One of the Factor Associated with Etiopathogenesis of Diabetes Mellitus: Intraerythrocyte Fluid Volume*

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Abstract

Aim: The aim of the present study was to investigate the levels of intraerythrocyte fluid volume, erythrocyte indices, and biochemical parameters and to evaluate the relationship between intraerythrocyte fluid volume and these parameters in patients with Diabetes Mellitus (DM) and healthy controls.

Method: The study included 42 patients with DM and 40 healthy controls. Biochemical parameters were measured using an automated analyzer. Complete blood counts were performed using an automated hematology analyzer, and intraerythrocyte fluid volumes were measured using the microcentrifugation method.

Results: Intraerythrocyte fluid volume, glycated hemoglobin (HbA1c), and glucose levels were higher in the patient group than in the control group, whereas mean corpuscular volume (MCV), potassium (K), and sodium (Na) values were lower in the patient group than in the control group. On the other hand, a negative correlation was found between intraerythrocyte fluid volume and mean corpuscular hemoglobin concentration (MCHC), magnesium (Mg), and K values in the patient group.

Conclusion: Studies findings indicated that intraerythrocyte fluid volume may be an effective hemodynamic parameter in the etiopathogenesis of DM. In line with these data, it can be suggested that intraerythrocyte fluid volume is an important factor that should be considered in monitoring the progression of the disease.

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ETHICAL STATEMENT: Ethics committee permission for the study was received from the University of Health Sciences, Haseki Training and Research Hospital Ethics Committee (Date: 04/08/2023, Issue: 198) and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Keywords: Diabetes mellitus, intraerythrocyte fluid volume, erythrocyte indices, biochemical parameters.

Diabetes Mellitus Etiyopatogenezinde İlişkili Faktörlerden Biri: Eritrosit İçi Sıvı Hacmi

Öz

Amaç: Bu çalışmanın amacı Diabetes Mellitus (DM) hastaları ve sağlıklı kontrollerde eritrosit içi sıvı hacmi, eritrosit indeksleri ve biyokimyasal parametrelerin düzeylerini araştırmak ve eritrosit içi sıvı hacmi ile bu parametreler arasındaki ilişkiyi değerlendirmektir.

Yöntem: Çalışmaya 42 DM'li hasta ve 40 sağlıklı kontrol dahil edildi. Biyokimyasal parametreler otomatik analizör kullanılarak ölçüldü. Otomatik hematoloji analiz cihazı kullanılarak tam kan sayımı yapıldı ve mikrosantrifüj yöntemi kullanılarak eritrosit içi sıvı hacimleri ölçüldü.

Bulgular: Hasta grubunda eritrosit içi sıvı hacmi, glikolize hemoglobin (HbA1c) ve glukoz düzeyleri kontrol grubuna göre daha yüksek, ortalama eritrosit hacmi (MCV), potasyum (K) ve sodyum (Na) değerleri ise hasta grubunda kontrol grubuna göre daha düşüktü. Öte yandan hasta grubunda eritrosit içi sıvı hacmi ile ortalama eritrosit hemoglobin konsantrasyonu (MCHC), magnezyum (Mg) ve K değerleri arasında negatif korelasyon saptandı.

Sonuç: Bulgular, eritrosit içi sıvı hacminin DM etyopatogenezinde etkili bir hemodinamik parametre olabileceğini göstermiştir. Bu veriler doğrultusunda eritrosit içi sıvı hacminin hastalığın seyrinin takibinde dikkate alınması gereken önemli bir faktör olduğu önerilebilir.

Anahtar Sözcükler: Diabetes mellitus, eritrosit içi sıvı hacmi, eritrosit indeksleri, biyokimyasal parametreler.

Introduction

Diabetes Mellitus (DM) is a long-term, chronic metabolic disorder that affects the conversion from food to energy, causing glucose levels to rise in the bloodstream when the pancreas cannot produce enough insulin or the body cannot use insulin appropriately. In line with the numerological information of the World Health Organization (WHO), it is predicted that ~642 million people will be affected by DM by 2040. Several factors affect the etiology of DM. It is a chronic metabolic disease characterized by hyperglycemia, including age, genetics, environmental factors, lifestyle, and high body mass index. Absolute or relative insufficiency of insulin release and action disrupts carbohydrate, lipid, and protein metabolism. Several limited studies in the literature have shown that some hemorheological parameters change and microcirculation is impaired in patients with DM. These parameters include erythrocyte aggregation, platelet aggregation, erythrocyte deformability, whole blood and plasma viscosity, and changes in erythrocyte structure and function. Because of microcirculation deterioration due to increased vascular resistance in DM, hypoxia develops in the tissue, leading to tissue damage and chronic complications occur[1,2].

Water is essential for life, constituting ~70% of body weight and forms the medium for many biological reactions. As humans gain water through drinking and metabolism and lose water through urinary excretion and evaporation, osmolarity changes are created in the tissues, and timely redistribution of water becomes important. Although systemic circulation takes less than a minute to complete, microcirculation flows much more slowly at ~1 mm/s[3-6]. Body fluids, which are essential for the basic physiological functions of the body, are divided into two types: extracellular and intracellular. The fluid found inside all cells in the body is called intracellular fluid; All fluids outside cells are also called extracellular fluid. The extracellular fluid compartment consists of blood plasma within the closed system of the heart and vessels[3-6]. The primary function of blood

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is redistributing critical molecules throughout the body. Erythrocytes, which occupy ~45% of blood volume, function to enhance this redistribution. The two main functions of erythrocytes, efficient transport of oxygen (O_2) and carbon dioxide (CO_2), have long been documented; O_2 diffuses passively across the lipid bilayer, whereas CO_2 transport is achieved by the transmembrane anion exchanger (anion exchanger 1 or band 3)[6-8].

Circulating erythrocytes equipped with aquaporin 1 increase the water-carrying ability of blood and regulate tissue osmolarity[6]. However, glucose oxidation and protein glycation caused by diabetes-related hyperglycaemia can cause various mechanical and rheological changes in erythrocytes. It is known that hyperglycaemia results in glycosylation of the erythrocyte membrane and hardening of glycosylated cell membranes, reducing the deformability of erythrocytes. In addition, it causes increased oxidative damage in diabetic erythrocytes because of intracellular reactive oxygen species (ROS) formation[1,2].

Erythrocytes, which are O_2 carriers, are highly sensitive to oxidative stress because of the absence of nuclei and mitochondria and the presence of iron-containing heme molecule. Oxidative stress is known to be the main reason behind the progression of DM, and it has been proven that a broad spectrum of damages occurs due to prolonged exposure of erythrocytes to increased glucose levels. High blood sugar levels lead to hemoglobin (Hb) glycation, and subsequent changes in erythrocyte deformability, adhesion, and membrane phospholipid asymmetry that promote aggregation and thus eryptosis. Pancreatic β-cells appear to be deficient against intracellular ROS production because of their poorly expressed endogenous antioxidant system. In addition, increased insulin production in patients with diabetes requires a higher amount of energy to keep β-cells metabolically active, these cells consume more O_2, making them more prone to ROS generation[9,10]. Chronic hyperglycemia in DM leads to increased glycosylation of erythrocytes and oxidative damage[11]. As a result of increased oxidative damage in hyperglycemia, complications related to heart diseases, atherosclerosis, stroke, diabetic retinopathy, and kidney disorders occur. DM is associated with a high mortality rate because of vascular complications[9-10].

Although erythrocytes play a decisive role in vascular complications, the mechanisms underlying the effects of glycation on erythrocyte structure and functionality have not been fully elucidated. In this study, we aimed to investigate the relationship between intraerythrocyte fluid volume, erythrocyte indices, and biochemical parameters in patients with DM and healthy controls.

Material and Methods

This study included 42 patients who were diagnosed with DM for the first time at the Internal Medicine Polyclinic of the University of Health Sciences Haseki Training and Research Hospital and had no diabetes complications and 40 healthy individuals. All participants were informed about the survey and freely signed and dated the consent form. The protocol was approved by the Ethics Committee of Haseki Training and Research Hospital of the University of Health Sciences and was conducted in accordance with the Declaration of Helsinki (Approval Number: 198/ date:04.08.2023).

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Smokers, regular alcohol users, pregnant and breastfeeding mothers, users of food supplements or vitamin complexes containing antioxidants, and those with known cardiovascular, renal, or liver diseases were excluded from the study.

Two 5 ml venous blood samples were taken in tubes, one with anticoagulant (EDTA) and the other without anticoagulant. Complete blood counts were measured in EDTA-containing samples. Blood samples without anticoagulants were centrifuged at 1500xg for 10 min, serum samples were separated, and biochemical parameters were analyzed.

Complete blood counts were measured by laser irradiation and the LED flow cell method on the CELL-DYN 3700 (Abbott Park-IL, 60064, USA) device. Biochemical parameters in serum samples were assessed using an Olympus AU5800 (Beckman Coulter, Inc. Brea, USA) AutoAnalyzer. The intraerythrocyte fluid volume was measured using the microcentrifugation method.

Statistical analysis was performed using SPSS 21.0 statistical software for Windows (SPSS, Chicago, IL, USA). Results were reported as mean ± SD. Distributions of the data were tested by Kolmogorov–Smirnov test. The means for normally distributed continuous variables were compared using Student's t-test. Non-normally distributed continuous variables were compared using the Mann-Whitney U test. Spearman or Pearson correlation analyses were performed to examine the relationship between the parameters. A value of $P<0.05$ was considered statistically significant.

Results

This study was conducted with 42 DM patients (28 females and 14 males) and 40 healthy subjects (24 females and 16 males). The mean±standard deviation of ages was 57.29±12.56 and 52.82±17.97 years in the patient and control groups, respectively. The age and gender of the patient and control groups did not differ significantly ($p>0.05$).

Table 1 presents the levels of studied variables, including intraerythrocyte fluid volume, glucose, glycated hemoglobin (HbA1c), erythrocyte, Hb, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) levels. Values of intraerythrocyte fluid volume, HbA1c, and blood glucose were increased in the DM group compared with the control group ($p<0.001$, for all). However, MCV, K, and Na values were observed to decrease in the patient group compared with the control group ($P=0.032$, $P=0.002$, and $P=0.003$, respectively). There were no significant differences in other studied parameters between the patient and healthy subjects (Table 1).

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Table 1. Comparison of intraerythrocyte fluid volume and some biochemical parameters between patients with diabetes mellitus and control subjects

	Patient (n=42)	**Control (n=40)**	**p**
Intraerythrocyte fluid volume (μm³)	67.510±17.530	51.810±15.730	**<0.001**
Glucose (mg/ dL)	196.20±96.520	100.900±13.030	**< 0.001**
HbA1c (%)	9.010±1.819	5.447±0.3963	**< 0.001**
Erythrocyte (M/Ul)	4.858±0.669	4.772±0.645	NS
Hb (g/dL)	13.810±1.673	14.070±1.777	NS
Hct (%)	41.580±4.597	42.180±4.826	NS
MCV (fL)	85.820±7.159	88.820±5.510	**0.032**
MCH (pg)	28.630±2.834	29.590±2.210	NS
MCHC (g/dL)	33.190±0.8022	33.300±0.847	NS
RDW (%)	13.980±1.023	13.840±0.8863	NS
Na (mEq/L)	138.300±2.884	139.900±1.813	**0.003**
K (mEq/L)	4.373±0.407	4.678±0.418	**0.002**
Ca (mg/dL)	9.492±0.4563	9.403±0.4693	NS
Mg (mg/dL)	1.808±0.334	1.908±0.116	NS
P (mg/dL)	3.311±0.530	3.418±0.639	NS

Data are presented as mean ± SD. Bold values indicate statistical significance. HbA1c, Glycated hemoglobin; Hb, Hemoglobin; Hct, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width; K, Potassium; Ca, Calcium; Mg, Magnesium; P, Phosphorus; NS: No significant.

When the relationship between intraerythrocyte fluid volume and biochemical markers was examined, a negative correlation was found between intraerythrocyte fluid volume and MCHC, K, and Mg levels in the patient group (Table 2).

Table 2. Correlation of intraerythrocyte fluid volume and biochemical parameters

	Correlation
Intraerythrocyte fluid volume vs. MCHC	p=0.040 r=-0.343
Intraerythrocyte fluid volume vs. Mg	p=0.020 r=-0.479
Intraerythrocyte fluid volume vs. K	p=0.007 r=-0.431

MCHC, Mean corpuscular hemoglobin concentration; Mg, Magnesium; K, Potassium; r, Correlation coefficient; P, Significance level.

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Discussion

In this study, we evaluated the relationship between intraerythrocyte fluid volume, erythrocyte indices, and some biochemical parameters in patients with DM and healthy controls. In the present study, intraerythrocyte fluid volume, HbA1c, and glucose values were higher but MCV, K, and Na values were lower in the patient group than in the control group. In addition, a negative correlation was found between intraerythrocyte fluid volume and MCHC, K, and Mg levels in the patient group.

In the literature, various studies conducted in patients with DM show that the structural, biophysical, physiological, and biochemical properties of erythrocytes change. A healthy erythrocyte is separated from the extracellular environment by its Hb-rich cytoplasm content and highly flexible viscoelastic membrane surrounding it. Under healthy conditions, a human erythrocyte takes the resting shape of a biconcave discoid with a diameter of approximately 7.5–8.0 μm. A single erythrocyte can contain 300 million Hb molecules and can therefore bind and transport up to 1.2 billion O_2 molecules. The flexibility of the membrane and the fluid structure of Hb make erythrocytes extremely deformable. They can easily squeeze and flow through capillaries with significantly smaller diameters. Deformability of erythrocytes, Hct and cell-to-vessel diameter ratio, among other factors, are responsible for the diameter dependence of the apparent viscosity of blood, often called the Fahraeus-Lindqvist effect. Under healthy conditions, the regulation of the microvascular network is optimized to maintain tissue perfusion and therefore O_2 delivery. However, in vivo studies have shown that the distribution of erythrocytes in a network is heterogeneous both spatially and temporally[12].

Human biconcave disk-shaped erythrocytes have a large surface-to-volume ratio. Although the membrane surface area in each cell type is conserved, erythrocyte volume can vary significantly due to the deformability of the membrane, the unrestricted transcellular cytoskeleton (excluding membrane skeleton), and extra space normally occupied by the nucleus and other organelles. Erythrocytes swell to become stomatocyte in hypoosmotic environments and shrink to become echinocytes in hyperosmotic environments. It is noteworthy that the stomatocyte volume is approximately three times the echinocyte volume. A large volume exchange capacity allows erythrocytes to more effectively transport and redistribute large amounts of circulating water than erythrocytes that do not have this capacity. The large volume change capacity and the absence of a nucleus, organelles, and especially an intercellular cytoskeleton allow erythrocytes to change their volume without encountering too much resistance. The monolayer spectrin-actin membrane skeleton can adapt to bending and anisotropic deformation, resulting in changes in cell volume. Other nucleated cells (e.g., endothelial cells) must overcome greater hydrostatic pressure when water enters or leaves the cell because the intracellular cytoskeleton resists changes in cell volume[6]. Erythrocytes must pass through capillaries to distribute O_2 throughout tissues to meet the O_2 requirement. Membrane phospholipid asymmetry, an important feature of functional erythrocytes, is interrupted in diabetic erythrocytes. The ratio of saturated and unsaturated fatty acid content in the erythrocyte membrane is important, and this ratio is high in hyperglycemic patients[13].

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Higher membrane fluidity has been observed in the early juvenile erythrocytes of patients with diabetes compared with healthy individuals[14]. The decreased cholesterol and phospholipid ratio, in parallel with the increased sphingomyelin and phosphatidylcholine ratios, may be the cause of altered diabetic erythrocyte membrane fluidity, as evidenced by spin labelling of fatty acids[15]. It has been proven that the morphology of erythrocytes changes in patients with diabetes due to membrane cholesterol modification[16]. It has been reported that there is a strong relationship between DM and changes in the lipid structure of the erythrocyte membrane in type 1 and type 2 diabetics compared with healthy individuals[17]. Scanning electron and atomic force microscopy studies visualised a smoother surface in diabetic erythrocytes than in healthy erythrocytes. This indicates that diabetic erythrocytes exhibit remarkable changes in surface roughness due to altered membrane cytoskeleton and superficial protein conformation and arrangements[18]. Erythrocytes from patients with diabetes have a higher Young modulus, indicating more rigid erythrocytes. In a study using scanning electron microscopy, it was observed that the fibrin network of diabetic erythrocytes was reshaped; Instead of forming individual visible fibres, fibrin has been observed to form a continuous layer of thinner fibres that give the appearance of an emboli[19]. It has been determined that increased Hb glycation induced by high glucose concentrations and impaired erythrocyte membrane integrity lead to a shortening of the erythrocyte lifespan. It is reported that there is high susceptibility to Glucose Transporter 1 (GLUT1), glycation in diabetic erythrocytes and that this glycation compromises glucose uptake and transport across the plasma membrane, eventually causing cellular damage. Additionally, high glucose concentrations have been shown to negatively affect Band 3, another membrane protein, which threatens the anion exchange capacity of erythrocytes[20-25]. Anemia occurs as a complication of DM due to the shortening of the lifespan of diabetic erythrocytes. The presence of a hypoxic environment in chronic hyperglycemia reduces erythrocyte formation by inhibiting erythropoietin secretion. Erythropoietin secretion may also be suppressed by interleukin 6 (IL-6) in DM, suggesting a role for inflammation in DM[26-28]. It has been shown that the diameter of erythrocytes increases with an increase in glucose concentration, and the area of erythrocytes decreases with an increase in irregularity of the erythrocyte membrane, and there are more acanthocytes (surface vesicular cells), distorted forms, and cup forms (stomatocytes) in patients with diabetes than in controls. As a result, the body's internal environment changes and the number of deformed erythrocytes gradually increases. Moreover, the number of biconcave disc erythrocytes decreases, further increasing the risk of diabetic complications[29]. In a previous study, we found that plasma viscosity, total protein, albumin, total cholesterol, low-density lipoprotein cholesterol (LDL-cholesterol), fasting blood sugar and insulin, homeostatic model assessment for insulin resistance (HOMA-IR), and HbA1c increased in the gestational diabetes mellitus (GDM) patient compared with the healthy pregnant and non-pregnant groups. It was determined that triglyceride levels increased in the GDM group compared with the healthy pregnant control group[30].

In the literature, studies on patients with DM have shown changes in serum and intraerythrocyte electrolyte levels. In addition, it has been shown in many studies that enzymatic and nonenzymatic antioxidant levels/activities in erythrocytes change in

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relation to oxidative stress[31-35]. In this study, MCV, K, and Na values were lower in the patient group than in the control group. The data obtained from this study show that further studies are needed to understand the mechanisms that play a role in the etiopathogenesis of the disease, including hemodynamic and biochemical parameters, inflammation, and oxidative damage processes.

Conclusion

Many mechanisms are involved in the etiopathogenesis of DM, and in these mechanisms, hemodynamic status is affected as a result of increased glycosylation of erythrocytes. In line with the data obtained from this study, intraerythrocyte fluid volume may be associated with disease progression in the etiopathogenesis of DM.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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