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## Article

# Correlation of Albumin, Red Cell Distribution Width and Other Biochemical and Hematological Parameters with Glycated Hemoglobin in Diabetic, Prediabetic and Non-Diabetic Patients

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**Abstract:** Diabetes mellitus is a chronic metabolic disease that affects over 10.5 percent of the global adult population. Biochemical and hematological parameters such as albumin (ALB) and red cell distribution width (RDW) have been shown to be altered in diabetic patients. This study aims to correlate hematological and biochemical parameters with glycated hemoglobin (HbA1c). 777 adult individuals (372 women and 405 men, aged 19-85 years) were divided into three groups: 218 participants with HbA1c < 5.7% (group A: non diabetic), 226 with HbA1c ≥ 5.7% and < 6.5% (group B: prediabetic) and 333 with HbA1c ≥ 6.4% (group C: diabetic). Biochemical and hematological parameters were compared among the 3 groups. Analysis of variance was performed to determine the correlations of the parameters among groups. ALB and sodium (Na) levels were significantly decreased in group C compared to groups A (ALB: 3.8 g/dL vs. 4.1 g/dL,  $p < 0.0001$ , Na: 138.4 mmol/L vs. 139.3 mmol/L,  $p < 0.001$ ), and B (ALB: 3.8 g/dL vs. 4.0 g/dL,  $p < 0.0001$ , Na: 138.4 mmol/L vs. 139.6 mmol/L,  $p < 0.0001$ ), while RDW-standard deviation (RDW-SD) and urea were increased in group C compared to group A (RDW: 45.8 vs. 43.9 fL,  $p < 0.0001$ , Urea: 55.6 mg/dL vs. 38.5 mg/dL,  $p < 0.0001$ ). Mean platelet volume (MPV) was increased in group C compared to group A (9.3 fL vs. 9.1 fL,  $p < 0.05$ , respectively). The increase of RDW-SD from group A towards B and C demonstrates the impact of hyperglycemia on red blood cells. RDW may be regarded as a potential innovative biomarker for improving risk assessment of developing diabetes. These results highlight the potential role of these parameters as an indication for prediabetes that would alert for measurement of HbA1c.

**Keywords:** albumin; diabetes; glycated hemoglobin; mean platelet volume; red blood cell distribution width; sodium; urea

## 1. Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia and affecting millions of people worldwide, results from either the body's inability to produce enough insulin (Type 1 diabetes) or the ineffective use of insulin by the body's cells (Type 2 diabetes) [1]. The prevalence of diabetes is rising, affecting over 10.5% of the global adult population [2]. Early diagnosis and management are crucial in preventing the severe complications associated with this disease, such as cardiovascular diseases, neuropathy, retinopathy, and nephropathy [3]. The diagnostic approach for diabetes typically includes several methods: fasting plasma glucose (GLU) tests, oral glucose tolerance tests, and the measurement of glycated hemoglobin (HbA1c), which

provides an average blood glucose level over the past two to three months [4–6]. This multi-faceted diagnostic approach is essential for the timely identification and effective management of diabetes.

The HbA1c test is particularly valuable in diagnosing and monitoring diabetes due to its ability to provide a long-term picture of blood glucose homeostasis. It is not influenced by short-term fluctuations in blood glucose levels caused by diet, stress, or illness. The American Diabetes Association recommends that an HbA1c level of 6.5% or higher on two separate tests confirms a diagnosis of diabetes, while levels between 5.7% and 6.4% indicate prediabetes, a state of elevated risk for developing diabetes [1].

Red cell distribution width (RDW) is a hematological parameter that quantifies the variability in the size of circulating erythrocytes. Typically reported as a percentage, RDW is calculated from the mean corpuscular volume (MCV) and is part of a standard complete blood count (CBC). Elevated RDW values indicate a greater degree of size variability among red blood cells, which can arise from various pathological conditions. In recent years, RDW has gained attention as a marker for several diseases beyond anemia [7,8]. Elevated RDW has been independently associated with increased mortality and adverse outcomes in patients with cardiovascular diseases, chronic kidney disease, and sepsis [9,10]. The underlying mechanisms linking RDW to these conditions are not entirely understood, but it is hypothesized that RDW reflects systemic inflammation and oxidative stress, both of which are common in chronic diseases. Inflammation can alter erythropoiesis and red blood cell survival, leading to increased heterogeneity in cell size. Similarly, oxidative stress can damage red blood cells, contributing to increased RDW [11].

In the context of diabetes, elevated RDW has been observed in patients with poor glycemic control and is associated with complications such as diabetic retinopathy, nephropathy and cardiovascular disease [12,13]. The exact relationship between RDW and diabetes is still under investigation, but it is believed that chronic hyperglycemia and associated metabolic disturbances contribute to the increased RDW seen in diabetic patients. Studies have suggested that RDW could serve as an early marker for prediabetes, as well as diabetic complications, providing clinicians with a valuable tool for risk stratification and management [14,15].

Albumin (ALB) is a vital protein in human plasma, accounting for approximately 60% of the total plasma protein content. It is primarily produced by the liver and has several important physiological functions, including maintaining colloidal osmotic pressure, binding and transporting various substances, and exhibiting antioxidant properties [16,17]. The measurement of albumin levels in serum and urine is an important aspect of clinical diagnostics, particularly in the context of chronic diseases such as diabetes. Hypoalbuminemia on admission or development of hypoalbuminemia during hospitalization has been associated with poor prognosis in hospitalized patients [18].

Moreover, serum albumin levels can also provide insights into the nutritional and inflammatory status of diabetic patients. Low serum albumin levels are associated with poor nutritional status, chronic inflammation, and increased risk of complications in diabetes [19,20]. Monitoring serum albumin levels, alongside urine albumin excretion, can therefore offer a more comprehensive assessment of a diabetic patient's health status and help guide therapeutic interventions [21,22].

Given the significance of HbA1c, albumin, and RDW as biomarkers in diabetes, exploring the correlations between these parameters can provide deeper insights into the disease's pathophysiology and its complications. HbA1c reflects long-term glycemic control and is a key diagnostic and monitoring tool for diabetes. Serum albumin levels provide additional information about nutritional and inflammatory status. Elevated RDW, on the other hand, is associated with systemic inflammation and oxidative stress, conditions commonly seen in diabetes and its complications.

Investigating the correlations between these biomarkers in diabetic and non-diabetic individuals can help identify patterns that may enhance our understanding of the disease. For instance, higher HbA1c levels may be associated with increased RDW and hypoalbuminemia, reflecting poor glycemic control and its impact on red blood cell morphology and kidney function. Conversely, non-diabetic individuals may exhibit lower RDW and stable albumin levels, indicating better overall health and less systemic inflammation.

Diabetes mellitus is a complex and multifaceted disease that requires a comprehensive approach for diagnosis and management. The use of HbA1c as a long-term marker of glycemic control, coupled with the measurement of routine hematological and biochemical parameters, offers a robust framework for understanding the disease and its complications.

Based on the above, this study aims to investigate the correlation of HbA1c with ALB, Urea, sodium (Na), MCV, RDW, RDW-SD, platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW) in diabetic, prediabetic and non-diabetic patients.

2. Results

Table 1 shows the mean values of hematologic and biochemical parameters in groups.

Table 1. Mean Values of Hematologic and Biochemical parameters in groups.

Hematologic parameters	Mean Values				p-values		
	Reference Range	Group A	Group B	Group C	Group A with Group B	Group A with Group C	Group B with Group C
HbA1c	<5.7 %	5.3	6.0	8.0	< 0.0001	< 0.0001	< 0.0001
MCV	80.0 - 95.0 fL	87.7	88.1	87.7	0.2634	0.4741	0.2484
RDW	11.5 - 14.5 %	14.6	15.0	15.2	0.0916	0.0201	0.2964
RDW-SD	40.0 - 55.0 fL	43.9	45.8	45.8	< 0.0005	< 0.0001	0.4480
PLT	150 -400 10 <sup>3</sup> /μL	241.1	241.5	242.5	0.4809	0.4315	0.4547
MPV	9.0 -13.0 fL	9.1	9.2	9.3	0.1628	< 0.05	0.2043
PDW	9.0 -17.0 fL	16.9	16.8	16.9	0.1709	0.2701	< 0.05
Biochemical parameters							
ALB	3.5-5.0 g/dL	4.1	4.0	3.8	0.2491	< 0.0001	< 0.0001
Urea	15.0-50.0 mg/dL	38.5	46.3	55.6	0.0008	< 0.0001	< 0.0005
Na	136.0 -146.0 mmol/L	139.3	139.6	138.4	0.1650	0.001	< 0.0001

Abbreviations of Table 1. ALB: albumin, HbA1c: glycated hemoglobin, MCV: mean corpuscular volume, MPV: mean platelet volume, Na: sodium, PDW: platelet distribution width, PLT: platelets, RDW: red cell distribution width, RDW-SD: red cell distribution width- standard deviation.

Participants were divided into three groups based on their HbA1c values: 218 participants with HbA1c < 5.7% (group A, 116 women and 102 men, 19-82 years), 226 with HbA1c ≥ 5.7% and < 6.5% (group B, 104 women and 122 men, 21-85 years) and 333 with HbA1c ≥ 6.4% (group C, 152 women and 181 men, 20-85 years).

The study found no statistically significant differences between the mean values of PLT (241.1 103/μL vs. 241.5 103/μL vs. 242.5 103/μL) and MCV (87.7 fL vs. 88.1 fL vs. 87.7 fL) on group A, group B and group C, respectively. The p-value for each parameter was greater than 0.05, indicating no significant difference.

There were statistically significant differences between the mean values of Urea (38.5 mg/dL vs. 46.3 mg/dL vs. 55.6 mg/dL) on group A, group B and group C, respectively. The p-value for each parameter was lower than 0.05, indicating a significant difference.

There was a statistically significant difference between mean values of RDW-SD (43.9 fL vs. 45.8 fL, p < 0.0005) on group A and group B, respectively. It was found statistically significant differences between the mean values of ALB (4.1 g/dL vs. 3.8 g/dL, p < 0.0001), Na (139.3 mmol/L vs. 138.4

mmol/L,  $p < 0.001$ ), MPV (9.1 fL vs. 9.3 fL,  $p < 0.05$ ), RDW (14.6 % vs. 15.2 %,  $p < 0.05$ ) and RDW-SD (43.9 fL vs. 45.8 fL,  $p < 0.0001$ ) on group A and group C, respectively. There were statistically significant differences between the mean values of ALB (4.0 g/dL vs. 3.8 g/dL,  $p < 0.0001$ ), Na (139.6 mmol/L vs. 138.4 mmol/L,  $p < 0.0001$ ) and PDW (16.8 fL vs. 16.9 fL,  $p < 0.05$ ) on group B and group C, respectively.

ANOVA was performed to test whether the outcomes of two or more groups differed from each other significantly (Tables 2 and 3).

**Table 2.** Analysis of variance between hematologic parameters and study groups.

Hematologic Parameters		Analysis of Variance				
	Source of Variation	Sum of Squares	Degrees of freedom	Mean Square	F	p-value
HbA1c	Between Groups	1091.72	2.00	545.86	393.25	0.00000000
	Within Groups	1074.37	774.00	1.39		1
	Total	2166.09	776.00			
MCV	Between Groups	28.91	2.00	14.46	0.28	0.75514700
	Within Groups	39825.57	774.00	51.45		2
	Total	39854.48	776.00			
RDW	Between Groups	37.22	2.00	18.61	2.10	0.12310780
	Within Groups	6858.61	774.00	8.86		9
	Total	6895.84	776.00			
RDW-SD	Between Groups	551.31	2.00	275.65	7.52	0.00058603
	Within Groups	27912.28	761.00	36.68		4
	Total	28463.58	763.00			
PLT	Between Groups	212.98	2.00	106.49	0.01	0.98516839
	Within Groups	4575244.06	642.00	7126.55		1
	Total	4575457.04	644.00			
MPV	Between Groups	4.50	2.00	2.25	1.81	0.16392600
	Within Groups	960.34	774.00	1.24		7
	Total	964.84	776.00			
PDW	Between Groups	1.50	2.00	0.75	1.42	0.24215858
	Within Groups	339.39	642.00	0.53		5
	Total	340.89	644.00			

Abbreviations of Table 2. HbA1c: glycated hemoglobin, MCV: mean corpuscular volume, MPV: mean platelet volume, PDW: platelet distribution width, PLT: platelets, RDW: red cell distribution width, RDW-SD: red cell distribution width- standard deviation.

In Table 2., an one-way ANOVA revealed that there was a statistically significant difference in mean HbA1c score between at least two groups ( $F(2, 774) = [393.25]$ ,  $p < 0.0001$ ). It was found a statistically significant difference in mean RDW - SD score between the study groups ( $F(2,761) = [7.52]$ ,  $p < 0.005$ ). The mean score of MCV, RDW, PLT, MPV and PDW did not differ on the three groups, significantly.



Table 3. presents the one-way ANOVA analysis of biochemical parameters between the three study groups. There was a statistically significant difference in mean ALB score between the groups (F (2,773) = [16.24],  $p < 0.0001$ ). Also, in mean GLU score between at least two groups (F (2,757) = [131.91],  $p < 0.0001$ ). It was noticed a statistically significant difference in mean Urea score between the study groups (F (2,751) = [20.61],  $p < 0.0001$ ). There was a statistically significant difference in mean Na score between the groups (F (2,721) = [8.72],  $p < 0.005$ ).

**Table 3.** Analysis of variance between biochemical parameters and study groups.

Biochemica		Analysis of Variance				
1						
Parameters						
	Source of Variation	Sum of Squares	Degrees of freedom	Mean Square	F	p-value
ALB	Between Groups	10.78	2.00	5.39	16.24	0.000000123
	Within Groups	256.44	773.00	0.33		0
	Total	267.22	775.00			
Urea	Between Groups	38545.47	2.00	19272.74	20.61	0.000000001
	Within Groups	702132.53	751.00	934.93	1	9
	Total	740678.00	753.00			
Na	Between Groups	189.65	2.00	94.82	8.72	0.000181939
	Within Groups	7844.30	721.00	10.88		0
	Total	8033.95	723.00			

Abbreviations of Table 3. ALB: albumin, Na: sodium, ANOVA analysis of variance.

3. Discussion

The present study aimed to explore the relationship between HbA1c levels and various hematological and biochemical parameters in a large cohort of participants. By categorizing the participants into three groups based on their HbA1c values, we were able to identify several significant differences in key parameters, shedding light on how glycemic control might influence various aspects of health.

*Hematological Parameters*

One of the primary findings of our study is that the mean values of platelet count (PLT) and mean corpuscular volume (MCV) did not differ significantly among the three groups, which suggests that HbA1c levels might not have a direct impact on these hematological parameters, in agreement with a recent study [23]. The consistency in PLT and MCV across groups A, B, and C (241.1  $\times 10^3/\mu\text{L}$  vs. 241.5  $\times 10^3/\mu\text{L}$  vs. 242.5  $\times 10^3/\mu\text{L}$  and 87.7 fL vs. 88.1 fL vs. 87.7 fL, respectively) reinforces the notion that these particular measures are stable regardless of glycemic status. This stability can be particularly relevant in clinical practice, where these parameters are often used to assess general health and diagnose various conditions.

However, our analysis revealed significant differences in red cell distribution width-standard deviation (RDW-SD) between groups A and B, and groups A and C. Specifically, RDW-SD was higher in groups B and C compared to group A (45.8 fL vs. 43.9 fL,  $p < 0.0005$  for group B vs. group A and 45.8 fL vs. 43.9 fL,  $p < 0.0001$  for group C vs. group A). These findings suggest that higher HbA1c levels might be associated with greater variability in red cell size, which could reflect underlying erythropoietic stress [24]. RDW values are shown to be increased in various inflammatory conditions such as inflammatory bowel disease, systemic lupus erythematosus rheumatoid arthritis and

psoriatic arthritis [25–28]. The underlying inflammation that is known to play a significant role in diabetic patients, could therefore affect RDW values [29]

Further, mean platelet volume (MPV) and platelet distribution width (PDW) were found to have significant differences when comparing groups A and C, and groups B and C, respectively. MPV was higher in group C compared to group A (16.9 fL vs. 16.9 fL,  $p < 0.05$ ), and PDW was higher in group C compared to group B (16.9 fL vs. 16.8 fL,  $p < 0.05$ ). These findings indicate that platelet activation and size variability are potentially more pronounced in individuals with higher HbA1c levels, and are in accordance with prior findings [30]. The increased reactivity of platelets in diabetic patients, however, is attributed to multiple factors, including hyperglycemia, hyperlipidemia, resistance to insulin, a more pronounced inflammatory and oxidative status along with increased expression of glycoprotein receptors and growth factors [31–35].

#### *Biochemical Parameters*

The study identified several significant differences in biochemical parameters among the groups. Urea levels showed marked increases with higher HbA1c groups (38.5 mg/dL vs. 46.3 mg/dL vs. 55.6 mg/dL). Elevated urea levels in higher HbA1c groups may indicate early kidney dysfunction, which is a common complication of diabetes. Another study suggested the use of urea nitrogen levels as a predictor for diabetes mellitus [36]. In this study, a concentration of urea nitrogen more than 25 mg/dL is directly associated with higher incidence of diabetes mellitus. Higher urea nitrogen levels in people with chronic kidney disease are shown to induce insulin resistance because of the activation of E3 ubiquitin ligases, which specifically conjugate ubiquitin to IRS-1 marking it for degradation in the ubiquitin-proteasome system [37]. Higher urea nitrogen levels have also been shown to be associated with complications such as retinopathy in type 2 diabetes patients [38].

ALB levels also showed significant differences between the groups. ALB levels were lower in group C compared to both groups A and B (4.1 g/dL vs. 3.8 g/dL,  $p < 0.0001$  for group A vs. group C, and 4.0 g/dL vs. 3.8 g/dL,  $p < 0.0001$  for group B vs. group C). This decrease in ALB with increasing HbA1c probably reflects the impact of chronic hyperglycemia on albumin permeability, as well as the chronic inflammation that is noted in diabetic patients [39]. The lower serum albumin levels can be partially attributed to the increased urinary albumin excretion due to hyperglycemia in type 2 diabetes patients [21].

Similarly, Na levels were lower in group C compared to both groups A and B (139.3 mmol/L vs. 138.4 mmol/L,  $p < 0.001$  for group A vs. group C, and 139.6 mmol/L vs. 138.4 mmol/L,  $p < 0.0001$  for group B vs. group C). Lower sodium levels in the higher HbA1c group could indicate a relative electrolyte imbalance, possibly due to altered renal function or shifts in fluid balance often seen in diabetes. In a study conducted among hypertensive individuals, lower Na levels were shown to be associated with increased risk of developing diabetes [40].

#### *Implications for Clinical Practice*

The findings of this study have several implications for clinical practice. First, the lack of significant differences in PLT and MCV across HbA1c groups suggests that these hematological parameters are not influenced by glycemic homeostasis and can be reliably used in clinical assessments without adjusting for HbA1c levels. However, the significant differences in RDW-SD, MPV, and PDW highlight the need for careful monitoring of hematological changes in patients with poor glycemic control, as these parameters might signal increased risks for complications such as anemia and thrombosis.

The biochemical parameters showing significant differences, particularly Urea, ALB, and Na, underline the importance of comprehensive metabolic monitoring in patients with varying degrees of glycemic control. The increase in urea levels with higher HbA1c groups reinforces the need for stringent glucose monitoring and management to prevent complications like nephropathy. Additionally, the decrease in ALB and Na levels with higher HbA1c groups suggests that routine liver and electrolyte monitoring could be beneficial in managing patients with poor glycemic control to prevent and manage potential complications early.

Furthermore, based on our study, decreased Na and/or decreased ALB, and/or increased urea and/or increased RDW-SD in routinely tested patients should alert the clinician to request measurement of HbA1c, as these findings may be associated with prediabetes or diabetes.

Limitations and Future Research

Despite the insightful findings, this study has limitations that should be acknowledged. The cross-sectional design limits the ability to establish causal relationships between HbA1c levels and the observed differences in hematological and biochemical parameters. Longitudinal studies are necessary to determine the temporal relationship and causality, as well as the prognostic value of the measured parameters in terms of diabetes complications and survival time. Additionally, while the study included a large and diverse sample, it did not account for potential confounding factors such as other medication use, diet, and comorbid conditions, which could influence the observed parameters.

Future research should focus on longitudinal studies to explore the causal relationships between glycemic control and the observed hematological and biochemical changes. Investigating the underlying mechanisms driving these changes, particularly the increase in RDW-SD, MPV, and PDW, as well as the decrease in ALB and Na, will provide deeper insights into the pathophysiology of diabetes and its complications. Furthermore, studies examining the impact of specific interventions, such as dietary changes, exercise, and medication adjustments, on these parameters in individuals with varying HbA1c levels will help develop targeted strategies to improve outcomes in diabetic patients.

4. Materials and Methods

4.1. Study Population

In this study, 777 samples were collected from adult patients after written consent (372 women and 405 men, aged 19-85 years), who were examined and hospitalized at the General Hospital of Thessaloniki. The patients underwent complete blood count, HbA1c and biochemical screening, including serum ALB, urea and sodium (Na). The samples were divided into three groups, based on their HbA1c levels, 218 participants with HbA1c < 5.7% (group A: non-diabetic, 116 women and 102 men, 19-82 years), 226 with HbA1c ≥ 5.7% and < 6.5% (group B: prediabetic, 104 women and 122 men, 21-85 years) and 333 with HbA1c ≥ 6.4% (group C: diabetic, 152 women and 181 men, 20-85 years). All participants were not on diabetes-related medication at the time of the study. The participant characteristics are summarized in Table 4.

Table 4. Participant characteristics.

Participant characteristics	Group A n=218 (%)	Group B n=226 (%)	Group C n=333 (%)	Total n=777 (%)
Gender				
Male	102 (25.19)	122 (30.12)	181 (44.69)	405 (100.00)
Female	116 (31.18)	104 (27.96)	152 (40.86)	372 (100.00)
	mean (±SD)			
Age	54 (±14.23)	63 (±13.56)	66 (±13.83)	62 (±14.63)

Abbreviations of Table 4. SD, standard deviation.

4.2. Hematologic Parameters

Fasted EDTA anticoagulated blood samples (2 mL) from each participant, in the morning hours, were collected and immediately analyzed. Samples with platelet clumps were excluded.

A complete blood count was performed using the automated Beckman Coulter- DxH 800 Hematology Analyzer (Beckman Coulter, Miami, FL, USA). The presented parameters include MCV, RDW, RDW-SD, PLT, MPV and PDW. The count and the size of particles were determined using



electrical impedance measurements, in accordance with the Coulter Principle. The average volume of individual erythrocytes (MCV) was derived from the RBC histogram, multiplied by a calibration factor and expressed in femtoliters (fL). The size distribution spread of the erythrocyte population (RDW and RDW-SD) was also derived from the RBC histogram. The RDW value is expressed as the coefficient of variation (%) and RDW-SD as a standard deviation in fL. The count of PLTs was determined based on the Coulter Principle and expressed as 10<sup>3</sup> cells/  $\mu$ L. The average volume of platelets (MPV) was derived from the PLT histogram, multiplied by a calibration factor and expressed in fL. The size distribution spread of PLT (PDW) was derived from the PLT histogram and expressed as the coefficient of variation (%).

#### 4.3. Biochemical Parameters

For biochemical analysis, whole-blood samples (10 mL) were collected from each participant in the morning hours, after overnight fasting. The samples were allowed to clot at room temperature for 20 min and were centrifuged at 3000 rpm for a total of 10 min. The separated serum samples were immediately analyzed and measured at least twice. Hemolyzed samples were excluded.

ALB, urea and Na concentrations were measured using Abbot Architect c16000 Analyzer (Abbott, Abbott Park, Chicago, IL, USA). ALB and urea were detected with colorimetric methods. ALB levels were expressed in gr/dL and urea in mg/dL. The determination of Na was performed with integrated chip technology (ICT) module based on a potentiometric method (ISE- Ion selective electrodes) and expressed in mmol/L.

For the measurement of HbA1c, EDTA anticoagulated blood samples (2 mL) from each participant, in the morning hours, were collected and immediately analyzed. Hemolyzed samples and samples with platelet clumps were excluded. The percentage of glycated hemoglobin was measured, based on HPLC technology, using Tosoh Automated Glycohemoglobin HLC-723G8 Analyzer (Tosoh Europe B.V., Rembrandt Toren, Amsterdam, The Netherlands).

#### 4.4. Statistical Analyses

For the statistical analyses, a statistical software package (IBM Corp. Released 2021. IBM Statistical Package for Social Sciences-SPSS for Windows, Version 28.0, Armonk, NY, USA) was used, calculating the mean, median, standard deviation (SD) and all correlations of glycated hemoglobin with hematologic and biochemical markers. ANOVA (Analysis of Variance) was used to investigate the relationships between the measured parameters. The significance level (p-value) was set at 0.05 for all analyses.

### 5. Conclusions

This study provides a comprehensive analysis of the relationship between HbA1c levels and various hematological and biochemical parameters. The significant differences in parameters such as RDW-SD, MPV, PDW, GLU, urea, ALB, and Na across different HbA1c groups highlight the complex interplay between glycemic homeostasis and overall health. The progressive increase of RDW-SD from normal glucose homeostasis group towards prediabetes and diabetes groups demonstrates the significant impact of hyperglycemia on red blood cells. Since Albumin and RDW are simple and inexpensive parameters, they may be regarded as a potential innovative biomarker for improving risk assessment of developing diabetes. These findings underscore the importance of holistic monitoring and management of patients with diabetes, emphasizing the need for regular and comprehensive assessments beyond just glucose levels to prevent and manage the wide range of complications associated with diabetes associated with diabetes.

**Author Contributions** Conceptualization, G.T., E.V. and E.L.; methodology, A.G., S.I., G.T. and E.L.; validation, A.G., S.I. and E.L.; formal analysis, A.G., S.I., K.K. and E.L.; investigation, A.G.; resources, K.K., E.L.; data curation, S.I.; writing—original draft preparation, A.G., S.I. and E.L.; writing—review and editing, A.G., S.I., K.K., E.V. AND E.L.; visualization, E.V. and E.L.; supervision, E.L.; project administration, E.L. All authors have read and agreed to the published version of the manuscript."

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**Institutional Review Board Statement:** The study was conducted according to the Declaration of Helsinki and its latest amendments. Ethical approval for the study was obtained from the Administration and the Scientific Committee of the General Hospital in Thessaloniki with protocol number 6/26/4/2018 on 26/4/2018. The confidentiality of the participants was meticulously maintained and personal privacy was thoroughly respected.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author due to ethical restrictions.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. ElSayed NA, Aleppo G, Aroda VR, et al. Addendum. 2. Classification and Diagnosis of Diabetes: Standards of Care in Diabetes-2023. *Diabetes Care* 2023;46(Suppl. 1):S19-S40. *Diabetes Care*. 2023;46(9):1715. doi:10.2337/dc23-ad08
2. Sun H, Saeedi P, Karuranga S, et al. Erratum to "IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045" [*Diabetes Res. Clin. Pract.* 183 (2022) 109119]. *Diabetes Res Clin Pract.* 2023;204:110945. doi:10.1016/j.diabres.2023.110945
3. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. *Phys Ther.* 2008;88(11):1254-1264. doi:10.2522/ptj.20080020
4. Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care.* 2000;23(3):295-301. doi:10.2337/diacare.23.3.295
5. Mannucci E, Ognibene A, Sposato I, et al. Fasting plasma glucose and glycated haemoglobin in the screening of diabetes and impaired glucose tolerance. *Acta Diabetol.* 2003;40(4):181-186. doi:10.1007/s00592-003-0109-8
6. Perry RC, Shankar RR, Fineberg N, McGill J, Baron AD; Early Diabetes Intervention Program (EDIP). HbA1c measurement improves the detection of type 2 diabetes in high-risk individuals with nondiagnostic levels of fasting plasma glucose: the Early Diabetes Intervention Program (EDIP). *Diabetes Care.* 2001;24(3):465-471. doi:10.2337/diacare.24.3.465
7. Aslam H, Oza F, Ahmed K, et al. The Role of Red Cell Distribution Width as a Prognostic Marker in Chronic Liver Disease: A Literature Review. *Int J Mol Sci.* 2023;24(4):3487. Published 2023 Feb 9. doi:10.3390/ijms24043487
8. Yu G, Ming L, Cao HL, Li Q. Correction to: Red Blood Cell Distribution Width as a Predictive Marker for Coronary Artery Lesions in Patients with Kawasaki Disease. *Pediatr Cardiol.* 2021;42(7):1662. doi:10.1007/s00246-021-02678-y
9. Deng X, Gao B, Wang F, Zhao MH, Wang J, Zhang L. Red Blood Cell Distribution Width Is Associated With Adverse Kidney Outcomes in Patients With Chronic Kidney Disease. *Front Med (Lausanne).* 2022;9:877220. Published 2022 Jun 9. doi:10.3389/fmed.2022.877220
10. Lu YA, Fan PC, Lee CC, et al. Red cell distribution width associated with adverse cardiovascular outcomes in patients with chronic kidney disease. *BMC Nephrol.* 2017;18(1):361. Published 2017 Dec 13. doi:10.1186/s12882-017-0766-4
11. Joosse HJ, van Oirschot BA, Kooijmans SAA, et al. In-vitro and in-silico evidence for oxidative stress as drivers for RDW. *Sci Rep.* 2023;13(1):9223. Published 2023 Jun 7. doi:10.1038/s41598-023-36514-5
12. Ma Y, Li S, Zhang A, et al. Association between Red Blood Cell Distribution Width and Diabetic Retinopathy: A 5-Year Retrospective Case-Control Study. *J Ophthalmol.* 2021;2021:6653969. Published 2021 Jul 6. doi:10.1155/2021/6653969
13. Malandrino N, Wu WC, Taveira TH, Whitlatch HB, Smith RJ. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. *Diabetologia.* 2012;55(1):226-235. doi:10.1007/s00125-011-2331-1
14. Hassan AA, Ahmed BE, Adam I. Red Cell Distribution Width and Prediabetes in Adults in Northern Sudan: A Case-Control Study. *Hematol Rep.* 2023;15(4):651-661. Published 2023 Nov 20. doi:10.3390/hematolrep15040066
15. Al-Kindi SG, Refaat M, Jayyousi A, Asaad N, Al Suwaidi J, Abi Khalil C. Red Cell Distribution Width Is Associated with All-Cause and Cardiovascular Mortality in Patients with Diabetes. *Biomed Res Int.* 2017;2017:5843702. doi:10.1155/2017/5843702
16. Hankins J. The role of albumin in fluid and electrolyte balance. *J Infus Nurs.* 2006;29(5):260-265. doi:10.1097/00129804-200609000-00004
17. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. *FEBS Lett.* 2008;582(13):1783-1787. doi:10.1016/j.febslet.2008.04.057

18. Barchel D, Almozni-Sarafian D, Shteinshnaider M, Tzur I, Cohen N, Gorelik O. Clinical characteristics and prognostic significance of serum albumin changes in an internal medicine ward. *Eur J Intern Med.* 2013;24(8):772-778. doi:10.1016/j.ejim.2013.08.004
19. Cai YW, Zhang HF, Gao JW, et al. Serum albumin and risk of incident diabetes and diabetic microvascular complications in the UK Biobank cohort. *Diabetes Metab.* 2023;49(5):101472. doi:10.1016/j.diabet.2023.101472
20. Hu Y, Wang J, Zeng S, et al. Association Between Serum Albumin Levels and Diabetic Peripheral Neuropathy Among Patients with Type 2 Diabetes: Effect Modification of Body Mass Index. *Diabetes Metab Syndr Obes.* 2022;15:527-534. Published 2022 Feb 22. doi:10.2147/DMSO.S347349
21. Nikolaidou B, Gkaliagkousi E, Anyfanti P, et al. The impact of hyperglycemia on urinary albumin excretion in recent onset diabetes mellitus type II. *BMC Nephrol.* 2020;21(1):119. Published 2020 Apr 6. doi:10.1186/s12882-020-01774-0
22. Wang GX, Fang ZB, Li JT, et al. The correlation between serum albumin and diabetic retinopathy among people with type 2 diabetes mellitus: NHANES 2011-2020. *PLoS One.* 2022;17(6):e0270019. Published 2022 Jun 16. doi:10.1371/journal.pone.0270019
23. Abass AE, Musa IR, Rayis DA, Adam I, Gasim I G. Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. *Clin Pract.* 2017;7(4):999. Published 2017 Jul 26. doi:10.4081/cp.2017.999
24. Ruan B, Paulson RF. Metabolic regulation of stress erythropoiesis, outstanding questions, and possible paradigms. *Front Physiol.* 2023;13:1063294. Published 2023 Jan 5. doi:10.3389/fphys.2022.1063294
25. Song CS, Park DI, Yoon MY, et al. Association between red cell distribution width and disease activity in patients with inflammatory bowel disease. *Dig Dis Sci.* 2012;57(4):1033-1038. doi:10.1007/s10620-011-1978-2
26. Cakal B, Akoz AG, Ustundag Y, Yalinkilic M, Ulker A, Ankarali H. Red cell distribution width for assessment of activity of inflammatory bowel disease. *Dig Dis Sci.* 2009;54(4):842-847. doi:10.1007/s10620-008-0436-2
27. Lee WS, Kim TY. Relation between red blood cell distribution width and inflammatory biomarkers in rheumatoid arthritis. *Arch Pathol Lab Med.* 2010;134(4):505-506. doi:10.5858/134.4.505.c
28. Conic RR, Damiani G, Schrom KP, et al. Psoriasis and Psoriatic Arthritis Cardiovascular Disease Endotypes Identified by Red Blood Cell Distribution Width and Mean Platelet Volume. *J Clin Med.* 2020;9(1):186. Published 2020 Jan 9. doi:10.3390/jcm9010186
29. Tsalamandris S, Antonopoulos AS, Oikonomou E, et al. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol.* 2019;14(1):50-59. doi:10.15420/ecr.2018.33.1
30. Sharpe PC, Trinick T. Mean platelet volume in diabetes mellitus. *Q J Med.* 1993;86(11):739-742.
31. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. *Diabetes Care.* 2003;26(7):2181-2188. doi:10.2337/diacare.26.7.2181
32. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. *Diabetes Care.* 2001;24(8):1476-1485. doi:10.2337/diacare.24.8.1476
33. Schneider DJ. Factors contributing to increased platelet reactivity in people with diabetes. *Diabetes Care.* 2009;32(4):525-527. doi:10.2337/dc08-1865
34. Kakouros N, Rade JJ, Kourliouros A, Resar JR. Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. *Int J Endocrinol.* 2011;2011:742719. doi:10.1155/2011/742719
35. Yngen M, Norhammar A, Hjemdahl P, Wallén NH. Effects of improved metabolic control on platelet reactivity in patients with type 2 diabetes mellitus following coronary angioplasty. *Diab Vasc Dis Res.* 2006;3(1):52-56. doi:10.3132/dvdr.2006.008
36. Xie Y, Bowe B, Li T, Xian H, Yan Y, Al-Aly Z. Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus. *Kidney Int.* 2018;93(3):741-752. doi:10.1016/j.kint.2017.08.033
37. Thomas SS, Zhang L, Mitch WE. Molecular mechanisms of insulin resistance in chronic kidney disease. *Kidney Int.* 2015;88(6):1233-1239. doi:10.1038/ki.2015.305
38. Zhong JB, Yao YF, Zeng GQ, et al. A closer association between blood urea nitrogen and the probability of diabetic retinopathy in patients with shorter type 2 diabetes duration. *Sci Rep.* 2023;13(1):9881. Published 2023 Jun 19. doi:10.1038/s41598-023-35653-z
- Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med.* 1984;310(6):356-360. doi:10.1056/NEJM198402093100605

39. Scalia R, Gong Y, Berzins B, Zhao LJ, Sharma K. Hyperglycemia is a major determinant of albumin permeability in diabetic microcirculation: the role of mu-calpain. *Diabetes*. 2007;56(7):1842-1849. doi:10.2337/db06-1198
40. Cheng Q, Liu X, Cai A, Zhou D, Huang Y, Feng Y. Serum sodium level is inversely associated with new-onset diabetes in hypertensive patients. *J Diabetes*. 2022;14(12):831-839. doi:10.1111/1753-0407.13338

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