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Prediabetes: Profile of Haematological Indices in Iraqi Patients

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Abstract

Prediabetes has been a target for research to understand good predictors of prediabetes. This cross-sectional study sought to analyze the predictive significance of WBC and neutrophil counts, as well as NLR prediabetes in Iraqi adults. Three groups of individuals have included: 30 apparently healthy individuals (normoglycemia), 125 prediabetics, and 30 patients with type 2 diabetes mellitus (T2DM).Results revealed that fasting blood glucose(FBG) median was significantly higher in prediabetics (106.6 [IQR: 103.7-112.5] *vs*.80.2 [IQR: 77.1-86.1] mg/dL; $p< 0.001$) or diabetics (157.14 [133.0-181.2]*vs*.80.2 [IQR: 77.1-86.1] mg/dL; $p< 0.001$) than in normoglycemic. The Hb1Ac showed a similar significant increase in prediabetics and diabetics compared to normoglycemic. It was also noted that both parameters were significantly elevated in diabetics compared to prediabetics. The total count of WBCs was significantly higher in prediabetics and diabetics compared to normoglycemic (7.5 ± 2.1 and 8.0 ± 2.4 *vs*. 6.5 ± 1.3 $\times 10^9$/L; $p = 0.020$), while there was no significant difference between prediabetics and diabetics in this context. A similar pattern of a significantly increased count of neutrophils was observed in prediabetics and diabetics compared to normoglycemic. The NLR was significantly higher in prediabetics (1.67 [IQR: 1.30-2.22]; $p = 0.016$) and diabetics (1.65 [IQR: 1.33-2.32]; $p = 0.021$) than in normoglycemic (1.40 [IQR: 1.12-1.72]), while the NLR showed no significant difference between prediabetics and diabetics ($p = 0.577$). ROC curve analysis revealed that the NLR had an approximated area under the curve in prediabetes and diabetes (0.641 and 0.674, respectively), but the sensitivity and sensitivity were slightly higher in prediabetes than diabetes (58.4 and 60. 0% *vs*. 55.2 and 56.7%, respectively). Logistic regression analysis revealed that the NLR was associated with a similar risk in the development of prediabetes (OR = 2.61; 95% CI = 1.12 - 6.07; $p = 0.027$) and diabetes (OR = 3.05; 95% CI = 1.07 - 8.68; $p = 0.067$), but the p-value in diabetes was not significant.

Keywords: Prediabetes, Diabetes, Haematological indices, Neutrophil-to-lymphocyte ratio

1. Introduction

It has been indicated that haematological indices are important predictors in determining numerical, structural, and functional changes in blood cells under various physiological, inflammatory, and pathological conditions [1]. One such condition is chronic hyperglycemia, and studies have linked it to microvascular and macrovascular complications due to damage to many components of blood cells [2, 3]. Therefore, haematological indices have been considered useful predictors in monitoring the vascular complications in patients with type 2 diabetes mellitus (T2DM) (Biadgo et al., 2016). For instance, persistently elevated glycated haemoglobin (HbA1c) in T2DM patients can cause functional and structural changes in the haemoglobin molecule, in addition to altering cytoplasmic viscosity and disturbing red blood cells (RBC) osmosis [4]. Besides, elevated HbA1c has been linked to impaired vascular endothelial responses, which are associated with an increased risk of developing hypertension and vascular diseases in T2DM patients [5].

In addition to RBCs, white blood cells (WBCs) can also be affected by chronic hyperglycemia, which has been correlated with elevated counts of these cells in T2DM patients [6]. Further, the elevated count of WBCs in diabetic patients has also been associated with vascular complications and the development of other clinical conditions such as retinopathy, peripheral arterial disease, and albuminuria [7]. The WBCs include two important cells, the neutrophils, and lymphocytes. The former mediate innate immunity, while the latter are key cells in adaptive immunity [8]. Regarding neutrophils, the functions of these cells are affected by chronic hyperglycemia; for instance, chemotaxis, phagocytosis, and killing capacity [9]. Similarly, dysregulated functions of

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lymphocytes have been observed in diabetic patients due to hyperglycemia [10]. Furthermore, it has been indicated that lymphopenia is one of the consequences of chronic hyperglycemia in T2DM patients, particularly those with microvascular and macrovascular complications [11].

Under normal physiological conditions, the number of neutrophils is higher than that of lymphocytes in humans, but these numbers can change during pathological conditions [8]. The numerical change of both types of WBCs is combined into a single parameter called the neutrophil-to-lymphocyte ratio (NLR), which is more sensitive than assessing either one separately [12]. The NLR is calculated by dividing the absolute count of neutrophils by the absolute count of lymphocytes and is considered a simply measured and reproducible biomarker in the evaluation of systemic inflammation [11]. Recent studies have disclosed the significance of assessing NLR in the prognosis of multiple inflammatory and cardiovascular diseases [13].

In T2DM, studies have also indicated that inflammatory pathways are activated in the patients, and thus the NLR may aid determine the degree of inflammation [14]. Further, it has also been proposed that elevated NLR in healthy people may indicate impaired glucose metabolism and predict the development of T2DM [15, 16].

The main concern of the current study was prediabetes and haematological data in this regard have not been well elaborated. Therefore, a retrospective study was conducted to determine haematological indices in prediabetics compared to normoglycemic and diabetics, with a special focus on the NLR. The predictive significance of NLR in prediabetes and diabetes was also evaluated.

2. Materials and Methods

Populations studied

A prospective study was performed on three groups of individuals from December 2020-May 2021. The first group included 30 apparently healthy individuals (Normoglycemia; mean age ± SD = 42.5 ± 12.9 years; 50% males), the second group included 125 prediabetics (mean age ± SD = 47.2 ± 11.1 years; 53.6% males) and the third group included 30 T2DM patients (mean age ± SD = 49.8 ± 13.1 years; 50% males). The definition of normoglycemia, prediabetes, and diabetes was according to the American Diabetes Association (ADA) criteria, which are based on assessing fasting blood glucose (FBG: Less than 100, 100-125 and ≥ 126 mg/dL, respectively) and HbA1c (Less than 5.7, 5.7-6.4 and ≥ 6.5%, respectively) [17]. Only people who met these criteria were included. Smokers, alcoholics, and patients with cardiovascular disease, kidney disease, and hepatic failure were excluded. Besides, pregnant women with gestational diabetes were also excluded.

Laboratory methods

Blood samples were collected by vein puncture after 10-12 hours of fasting. The blood sample (5 mL) was divided into two aliquots. The first aliquot (3 mL) was dispensed in a tube containing ethylene-diamine tetra-acetic acid (EDTA). This blood was processed in less than three hours and used for HbAlc estimation and the determination of complete blood count (CBC) using the CELL-DYN Emerald Haematology Analyzer (Abbott, U.S.A). The remaining blood (2 mL) was transferred to a plain tube, and after clotting, the tube was centrifuged (3000 rpm for 15 minutes) to collect serum. The serum was assessed for fasting blood glucose (FBG) using Cobas c 311analyzer (Cobas-Roche, Germany) preloaded with the required reagents.

Statistical analysis

Parametricvariables (normally distributed) were given as mean ± standard deviation (SD), and significant differences between means were assessed using analysis of variance (ANOVA) followed by least significant difference (LSD) or Duncan multiple range test. Non-parametric variables (skewed) were expressed as the median and inter quartile range (IQR: 25 – 75%), and significant differences between medians were assessed using Kruskal-Wallis test followed by Mann-Whitney *U* test. Receiver operating characteristic (ROC) curve analysis was applied to determine the predictive significance of NLR, and it was addressed in terms of area under the curve (AUC), 95% confidence interval (CI), cut-off value, sensitivity and specificity. To estimate odds ratio (OR) and 95% CI, the participants were distributed into two groups according to the median of normoglycemic (> and ≤ median), and then logistic regression analysis was performed. A probability (p) value ≤ 0.05 was considered significant. The statistical package IBM SPSS version 25 (Armonk, NY: IBM Corp.) was used to perform statistical analyses.

3. Results

Fasting blood glucose and Hb1Ac

To define the glycemic status of the subjects investigated, the ADA criteria were followed to categorize them into normoglycemia, prediabetes, and diabetes. Two parameters were assessed in this context; FBG and Hb1Ac. The FBG median was significantly higher in prediabetics (106.6 [IQR: 103.7-112.5] *vs*.80.2 [IQR: 77.1-86.1] mg/dL; $p< 0.001$) or diabetics (157.14 [133.0-181.2] *vs.80.2* [IQR: 77.1-86.1] mg/dL; $p< 0.001$) than in normoglycemic. The Hb1Ac showed a similar significant increase in prediabetics and diabetics compared to normoglycemic. It was also noted that both parameters were significantly elevated in diabetics compared to prediabetics (Figure 1).

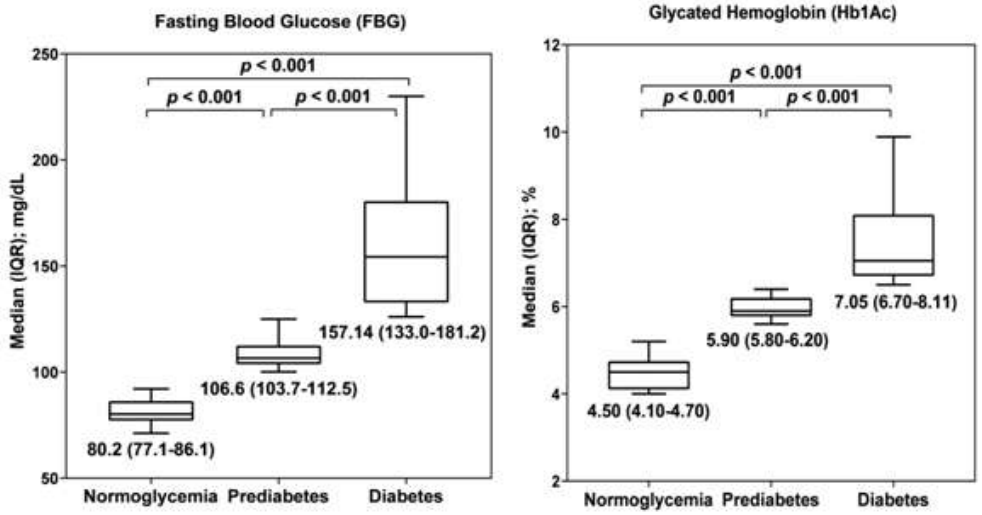

Figure 1: Fasting blood glucose and glycated hemoglobin in normoglycemic, prediabetics, and diabetics

Haematological parameters

The hematological parameters included hemoglobin (Hb), packed cell volume (PCV), and total and absolute counts of WBCs. Of these indices, only total WBC and neutrophils showed significant differences.The total count of WBCs was significantly higher in prediabetics and diabetics compared to normoglycemic (7.5 ± 2.1 and 8.0 ± 2.4 vs. 6.5 ± 1.3×10^9/L; $p = 0.020$), while there was no significant difference between prediabetics and diabetics in this context. A similar pattern of a significantly increased count of neutrophils was observed in prediabetics and diabetics compared to normoglycemic (Table 1).

Table 1: Haematological parameters in normoglycemic, prediabetics, and diabetics

Variable	Mean ± SD			*p*-value
	Normoglycemia (N = 30)	Prediabetes (N = 125)	Diabetes (N = 30)	
Hb (g/dL)	14.2 ± 1.7^{A}	14.0 ± 1.9^{A}	14.1 ± 1.8^{A}	0.929
PCV (%)	42.1 ± 4.9^{A}	42.4 ± 6.5^{A}	41.7 ± 5.2^{A}	0.821
Platelets (×10^9/L)	244.2 ± 54.9^{A}	276.3 ± 69.2^{A}	253.9 ± 95.3^{A}	0.051
WBC (×10^9/L)	6.5 ± 1.3^{A}	7.5 ± 2.1^{B}	8.0 ± 2.4^{B}	**0.020**
Neut (×10^9/L)	3.5 ± 0.9^{A}	4.3 ± 1.8^{B}	4.7 ± 2.2^{B}	**0.025**
Lymp (×10^9/L)	2.5 ± 0.6^{A}	2.4 ± 0.7^{A}	2.5 ± 0.7^{A}	0.959
Mono (×10^9/L)	0.51 ± 0.11^{A}	0.56 ± 0.26^{A}	0.55 ± 0.22^{A}	0.563
Eosi (×10^9/L)	0.19 ± 0.05^{A}	0.20 ± 0.24^{A}	0.16 ± 0.10^{A}	0.551
Baso (×10^9/L)	0.03 ± 0.02^{A}	0.18 ± 0.19^{A}	0.02 ± 0.02^{A}	0.797

Similar letters indicate no significant differences between means in rows (p-value >0.05).Different letters indicate significant differences between means in rows (p-value ≤ 0.05).

Neutrophil to lymphocyte ratio

The NLR was significantly higher in prediabetics (1.67 [IQR: 1.30-2.22]; $p = 0.016$) and diabetics (1.65 [IQR: 1.33-2.32]; $p = 0.021$) than in normoglycemics (1.40 [IQR: 1.12-1.72]), while the NLR showed no significant difference between prediabetics and diabetics ($p = 0.577$) (Figure 2). ROC curve analysis revealed that the NLR had an approximated AUC in prediabetes and diabetes (0.641 and 0.674, respectively), but the sensitivity and sensitivity were slightly higher in prediabetes than diabetes (58.4 and 60. 0%*vs*. 55.2 and 56.7%, respectively)

(Figure 3 and Table 2). Logistic regression analysis revealed that the NLR was associated with a similar risk in the development of prediabetes (OR = 2.61; 95% CI = 1.12 - 6.07; $p = 0.027$) and diabetes (OR = 3.05; 95% CI = 1.07 - 8.68; $p = 0.067$), but the p-value in diabetes was not significant (Table 3)

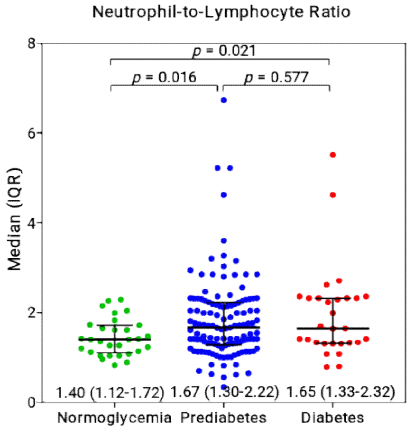

Figure 2: Neutrophil-to-lymphocyte ratio in normoglycemic, prediabetics, and diabetics

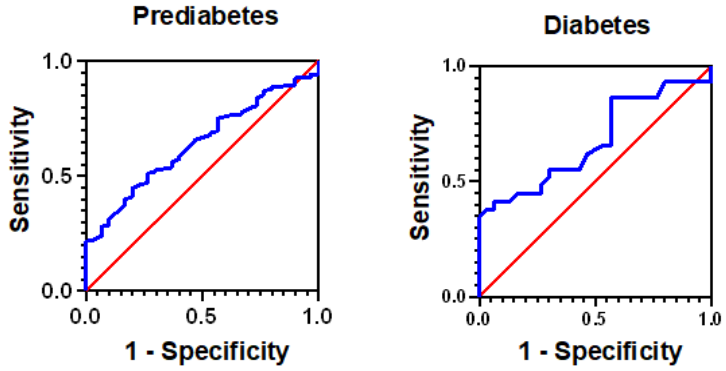

Figure 3: ROC curve analysis of neutrophil-to-lymphocyte ratio in prediabetics and diabetics versus normoglycemic (The figure data are given in Table 2).

Table 2: ROC curve analysis of neutrophil-to-lymphocyte ratio in prediabetics and diabetics *versus* normoglycemics

Group	AUC	95% CI	*p*-value	Cut-off value	Sensitivity (%)	Specificity (%)
Prediabetes	0.641	0.544-0.739	0.017	1.43	58.4	60.0
Diabetes	0.674	0.534-0.814	0.022	1.42	55.2	56.7

Table 3: Logistic regression analysis of neutrophil-to-lymphocyte ratio in prediabetes and diabetes *versus* normoglycemia

Group	Neutrophil/lymphocyte ratio				OR (95% CI)	*p*-value
	> Median		≤ Median			
	N	%	N	%		
Normoglycemia (N = 30)	9	30.0	21	70.0	Reference	
Prediabetes (N = 125)	66	52.8	59	47.2	2.61 (1.12 - 6.07)	**0.027**
Diabetes (N = 30)	17	56.7	13	43.3	3.05 (1.07 - 8.68)	0.067

4. Discussion

The present study results indicated that some of the haematological indices, particularly WBC and neutrophil counts, might be considered good predictors of prediabetes. Both indices were influenced by hyperglycemia, and their counts were significantly elevated in prediabetics and diabetics. In this study, the NLR was also determined, and it was significantly elevated, particularly in prediabetic

patients. ROC curve analysis confirmed this significance and the estimated AUC exceeded 0.6. Besides that, regression analysis revealed that the NLR was associated with a higher risk of hyperglycemia by 2.61-folds.These data may indicate that the transition from normoglycemia to prediabetes and then to diabetes paralleled with changes in the dynamics of these biomarkers, with an increase in WBCs and neutrophils, as well as NLR. These biomarkers have been observed to be influenced by the glucose status in the blood [18]. In line with these findings, [19] have reported a higher count of WBCs levels during the development of prediabetes and diabetes than normoglycemia. It has also been noticed that the increased counts of WBCs are linked to an increased risk of developing impaired fasting glucose (IFG), and therefore, chronic inflammation is suggested to play a role in the development and progression of IFG [20]. The data also suggest that immunological activation might have occurred during the prediabetic stage, particularly when inflammatory markers such as NLR, are up-regulated [21]. Davison et al. (2017) [22] found that prediabetes and diabetes had higher levels of monocytes, neutrophils, and platelets than in normoglycemia, and monitoring their activation in confirmed diabetic patients might aid in the management and risk assessment of disease. In diabetes mellitus (DM), the NLR is thought to be a measure of low-grade inflammation (DM), but other factors have to be taken into account. Among these factors are age, vitamin D status, and pre-existing morbidity, as these factors have been described to affect the clinical interpretation of NLR as a predictive marker of prediabetes and DM-related inflammation [23]. Further, the NLR is regarded as an independent predictor of renal dysfunction in people with diabetes and prediabetes [24]. In spite of these complications, the NLR remains a simple test to calculate and it is affordable, and therefore it is expected to be utilized as an effective indication in the clinical follow-up of people with diabetes and prediabetes [25]. The current study results sustained this significance of NLR, especially in the evaluation of prediabetic status. Further data suggest that elevated NLR is also linked to higher HbA1c and poor glycemic control in diabetic patients, and it can be utilized as a disease monitoring tool during diabetic patient follow-up [26]. It has also been strengthening that NLR is a significant predictor of subclinical inflammation or low-grade inflammation in prediabetics [27].

5. Conclusion

In conclusion, the current study indicated that elevated total WBCs and neutrophil counts are good prediabetes predictors. The neutrophil to lymphocyte ratio (N/LR), on the other hand, is favorably related to the disease and has been shown to be even more reliable than WBCs and neutrophils

6. References

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مرحلة ماقبل السكري: لمحة عن مؤشرات الدم في المرضى العراقيين

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الخلاصة

تعد مرحلة ماقبل السكري هدفًا للبحث لفهم عوامل الاختطار التي قد تتنبأ به. سعت هذه الدراسة المقطعية لتحليل الأهمية التنبؤية لبعض مؤشرات الدم ، وخاصة عدد خلايا الدم البيضاء لمرحلة ماقبل السكري في البالغين العراقيين. تضمنت هذة الدراسة ثلاث مجموعات: 30 فردًا من الاصحاء ظاهريا و 125 فردا بمرحلة ماقبل السكري و 30 فردا من مرضى السكري النمط الثاني. أظهرت النتائج أن متوسط جلوكوز الدم أثناء الصيام كان قد ازداد معنويا في مجموعة ماقبل السكري (106.6 [IQR: 112.5-103.7]مقابل 80.2 [IQR: 86.1-77.1] ملغرام/ديسي لتر p< 0.001؛) و مرضى السكري(157.14 [181.2-133.0] مقابل 80.2 [IQR: 86.1-77.1] ملغرام/ديسي لتر؛ p< 0.001) مقارنة بذوي سكر الدم الطبيعي.وكذلك مستوى النسبة المئوية للهيموغلوبين السكري HbA1c% ازداد معنويا في مجموعة ماقبل السكري ومرضى السكري مقارنه بذوي السكر الطبيعي كما لوحظ أن كلا البارامترات كانت مرتفعة معنويا بشكل ملحوظ في مرضى السكري مقارنة مع مجموعه ماقبل السكري حيث كان العدد الإجمالي لكريات الدم البيضاء أعلى بشكل ملحوظ في مرضى مرحلة ماقبل السكري ومرضى السكري مقارنةً بذوي السكر الطبيعي (7.5 ± 2.1 و 8.0 ± 2.4 مقابل 6.5 ± 1.3 ×109 / لتر ؛ p = 0.020) ، بينما لم يكن هناك فرق كبير بين مرضى السكري ومرحلة ماقبل السكري في هذا. لوحظ نمط مشابه من زيادة عدد كريات الدم العدلة بشكل ملحوظ في مرضى السكري ومرضى ماقبل السكري مقارنة بذوي السكر الطبيعي. كان NLR أعلى بشكل ملحوظ في مرضى السكري (1.67 [[2.22-1.30 :IQR؛ p = 0.016) ومرضى السكر (1.65 [[2.32-1.33 :IQR؛ p = 0.021) عنه في معياري السكر (1.40 [IQR: 1.12-1.72]) بينما لم يظهر نسبة العدلات للمفاويات NLR فرق معنوي بين مرضى السكري ومجموعه ماقبل السكري (p = 0.577). كشف تحليل منحنى ROC أن NLR كان لها منطقة تقريبية تحت المنحنى في مرض السكري ومرحلة ماقبل السكري (0.641 و 0.674 ، على التوالي) ، والحساسية كانت أعلى قليلاً في مرحلة ماقبل السكري من مرض السكري (58.4 و 60.0٪ مقابل 55.2 و 56.7. ٪، على التوالى). أظهر تحليل الانحدار اللوجستي أن NLR ارتبطت بزيادة خطر الإصابة بمرحله ماقبل السكري (OR = 2.61؛ 95٪ CI = 1.12 - 6.07؛ p = 0.027) ومرض السكري (OR = 3.05؛ 95٪ CI = 1.07 - 8.68؛ p = 0.067) ، لكن القيمة الاحتمالية في مرض السكري لم تكن مهمة.

الكلمات المفتاحية: مرحلة ماقبل السكري ، السكري ، مؤشرات الدم ، نسبة العدلات للمفاويات