1C% (r= -.756 & -.205 respectively). PLR was positively correlated with NLR and neutrophil (r= .614 &.721 respectively), and negatively correlated with Lymphocyte(r=-.726). Result of a multiple linear regression shows that HbA1C% & Neutrophil were the main predictors of NLR. The study showed that NLR and PLR were significantly higher in type 1 diabetic children compared to healthy subjects. Evaluation of CBC, differential count, PLR and NLR could be valuable and helpful in management of T1DM in the future,

Keywords: CBC, HbA1C%, T1DM, PLR. NLR

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INTRODUCTION

Type 1Diabetes mellitus (T1DM) is a global metabolic disorder, characterized by persistent hyperglycemia and increased risk of macrovascular and microvascular complications. Its incidence continues to rise [1]. High levels of glycated hemoglobin (HbA1c) have shown to attributed to an increase in erythrocyte internal viscosity and can lead to functional changes in hemoglobin molecule [2&3]. Hematological parameters include White blood cells (WBC) and platelet parameters may be affected with high levels of HbA1c [4]. However, less is known about other changes in blood cells indices due to diabetes mellitus [5]. Increase in the White blood cell count gives an inflammatory marker associated with Cardiac diseases [4].

Hematological changes in T1DM could be used to monitor the disease progression in diabetic patients [6, 7]. Recent epidemiological studies indicate that T1DM is as great a risk factor for cardiovascular mortality and that these complications may occur at a young age [7].

There is little information about the correlation between HbA1c and neutrophil lymphocyte ratio (NLR) in type 2 diabetes mellitus [7].

Recently, studies have reported that platelets and lymphocytes play important roles in the inflammatory process. The platelet-to-lymphocyte ratio (PLR) is an inexpensive index, PLR is useful in predicting the development and control levels of type 2 diabetes mellitus [8].

To our knowledge, there is few published study concerning the relationship between NLR and PLR and HbA1c in children with T1DM. This study was done to evaluate the hematological parameters among T1DM patients and to evaluate NLR, PLR and to investigate the relationship between NLR, PLR and elevated HbA1c in children with T1DM.

SUBJECTS AND METHODS

This was a case-control study which was conducted on 101 type1DM patients: (51 male, 50 female). They were recruited from the Pediatric Diabetic Clinic in Centre of Excellence in National Research Centre during October 2019 to April 2021. A control group included 59 children (49 male, 13female); they were age-matched healthy subjects. The study protocol was approved by the Human Ethics Committee of National Research Centre, and written informed consent was obtained from all children and their parents, after full discussion about the aim of the study. This study is a part from a project done in the National Research Centre, Cairo, Egypt. Ethical Approval No 19 – 223.

The inclusion criteria for cases were noted as T1DM for at least 6 months, concomitant use of insulin, and need for glycemic control. Exclusion criteria: Acute complications of T1DM such as diabetic ketoacidosis (DKA), hypoglycemia, or use nonsteroidal anti-inflammatory drug& any conditions that could alter hematological parameters. They were excluded if they had PLTs count $>450\times10^3$ or $<150\times10^3$, inherited or acquired diseases which affect PLTs count and function, maturity onset diabetes of the young or using insulin pump.

All children were subjected to history taking and clinical examination to fulfill needed data: Insulin therapy, regarding dose in units/kg and type. History suggestive of acute metabolic complications, or chronic diabetic complications was included. Blood pressure was measured according to American Heart Association guidelines; three times for patients and controls after 5-min rest in sitting position with the use of mercury sphygmomanometer. The mean value of 2nd and 3rd measurement was calculated. SBP was defined as the onset of the Korotkoff sound (K1), and DBP was defined as the fifth Korotkoff sound (K5). Anthropometric indices: Body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. Body mass index was calculated as weight divided by height squared ($kg/^{m2}$). Waist circumference (WC) was measured at the level midway between the lowest rib margin and the iliac crest. Hip circumference (HIP C) was measured at the widest level over the greater trochanters in a standing position by the same examiner; then waist to hip ratio (WHR) were calculated [9]. The landmarks,

instruments used, and techniques followed were those recommended by the international biological program [10].

Laboratory measurements

Investigations were done for both patients and controls.

Blood samples from all participants were withdrawn under complete aseptic conditions. Laboratory investigations for all participants at the time of study including complete blood picture by CELL-DYN, Glycosylated Hb (HbA1c) was measured using ion exchange HPLC (high purified liquid chromatography) Kit supplied by Crystal Chem, USA Hemoglobin and differential WBC count were done . Neutrophil to lymphocyte ratio (NLR) and PLR were calculated [11]. Complete blood picture including hemoglobin, total and differential WBC count (neutrophile) & platelets.

Statistical analysis

Data analysis was carried out using the standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 22.0 (SPSS Inc., USA). All numeric variables were expressed as mean ± standard deviation (SD). The intergroup comparisons were performed by using an independent-sample t test and a one-way analysis of variance and Chi-Square tests for categorical variables. Pearson's and Spearman's correlation tests (r=correlation coefficient) were used for correlating normal and nonparametric variables, respectively. For all tests, a P-value of less than 0.05 was considered significant. Linear multiple regression was run to predict PLR and NLR. A receiver operating characteristic curve was configured to test the validity of HbA1C, NLR and PLR in detecting the uncontrolled T1DM cases.

RESULTS

Demographic data

One hundred one T1DM patients and fifty-nine apparently healthy controls were studied, with mean age of (12.85 ± 3.24, 11.13±4.06 years respectively). As regard gender: cases (F=49, M=52) and controls (F=13, M=46), with was no statistically significant difference between the 2 groups (P > 0.05). No gender differences were seen for HbA1C%, Neutrophil, Lymphocyte, platelet, PLR & NLR (P<.05). All demographic data of the studied groups are summarized in the Table 1. Table2 shows Hematologic Indices of Children with Diabetes& Controls. The mean values of the children with T1DM for neutrophil % was 56.29, mean platelet count was 299.32(± 72.39) × 10^3/µL , and lymphocyte % was 34.22 . The mean value for NLR was 1.74 ± .67, PLR was 181.84 ± 60.3.

In the present study, there was significant difference in studied laboratory parameters between patients and control groups except in total Leucocyte count and platelet count in diabetic patients in comparison with their healthy controls. Our data showed that NLR and PLR were significantly higher in type 1 diabetic children compared to healthy subjects.

Table 1: Demographic data of the studied T1DM cases & Controls

	CASES CONTROLS	N	Mean	Std. Deviation	Sig. (2-tailed)
Age (y)	CASES	101	12.8596	3.24141	.05
	CONTROLS	59	11.1333	4.06612	
BMI (kg/m2)	CASES	101	21.3462	4.52995	.183
	CONTROLS	59	23.1061	10.41064	
Systolic BP	CASES	101	109.8485	10.70129	.042
	CONTROLS	59	105.0000	12.01850	
Diastolic BP	CASES	101	70.7071	7.82494	.037
	CONTROLS	59	67.1429	8.09991	
WC CM	CASES	101	73.9394	12.58426	.013
	CONTROLS	59	67.4667	11.30070	
WH R	CASES	101	.8667	.05717	.433
	CONTROLS	59	.8760	.05481	

(BMI) Body mass index, (WC) Waist circumference, (WHR) waist to hip ratio

Table 2: Hematologic Indices of Children with Diabetes& Controls

	CASES CONTROLS	N	Mean	Std. Deviation	Sig. (2-tailed)
Hemoglobin(g/dL)	CASES	101	13.38	1.27	.000
	CONTROLS	59	12.54	1.24	
HbA1C%	CASES	101	7.86	1.13	.000
	CONTROLS	59	5.43	.67	
Total Leucocytic count (10^3/ µL)	CASES	101	6.55	2.02	.745
	CONTROLS	59	6.68	1.90	
Neutrophil %	CASES	101	56.29	7.86	.009
	CONTROLS	59	52.31	5.86	
Lymphocyte %	CASES	101	34.22	7.58	.001
	CONTROLS	59	39.06	6.14	
NLR	CASES	101	1.7432	.67	.000
	CONTROLS	59	1.2466	.78	
PLATELT ($\times 10^3$/µL)	CASES	101	299.32	72.39	.181
	CONTROLS	59	283.94	63.43	
PLR	CASES	101	181.84	60.39	.000
	CONTROLS	59	100.42	49.89	

(NLR) neutrophil lymphocyte ratio (PLR) The platelet-to-lymphocyte ratio

Diabetics patients were divided into two groups, one with HbA1c >= 7.0 (group I) which included 73 subjects and the other with HbA1c < 7.0 (group II) which included 28 subjects. Table 3 shows hematologic indices of the patients according to HbA1c%. In this study, there was no significant difference in demographic characteristics and hematologic indices, in Group I and II children with diabetes.

Table 3: Hematologic Indices of the Patients According to HbA1c%

	HbA1C% I >= 7.00 II< 7.00	N	Mean	Std. Deviation	Sig. (2-tailed)
Total Leucocytic count(10^3/ µL)	>= 7.00	73	6.52	1.958	.790
	< 7.00	28	6.64	2.21	
PLATELT (×10^3/µL)	>= 7.00	73	295.63	69.70	.418
	< 7.00	28	308.78	79.42	
Neutrophil%	>= 7.00	73	55.91	8.05	.436
	< 7.00	28	57.28	7.38	
Lymphocyte %	>= 7.00	73	34.44	7.83	.636
	< 7.00	28	33.64	6.97	
PLR	>= 7.00	73	178.22	60.25	.333
	< 7.00	28	191.28	60.83	
NLR	>= 7.00	73	1.69	.681	.275
	< 7.00	28	1.86	.646	

(NLR) neutrophil lymphocyte ratio (PLR) The platelet-to-lymphocyte ratio

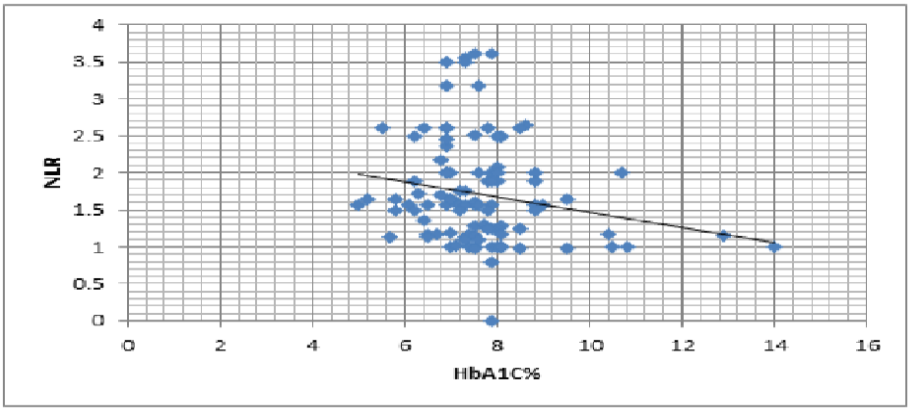

Figure 1: Relation between NLR of the patients and HbA1c %

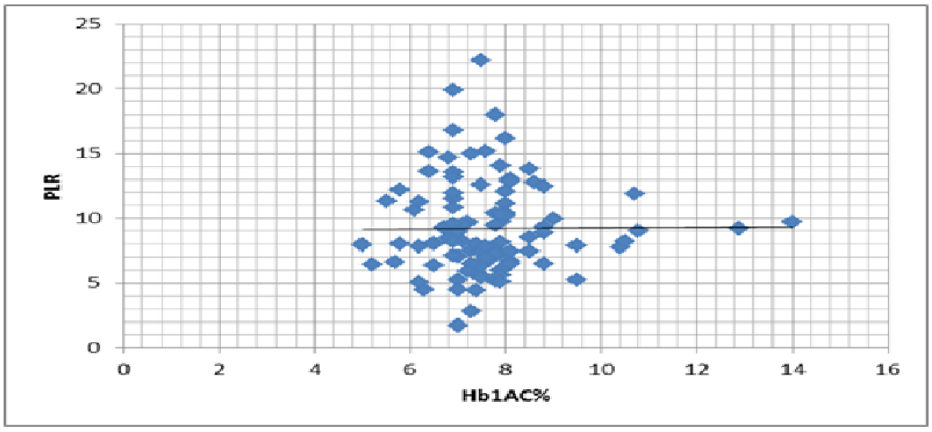

Figure 2: Relation between PLR of the patients and HbA1c %

There were positive correlation between NLR & PLR(r= .614), Neutrophil r(=.774),and negative correlation between NLR, Lymphocyte, and HbA1C% (r= -.756& -.205 respectively). PLR was positively correlated with NLR and neutrophil (r= .614 &.721 respectively), and negatively correlated with Lymphocyte(r=-.726)). Table 4 shows correlations between NLR & PLR and Hematologic Indices in children with T1DM.Fig 1&2 show the relation between HbA1G% & NLR and HbA1G% & PLR.

Table 5 shows result of a multiple linear regression to predict NLR from Predictors: (Constant). Result of a multiple linear regression shows that HbA1C% & Neutrophil were the main Predictors of NLR.

Table 4: correlations between NLR & PLR and Hematologic Indices in children with T1DM.

		NLR	PLR	Neutrophil	Lymphocyte	HbA1C%	Insulin unit /KG
NLR	Pearson Correlation	1	.614**	.774**	-.756**	- .205*	-.080
	Sig. (2-tailed)		.000	.000	.000	.039	.438
	N	102	101	102	102	102	96
PLR	Pearson Correlation	.614**	1	.721**	-.726**	.032	-.061
	Sig. (2-tailed)	.000		.000	.000	.747	.555
	N	101	101	101	101	101	101
(NLR) neutrophil lymphocyte ratio (PLR) The platelet-to-lymphocyte ratio **. Correlation is significant at the 0.01 level (2-tailed).							
*. Correlation is significant at the 0.05 level (2-tailed).							

Table 5: Result of a multiple linear regression to predict NLR & PLR

Model1 Dependent Variable: NLR Model2 Dependent Variable: PLR		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-1.658	.433		-3.828	.000
	HbA1C%	-.056	.033	-.106	-1.673	.098
	Neutrophil	.067	.006	.760	12.017	.000
2	(Constant) Neutrophil HbA1C%	-11.171 .326 .277	2.061 .030 .151	 .709 .120	-5.420 10.804 1.834	.000 .000 .069
(NLR) neutrophil lymphocyte ratio (PLR) The platelet-to-lymphocyte ratio						

Comparison of receiver-operating characteristics (ROC) analysis of: HbA1C%, NLR, and PLR for predicting T1DM (fig 3). The area under the curve of NLR was found as 0. 623 (P = 0.037) while that of PLR was 0.657 (P = 0.007). Indicating that predictability of PLR is higher when compared to NLR (table6).

Table 6: Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
HbA1C%	.989	.008	.000	.973	1.004
NLR	.623	.053	.037	.516	.739
PLR	.657	.043	.007	.555	.760
(NLR) neutrophil lymphocyte ratio (PLR) The platelet-to-lymphocyte ratio					
a. Under the nonparametric assumption					
b Null hypothesis: true area = 0.5					

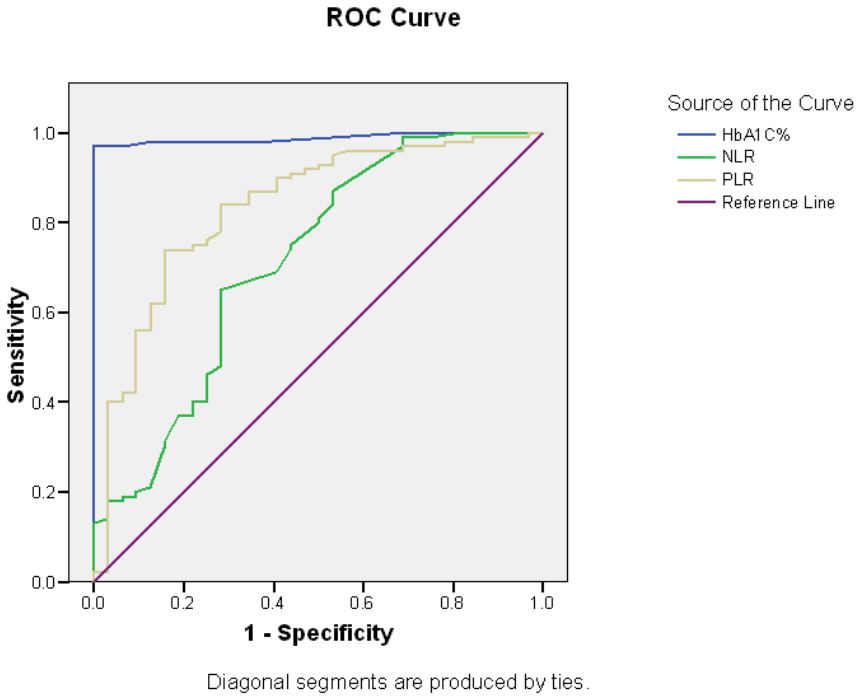

Figure 3: Comparison of receiver-operating characteristics (ROC) analysis of HbA1C%, Neutrophil-to-Lymphocyte (NLR), and Platelet-to-Lymphocyte Ratio (PLR) for predicting T1DM.

DISCUSSION

Type 1 diabetes mellitus is one of the most common chronic endocrinal diseases in children. In this study, we selected a group of patients with diabetes and a group of healthy children to compare hematological parameters, PLR and NLR between them. In our study, we observed that the white blood cell count was lower in children with type 1 diabetes mellitus than controls with no statistical difference. This finding is consistent with the results mentioned by others [12-14]. On other hand, several authors reported that type 1 diabetes patients had higher WBC counts [15&16]. The discrepancy in reported white blood cell counts in different studies might be explained by the fact that the data are related to different ethnic groups or WBCs was evaluated in different stages of diabetes. Therefore, further studies are required to explain these differences in WBC counts.

Neutrophils are the most abundant WBCs circulating in the body. Neutrophils are most involved WBC in the pathogenesis of type 1DM; thus, evaluation of neutrophil count is very important [15]. Our data showed that children with type 1 diabetes mellitus had significantly higher neutrophil numbers than the controls. Our result was in agreement with those of previous study [15]

Lymphocytes are known as the immune regulators of human disease, and they participate in the regulation of inflammation and infection [17]. In this study, there were statistically less lymphocytes than in control children. Khodabandehlou et.al, reported that lymphocyte levels were reduced as a result of hyperglycemia in patients with type 1 diabetes mellitus [13].

PLTs play important roles in many physiologic and pathologic processes such as thrombosis, inflammatory and hemostasis. Insulin deficiency can induce PLT activation [4] but researches to evaluate this in T1DM are limited. The current study showed higher platelet count in children with type 1 diabetes mellitus than the controls with no significant difference. Previous studies have shown that there is increased production of platelets in T1DM [7]. Khodabandehlou et.al, reported that the number of platelets was significantly decreased due to the hyperglycemic spike [13].

The HbA1c is a reliable biomarker for the diagnosis and prognosis of diabetes. HbA1c levels are depending on the management of diabetes and whether they are on long-term and/or short-term insulin dosage [18].

Currently, hematological indicators of NLR and PLR have extensive diagnostic and prognostic value in human diseases [19-22]. The present study showed that NLR and PLR were significantly higher in type 1 diabetic children compared to healthy subjects. This result was Similar to a previous study [23]. Another interesting finding was the positive correlation between HbA1c% and PLR in diabetic children. This finding is consistent with the results mentioned by Malachowska et al and Demitras et al, found correlation between HbA1c and some of PLT markers [24&4], In contrast, with the results mentioned by others [8, 25]. Elevated PLR in children with type 1 diabetes mellitus may reflect the underlying inflammatory burden of the disease. As HbA1c worsens due to poor diabetes control, increases inflammatory markers, including PLR, increase [8]. In the present study, in contrast to what may have been expected, our data showed that NLR correlated negatively with HbA1c levels. NLR is an essential marker of systemic inflammation. Another study found a relationship between NLR and diabetes mellitus in a patient group with chronic complications [26] NLR may be further increased in patients with diabetes due to hyperglycemia and complications, but we did not include in the study.

In this study, Diabetics patients were divided into two groups, one with HbA1c >= 7.0 (group 1) which included 73 subjects and the other with HbA1c < 7.0 (group 2) which included 28 subjects, there were no significant differences between demographic characteristics and hematologic indices in both groups. This night be due to the relatively small study population.

Based on ROC, accuracy is measured by the area under the ROC curve, it was found that area under the curve (AUC) of NLR and PLR were 0 .623, 0 .657 and respectively which indicates that PLR and NLR were not as good as Hb1AC% for diabetes management.

Limitations of the present study are the relatively small study population; glucose blood levels were not included and we did not assess the diabetic complications.

CONCLUSION

Based on our study results some of the parameters of CBC and PLR and NLR could be useful tool in following up T1DM. However, their use in clinical practice is often limited, due to lacking clinical validation. Complications of T1DM and PLR and NLR need further evaluation**.**

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