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Study of Neutrophil/Lymphocyte Ratio as a New Marker in Early Prediction of the Occurrence of Diabetic Ketoacidosis in Type 1 Diabetes Mellitus Patients without Infection

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ABSTRACT

Background: Diabetic ketoacidosis (DKA) is a severe, acute, life-threatening consequence of diabetes, characterized by hyperglycemia, ketoacidosis, as well as ketonuria. DKA in type 1 diabetes mellitus (T1DM) cases without infection represents an uninfected variant of systemic inflammatory response syndrome. Neutrophil-to-lymphocyte ratio (NLR) stands as a novel, simple and cost-effective marker for the systemic inflammatory response.

Objective: This study aims to investigate NLR role in predicting the DKA among T1DM cases without infection.

Patients and Methods: This observational cross-sectional study included 90 participant. They exhibited the clinical criteria of T1DM without infection in the presence or absence of DKA. Participants were grouped into: Group (I): Healthy controls, group (II) included non-DKA patients with T1DM and group (III) that consisted of DKA patients with T1DM.

Results: TLC was positively correlated with glycated hemoglobin (HbA1c), NLR, and C-reactive protein (CRP). Also, NLR was positively correlated with disease duration, HbA1c, TLC, hemoglobin, platelets and CRP. According to NLR, HbA1c and TLC for predicting diabetic ketoacidosis in patients with T1DM without infection: At a cut-off value of ≤ 2.97, ≤ 8.35 and ≤ 6.9 respectively, the area under the curve was 0.887, 0.695 and 0.667 respectively and the sensitivity was 86.7%, 83.3% and 83.3% respectively, the specificity was 80.0%, 60% and 73.3% respectively.

Conclusions: NLR was elevated among T1DM cases who developed DKA and an uninfected state. NLR could be a simple, easily obtainable and cheap biomarker for prediction of DKA in patients with T1DM.

Keywords: Neutrophil/Lymphocyte, Prediction, Diabetic Ketoacidosis, Type 1 Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus (DM) stands as a fast-growing worldwide condition with major social, health as well as financial implications. It is escalating to an alarming epidemic level [1]. DM is described as a collection of metabolic disorders characterized by persistent hyperglycemia arising from abnormalities in insulin production, insulin action, or both. It has several subclassifications, but Type 1 and 2 DM represent the primary subtypes [2].

Diabetic ketoacidosis (DKA) is a severe, acute, life-threatening consequence of diabetes, characterized by hyperglycemia, ketoacidosis, as well as ketonuria [3]. It is mostly seen among individuals developing T1DM [4]. The primary etiology for DKA is infection, however, a missed or inadequate insulin dosage could contribute to it [5]. Nevertheless, DKA among T1DM cases without infection represents an uninfected variant of systemic inflammatory response syndrome (SIRS), characterized by markedly elevated proinflammatory markers despite the lack of infection [6].

A prior research has shown a significant rise in IL-6, IL-1B, IL-8, and TNF-α, along with elevated counter-regulatory hormone levels among individuals developing uncontrolled diabetes and ketoacidosis [7]. The increase of circulating proinflammatory cytokines is rapidly reduced after insulin treatment and the blood glucose level normalization [8]. The secretion of inflammatory factors diminishes the pancreatic β cells functionality, whereas insulin insufficiency in DKA exacerbates the elevation of inflammatory factors via enhancing the acute-phase response, thus creating a vicious circle [9].

Neutrophils as well as lymphocytes are the first inflammatory and regulatory indicators, respectively, identified in damaged sites. They stimulate primary cell types included in acute and chronic inflammation [10].

The neutrophil-to-lymphocyte ratio (NLR) represents a novel, straightforward, and cost-effective indicator for the systemic inflammatory response, with prior research that documented its use as an inflammatory marker [11]. Recent studies indicate that NLR is significantly associated with the incidence, severity, as well as outcomes of several acute conditions, including acute coronary syndrome, [12] acute ischemic stroke (AIS) [13], and acute pancreatitis [14]. Thus, this work aimsinvestigate the neutrophil/lymphocyte ratio role in predicting the DKA occurrence among T1DM cases without infection.

PATIENTS AND METHODS

Study Design and Participants: This observational cross-sectional study included 90 participants, with ages between 16 and 32 years from both sexes. They had the clinical criteria of T1DM without infection in the presence or absence of DKA. Patients with DKA showed a plasma glucose level > 200 mg/dl (11 mmol/L), a positive urinary dipstick for ketone bodies, as well as an arterial pH value < 7.30 upon admission.

Participants were selected from the inpatient and outpatient clinics of The Internal Medicine Department

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at Tanta University Hospitals during the study period from November 2022 to November 2023.

Exclusion criteria: Patients diagnosed with type 2 diabetes mellitus, severe cardiovascular conditions such as acute myocardial infarction, cachexia, immune disorders, hepatic dysfunction, or renal impairment (defined as eGFR < 90 mL/min/1.73 m² or an ACR > 30 mg/g). Those with active inflammation or infection, use of anti-inflammatory medications, pregnancy, aspirin or statin therapy, and any history of malignancy.

Grouping: All participants underwent an equal categorization into three groups: Group (I): healthy controls, group (II) included non-DKA patients with T1DM and group (III) that composed of DKA patients of T1DM, and it was further subdivided, based on the severity of DKA, into 3 subgroups; A, B and C as mild, moderate, and severe DKA respectively according to the following parameters: Serum bicarbonate was 15-18, 10 to <15 and < 10mEq/L respectively, arterial pH was 7.25-7.30, 7.00 to < 7.24 & < 7 respectively, and clinically diagnosed with Oriented, alert yet fatigued among mild cases, Kussmaul respirations; oriented yet sleepy; arousal among moderate cases, while Kussmaul or depressed respirations; sleepy to depressed sensorium to coma among severe cases.

Our team gathered a comprehensive medical history from all participants, then conducted clinical examination and systemic exams, followed by electro-cardiography (ECG), routine imaging (Chest X-ray and pelvi-abdominal ultrasound) and laboratory investigations [Urine, stool analysis, complete blood count (CBC), complete liver function, serum urea, creatinine, albumin-creatinine ratio (ACR), sodium, potassium levels (Na and K), blood glucose level (fasting blood glucose level (FBG) and 2-hour postprandial blood glucose level (2HPP), lipid profile [total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c)], glycated hemoglobin (HbA1c), arterial blood gases (ABG) and NLR = neutrophil count/lymphocyte count].

Blood collection and laboratory analysis: Laboratory analyses were performed in the Clinical Pathology Department, Faculty of Medicine, Tanta University. Our team collected fasting venous blood samples (10 mL) to assess hematological parameters while conducting biochemical analysis. We utilized vacutainer tubes with and without EDTA for blood specimens. In addition, we collected arterial blood samples in heparinized tubes for measuring arterial blood gases. We also obtained blood samples upon admission prior to the initial therapy. The samples were collected in EDTA tubes for differential WBC counts then analyzed employing an automated Sysmex blood counting analyzer. Fasting plasma glucose (FBG), 2-hour postprandial blood glucose level (2HPP) levels, liver function tests (S. bilirubin, S. total protein, S. albumin, AST, ALT), blood urea nitrogen (BUN), serum creatinine, CRP, and urinary albumin creatinine ratio (ACR) were determined using an automated biochemical analyzer. HbA1c was quantified utilizing a hemoglobin A1c analyzer, while lipid parameters (total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were assessed through standard enzymatic techniques employing an automated biochemical analyzer, and LDL concentration was assessed by the Friedewald equation. Na and K levels were assessed utilizing the ion-selective electrode (ISE) technique. Morning urine samples were collected in sterile cups and immediately sent to the lab for laboratory physical, biochemical, and microscopical examination. Stool samples were collected and sent immediately to the lab for laboratory examination.

Ethical considerations: The study was done after being accepted by The Research Ethics Committee, Tanta University. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Data Management:

Our team conducted a statistical analysis utilizing SPSS version 26 (IBM Inc., Chicago, IL, USA). Quantitative variables were illustrated as mean and standard deviation (SD), and comparisons were carried out among the three groups utilizing ANOVA (F) test with post hoc test (Tukey). Qualitative variables were illustrated as frequency and percentage (%) and analysis was carried out utilizing the Chi-square test. A two tailed P value ≤ 0.05 was deemed statistically significant. Pearson correlation was employed for estimating the degree of correlation between two quantitative variables. Spearman correlation was also employed for estimating the degree of correlation among quantitative and qualitative variables. Receiver operating characteristic curve (ROC) was utilized for comparing the variables' ability in distinguishing between specific groups of cases. It is generated utilizing plotting sensitivity (TP) on the Y axis versus 1-specificity (FP) on the X axis at different cut-off values. The area under the curve (ROC) indicated the test's diagnostic performance. Sensitivity: (true positive rate) denoted the probability of a positive test, conditioned on truly being positive. Sensitivity= True positive/(True positive + false negative). Specificity (true negative rate) denoted the probability of a negative test, conditioned on truly being negative. Specificity= True negative/(True negative + false positive).

RESULTS

Table (1) showed no significant variance among all groups as regards demographic data, AST, ALT, serum albumin, cholesterol, triglycerides, HDL, LDL, BUN, creatinine and ACR. Also, no significant variance was documented among the DKA and the Non-DKA groups as regards disease duration.

Table (1): Comparison among all groups regarding demographic data, liver function tests, lipid profiles and kidney function tests

Groups / Parameters		Groups			P- Value
		Group I Control	**Group II Non-DKA**	**Group III DKA**	
Age (Years)		22.63±3.68	22.03±4.0	23.48±4.01	F=1.046, P=0.356(a)
Disease duration (Years)			5.48±3.37	5.93±3.05	t= -0.532, P=0.597(b)
Sex	**Male**	12(29.3%)	15(36.6%)	14(34.1%)	X^2=0.627, P=0.731(c)
	Female	18(36.7%)	15(30.6%)	16(32.7%)	
AST (U/L)		22.5±5.96	22.2±6.26	22.57±5.49	F=0.033, P=0.968(a)
ALT (U/L)		21.77±5.29	21.83±5.23	22.67±5.07	F=0.280, P=0.757(a)
Serum albumin (g/dL)		4.52±0.72	4.53±0.71	4.49±0.71	F=0.025, P=0.975(a)
Cholesterol (mg/dL)		128.73±14.36	129.2±14.23	126.8±12.63	F=0.249, P=0.780(a)
Triglycerides (mg/dL)		119.5±18.29	119.8±18.24	121.4±7.24	F=0.137, P=0.872(a)
HDL (mg/dL)		73.57±6.47	73.26±6.51	74.1±5.85	F=0.135, P=0.874(a)
LDL (mg/dL)		79.46±8.85	79.3±8.75	79.23±8.58	F=0.006, P=0.994(a)
BUN (mg/dL)		16.77±3.79	16.33±3.78	17.4±2.92	F=0.693, P=0.503(a)
Creatinine (mg/dL)		0.81±0.15	0.82±0.13	0.82±0.14	F=0.037, P=0.963(a)
ACR (mg albumin/g creatinine)		18.03±3.64	17.93±3.65	18.83±3.46	F=0.567, P=0.570(a)
CRP (mg/L)		2.44±1.27	5.83±0.79	18.47±5.32	**F=210.485, P<0.001**(a) P1A<0.001** P2B<0.001** P3C<0.001****

Data are presented as Mean ± SD. DKA: Diabetic ketoacidosis, (a): one-way anova test, **(**b): Independent T-test, (c): Chi-Square Test, P: P-value between groups, ALT: Alanine aminotransferase, AST: aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, BUN: Blood urea nitrogen, ACR: Albumin to creatinine ratio. P: P-value between groups, P1A: p-value for comparing between group I & group II, P2B: p-value for comparing between group I & group III, P3C: p-value for comparing between group II & group III.

Table (2) showed no significant variance between the control and the non-DKA groups as regards PH, PCO_2, HCO_3, sodium, hemoglobin, TLC and platelets count. No significant variation was documented among all groups as regards potassium. A significant variance was observed among all groups as regards TLC, absolute neutrophils and NLR (p-value < 0.001). The control group showed lower TLC, absolute neutrophils and NLR than the non-DKA group and both groups showed lower TLC, absolute neutrophils and NLR than the DKA group. A significant variance was documented among all groups regarding absolute lymphocytes (p-value < 0.001). The control group showed higher absolute lymphocytes as opposed to the non-DKA group. Additionally, both groups showed higher absolute lymphocytes than the DKA group. A statistically significant variation was documented between the DKA group and the other two groups; (the control group and the non-DKA group). As regards PH, PCO_2, HCO_3 and sodium (p-value < 0.001) for all the DKA group showed lower PH, PCO_2, HCO_3 and sodium than other two groups. A statistically significant variation was documented among the DKA group and the other two groups; (the control group and the non-DKA group). As regards hemoglobin level and platelets count (p-value < 0.001), the DKA group showed higher hemoglobin level and platelets count, than the other two groups.

Table (2): Comparison among all groups regarding ketoacidosis parameters, sodium, potassium, complete blood count and TLC derivatives

	Groups	**P- Value**

	Group I Control	Group II Non-DKA	Group III DKA	
PH	7.37±0.024	7.37±0.025	7.19±0.103	**F=65.227, P<0.001**(a)** P1A=0.727 **P2B<0.001** **P3C<0.001**
PCO2 (mmHg)	38.13±2.04	38.73±2.03	23.58±6.58	**F=128.24, P<0.001**(a)** P1A=0.577 **P2B<0.001** **P3C<0.001**
HCO3 (mEq/L)	24.53±1.47	25.0±1.41	10.53±4.91	**F=214.94, P<0.001**(a)** P1A=0.558 **P2B<0.001** **P3C<0.001**
Sodium(mEq/L)	138.23±2.24	137.87±1.86	134.4±2.31	**F=29.193, P<0.001**(a)** P1A=0.510 **P2B<0.001** **P3C<0.001**
Potassium (mmol/L)	4.03±0.36	3.91±0.31	4.13±0.79	F=1.380, P=0.257(a)
Hemoglobin (g/dL)	11.94±1.16	12.15±1.04	13.12±1.13	**F=9.564, P<0.001**(a)** P1A=0.468 **P2B<0.001** **P3C<0.001**
TLC (10^3/µL)	6.17±1.03	7.68±1.06	18.45±3.49	**F=281.45, P<0.001**(a)** **P1A=0.009*** **P2B<0.001** **P3C<0.001**
Platelet count (10^3/µL)	298.88±59.18	311.8±64.32	370.3±72.03	**F=10.269, P<0.001**(a)** P1A=0.427 **P2B<0.001** **P3C<0.001**
Absolute neutrophil (10^3/µL)	3.23±0.51	4.68±1.15	6.8±2.73	**F=32.112, P<0.001**(a)** **P1A<0.001** **P2B<0.001** **P3C<0.001**
Absolute lymphocyte (10^3/µL)	4.22±0.33	2.99±0.53	2.55±0.45	**F=114.54, P<0.001**(a)** **P1A<0.001** **P2B<0.001** **P3C<0.001**

Data are presented as Mean ± SD. DKA: Diabetic ketoacidosis, PH: potential of hydrogen, PCO2: partial pressure of carbon dioxide, HCO3: Bicarbonate, TLC: total leucocytic count, NLR: neutrophil to lymphocyte ratio, (a): one-way anova test, (c): Chi-Square Test. P: P-value between groups, P1A: p-value for comparing between group I & group II, P2B: p-value for comparing between group I & group III, P3C: p-value for comparing between group II & group III.

Table (3) showed a statistically significant variance among the DKA subgroups as regards PH (p-value < 0.001). The mild-DKA group showed higher PH than the moderate-DKA group and the severe-DKA group. Additionally, a statistically significant variance was documented among the DKA subgroups regarding HCO_3 (p-value < 0.001). The mild-DKA group showed higher HCO_3 than the moderate-DKA group and both groups showed higher HCO_3 than the severe-DKA group. Additionally, a statistically significant variance was documented among the DKA subgroups as regards NLR (p-value < 0.001). The mild-DKA group showed a lower NLR than the moderate-DKA group and both groups showed lower NLR than the severe-DKA group.

Table (3): Comparison among the studied DKA subgroups as regards PH, HCO_3 and NLR

	Group A	Group B	Group C	P- Value
	10(33.33%)	**18(60.0%)**	**2(6.7%)**	

PH	7.27±0.02	7.17±0.05	6.89±0.1	**F=51.74, P<0.001**(a) P1A<0.001** P2B<0.001** P3C<0.001****
HCO_3 (mEq/L)	14.51±3.26	8.69±3.59	3.0±1.13	**F=14.088, P<0.001**(a) P1A<0.001** P2B<0.001** P3C=0.034***
NLR	2.98±0.39	4.07±0.48	5.5±2.12	**F=18.99, P<0.001**(a) P1A<0.001** P2B<0.001** P3C=0.004***

Data are presented as Mean ± SD. DKA: Diabetic ketoacidosis, PH: potential of hydrogen, HCO_3: Bicarbonate, NLR: neutrophil to lymphocyte ratio, (a): one-way anova test, (c): Chi-Square Test, P: P-value between groups, P1A: p-value for comparing between group I & group II, P2B: p-value for comparing between group I & group III, P3C: p-value for comparing between group II & group III.

Table (4) showed NLR was negatively correlated with PH, PCO_2, HCO_3 and sodium. Also, NLR was positively correlated with disease duration, HbA1c, TLC, hemoglobin, platelets and CRP. NLR was not correlated with age, sex, AST, ALT, serum albumin, BUN, creatinine, ACR, cholesterol, triglycerides, HDL, LDL and potassium. As the diabetes duration exhibited a significant association with NLR among T1DM cases. To prevent the diabetes duration impact on NLR levels, while eliminating potential confounding factors, we conducted a diabetes duration adjustment. Following adjustment, NLR was negatively correlated with PH, PCO_2 and HCO_3. Also, NLR was positively correlated with HbA1c, hemoglobin, platelets, TLC and CRP. NLR was not correlated with age, sex, AST, ALT, serum albumin, BUN, creatinine, ACR, cholesterol, triglycerides, HDL and LDL.

Table (4): Correlations between NLR and other parameters

	NLR		**Adjusted NLR for disease duration**	
	r	**p**	**r**	**P**
Age	0.061	0.567	-0.119	0.371
Sex	-0.112	0.295	-0.201	0.126
Disease duration	**0.357**	**0.005***		
HbA1C	**0.830**	**<0.001****	**0.568**	**<0.001****
PH	**-0.496**	**<0.001****	**-0.828**	**<0.001****
PCO_2	**-0.745**	**<0.001****	**-0.707**	**<0.001****
HCO_3	**-0.780**	**<0.001****	**-0.735**	**<0.001****
Hemoglobin	**0.400**	**<0.001****	**0.324**	**0.012***
Platelets	**0.322**	**<0.001****	**0.296**	**<0.001****
TLC	**0.410**	**<0.001****	**0.369**	**<0.001****
AST	-0.012	0.909	-0.029	0.826
ALT	0.02	0.851	-0.068	0.611
Serum albumin	0.055	0.605	0.082	0.536
BUN	0.065	0.543	-0.049	0.714
Creatinine	-0.058	0.589	-0.122	0.356
ACR	0.101	0.345	0.075	0.575
Sodium	**-0.569**	**<0.001****	**-0.552**	**<0.001****
Potassium	0.079	0.461	-0.082	0.572
Cholesterol	-0.047	0.661	0.008	0.952
Triglycerides	0.104	0.330	0.114	0.389
HDL	0.095	0.391	0.130	0.327
LDL	0.074	0.491	0.085	0.521
CRP	**0.745**	**<0.001****	**0.525**	**<0.001****

*: Statistically significant at $p \leq 0.05$ **: Statistically significant at $p < 0.001$. r: Pearson correlation and Spearman rho, NLR: Neutrophil to lymphocyte ratio, PH: potential of hydrogen, PCO_2: partial pressure of carbon dioxide, HCO_3: Bicarbonate, TLC: Total leucocytic count, BUN: Blood urea nitrogen, ACR: Albumin to creatinine ratio, ALT: alanine transaminase, AST: aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CRP: C-reactive protein.

Table (5) showed TLC was negatively correlated with PH, PCO_2 and HCO_3. Also, TLC was positively correlated with HbA1c, NLR, and CRP. TLC was not correlated with age, sex, disease duration, hemoglobin, platelets,

AST, ALT, serum albumin, BUN, creatinine, ACR, cholesterol, triglycerides, HDL, LDL, sodium and potassium. TLC was negatively correlated with PH, PCO_2 and HCO_3.

Table (5): Correlations between TLC, and other parameters

Variables	TLC	
	r	P
Age	0.062	0.564
Sex	0.023	0.831
Disease duration	-0.113	0.390
HbA1C	**0.478**	**<0.001****
PH	**-0.370**	**<0.001****
PCO_2	**-0.294**	**0.005***
HCO_3	**-0.283**	**0.007***
Hemoglobin	0.113	0.291
Platelets	0.143	0.180
AST	-0.041	0.699
ALT	0.013	0.905
Serum albumin	0.166	0.118
NLR	**0.410**	**<0.001****
BUN	-0.154	0.148
Creatinine	0.114	0.283
ACR	-0.191	0.393
Sodium	-0.118	0.268
Potassium	-0.061	0.566
Cholesterol	-0.009	0.936
Triglycerides	-0.19	0.859
HDL	-0.063	0.554
LDL	-0.190	0.073
CRP	**0.228**	**0.027***

*: Statistically significant at $p \leq 0.05$ **: Statistically significant at $p < 0.001$. r: Pearson correlation and Spearman rho, NLR: Neutrophil to lymphocyte ratio, PH: potential of hydrogen, PCO2: partial pressure of carbon dioxide, HCO3: Bicarbonate, TLC: Total leucocytic count, BUN: Blood urea nitrogen, ACR: Albumin to creatinine ratio, ALT: alanine transaminase, AST: aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, CRP: C-reactive protein.

Table (6) showed the regression analysis that showed that NLR and HbA1c exhibited a significant association with predicting T1DM cases developing DKA.

Table (6): Linear regression analysis of factors related to blood PH in T1DM patients

PH	B	95% CI for B		Sig.	SE B	β	R^2	ΔR^2
		LL	UL					
Model							0.744	0.732
Age	0.005	-0.019	0.028	0.680	0.012	0.023		
Sex	0.110	-0.076	0.295	0.243	0.093	0.067		
HbA1C	0.062	0.013	0.111	**0.014***	0.025	0.248		
NLR	0.487	0.340	0.634	**<0.001****	0.074	0.651		

T1DM: Type 1 diabetes mellitus, B: unstandardized regression coefficient, CI: confidence of interval, LL: lower limit, UL: upper limit, Sig: significance, SE B = standard error of the coefficient, ß = standardized coefficient, R2 = coefficient of determination, ΔR2 = adjusted R2. * $p< 0.05$, ** $p<0.001$.

According to NLR for predicting diabetic ketoacidosis in patients with T1DM without infection: At a cut-off value of ≤ 2.97; the area under the curve was 0.887, the sensitivity was 86.7%, the specificity was 80.0%, the positive predictive value (PPV) was 81.25%, and the negative predictive value (NPV) was 85.71%. Regarding HbA1c for predicting diabetic ketoacidosis in patients with T1DM without infection:

At a cut-off value of ≤ 8.35; the area under the curve was 0.695, the sensitivity was 83.3%, the specificity was 60%, the positive predictive value (PPV) was 67.6%, and the negative predictive value (NPV) was 78.3%. According to the TLC for predicting diabetic ketoacidosis patients with T1DM without infection: At a cut-off value of ≤ 6.9; the area under the curve was 0.667, the sensitivity was 83.3%, the specificity was 73.3%, the positive predictive value (PPV) was 75.75%, and the negative predictive value (NPV) was 81.48% (Figure 1).

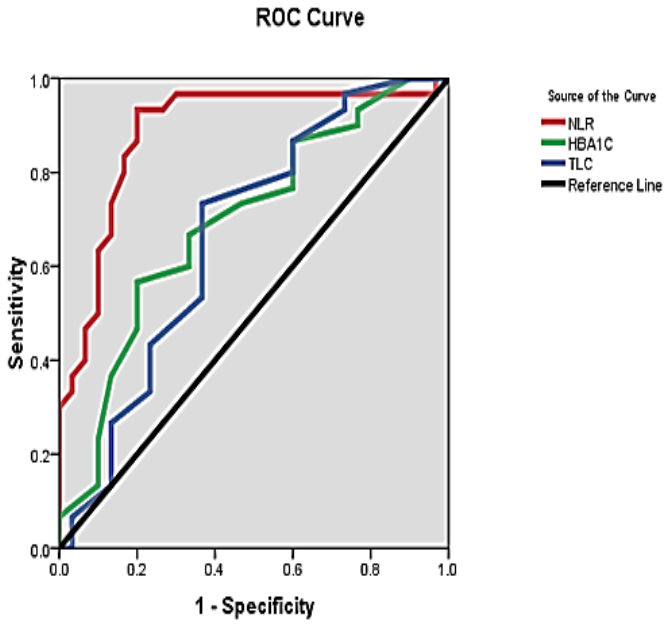

Figure (1): ROC curve for NLR, $HbA1_C$, and TLC for predicting DKA in T1DM without infection,

DISCUSSION

DM stands as a major health issue globally. Its burden increases progressively over years [15]. Type 1DM accounts for 5-10% of diabetic patients and affects mainly children and young adults [16]. DKA is the most popular and severe acute diabetic complication, occurring mainly in T1D patients and its incidence is increasing progressively [17, 18]. Infection represents the most precipitating cause for DKA [19]. Nevertheless, DKA among T1DM cases who do not exhibit an infection is a variant of systemic inflammatory response syndrome characterized by markedly elevated proinflammatory markers [20].

NLR was correlated with many inflammatory-associated diseases for example but not inclusively; **Adamstein *et al.*** [21] reported that NLR is deemed an independent predictor for cardiovascular disease (CVD) risks as well as all-cause mortality. Also, **Umarani *et al.*** [22] studied NLR as an early prognostic indicator of microvascular adverse events among diabetes cases, addressing that diabetes cases with complications showed higher NLR as opposed to others developing controlled DM [22]. Another study by **Wan *et al.*** [23] found that an increased NLR level was linked to an elevated incidence of CVDs as well as diabetic kidney diseases (DKDs) in diabetic adults. However, very little studies describe NLR levels among T1DM cases or in acute complications of DM.

Our current study investigated NLR among normal individuals and within T1DM cases with non-DKA, mild-DKA and moderate-DKA as well as severe-DKA in an uninfected condition. In our study, being at an uninfected condition, TIDM patients exhibited a significant increase as regards serum NLR levels as opposed to controls (with a mean of 2.60 ± 0.55 vs 1.49 ± 0.25, p <0.001). Furthermore, TIDM cases developing DKA exhibited a significant increase as regards serum NLR as opposed to non-DKA group (with a mean of 3.83 ± 0.99 for DKA group). Also, NLR was elevated in severe DKA, with means of 2.98 ± 0.98, 4.07 ± 0.48 and 5.5 ± 2.12 for mild, moderate and severe DKA groups respectively.

Furthermore, TIDM cases developing DKA and an uninfected state exhibited a significant increase as regards WBC counts with a mean of 18.45 ± 3.49 $10^3/\mu L$ as opposed to the non-DKA group with a mean of 7.68 ± 1.06 $10^3/\mu L$ and the control group with a mean of 6.17 ± 1.03 $10^3/\mu L$, and WBC counts were elevated in severe DKA. This supports **Cheng *et al.*** [9] and **Scutca *et al.*** [24] where both studies discussed the NLR role in DKA among T1DM cases without infection, reporting that NLR scores were elevated in comparison with those who did not exhibit ketoacidosis to mild, moderate, and severe ketoacidosis groups.

Our previous results can be explained that T1DM represents a chronic inflammatory condition, which is aggravated when DKA develops and NLR is a predictor of the state of systemic inflammation. This is supported by **Erbağci *et al.*** [25] who studied mediators of inflammation in children with T1DM and showed that the elevated secretion of inflammatory mediators (e.g., TNF-a, IL-6, C-reactive protein) and the diminished anti-inflammatory cytokines production (e.g., IL-10) might result in enhanced adipose tissue activation, higher insulin resistance, as well as subsequent diabetes progression. Another research by **Stentz *et al.*** [26] proposed that DKA remains linked to oxidative stress as well as inflammatory responses in a hyperglycemic condition. In addition to the evident impact of haemoconcentration on the NLR as well as WBC counts resulting from hyperglycaemia, we hypothesized that the elevated NLR would also be associated with inadequate blood glucose management. Similarly, **Sefil *et al.*** [27] addressed that he NLR was elevated among T2DM individuals with HbA1c levels beyond 7% compared to those at or below 7%. Similar results were documented by **Nurahmi *et al.*** [28]. Similarly, we observed a positive association between NLR and HbAlc in TIDM cases after adjustment for diabetes duration. Another explanation is that the autonomic nervous system regulates the neutrophils and lymphocytes number. Additionally, **Abo *et al.*** [29]

addressed that adrenergic receptors remain evident on neutrophils, with their quantity and function modulated by sympathetic neurons, while cholinergic receptors on lymphocytes are controlled by parasympathetic nerves. Elevated sympathetic nerve activity may lead to the production of more neutrophils as well as proinflammatory substances.

Our research addressed a positive association between NLR, hemoglobin and platelet levels. This could be explained by the hemoconcentration effect of DKA on blood components due to hyperglycemia. Furthermore, thrombocytosis was evident in DKA as a response to the associated inflammatory reaction as reported by **Putradi *et al.* [30]** who found a statistically significant variation as regards platelet counts among DKA and Non-DKA groups. Platelet counts increased in the DKA group (451,000/mm^3 vs 285,500/mm^3 for the non-DKA population). This comes in agreement with **Mousa *et al.* [31]** who assessed the platelets' morphological changes in children with DKA and reported a significant increase in platelet count in DKA group in comparison with non-DKA one. Those findings could explain the hypercoagulable state in DKA. These findings came in disagreement with **Cheng *et al.* [9]** who found that NLR and albumin levels were negatively correlated and that NLR and creatinine levels were positively correlated. This result could be caused due to limitations in this study, so more research including larger populations is necessary to identify the exact correlation of NLR to serum creatinine and albumin levels.

NLR and HbA1c were found to be significantly associated with predicting diabetic ketoacidosis (DKA) in T1DM patients using linear regression analysis of factors associated with blood PH. Our study also demonstrated the high specificity and sensitivity of NLR in DKA prediction, as demonstrated utilizing ROC curve analysis. When used to predict diabetic ketoacidosis among T1DM cases without infection, NLR at a cut-off value of ≤ 2.97 exhibited a superior diagnostic efficacy, indicating an area under the curve (AUC) of 0.887, sensitivity of 86.7%, specificity of 80.0%, PPV of 81.25%, and NPV of 85.71%. Furthermore, NLR was not correlated with serum albumin and creatinine. These findings are supported by **Cheng *et al.* [9]** who showed that the addition of the ROC curve of predicted variables related to DKA occurrence among T1DM cases in an uninfected state could show a significant enhancement as regards the predictive model for DKA incidence in T1DM. In the same context by applying ROC curve analysis, and as reported by **Scutca *et al.* [24]**, the statistical threshold value of the NLR for predicting DKA reached 1.84, exhibiting a sensitivity of 80.2%, as well as a specificity of 80%. Our findings are also supported by **Hamza *et al.* [32]** who found that by using ROC curve, The NLR's statistical threshold value for DKA prediction in T1DM reached 2.05, exhibiting a sensitivity of 100%, while specificity was 50%.

Limitations of the study: The total number of patients was small, and our data was only from one hospital. The results could be more conclusive and more propagative if carried out with a larger population.

CONCLUSION

NLR was elevated among T1DM cases who develop DKA and an uninfected state. Additionally, it was present at high levels in severe DKA. NLR could be a simple, easily obtainable and cheap biomarker for prediction of DKA in patients with T1DM, particularly in pre-hospital cases as blood gas test is not a routine test. Clearly, more well-designed prospective studies are needed to support this theory.

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