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The prevalence of the red cell morphology changes in patients with type 2 diabetes mellitus

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Abstract

Previous studies have shown that hematological alterations are a common finding in patients with diabetes. The aim of our study was to estimate the prevalence of the red cell morphology changes in diabetic patients and their correlation with markers of glycemic control. Thirty patients with type 2 diabetes mellitus were recruited for this study. Patient demographics, relevant concomitant illnesses and medical history were recorded. Anthropometric, biochemical parameters (fasting plasma glucose – FPG, glycated hemoglobin – HbA1c, glomerular filtration rate – GFR) and morphology of blood smear were assessed. Results were compared with the same measurements in 30 subjects without diabetes mellitus. The groups were similar in terms of age and gender but there were statistically significant differences for the recorded parameters in patients of study group and control subjects. Regarding the assessment of FPG, in the study group were recorded averages of 217.70±73.20 mg/dL compared with controls that compared with controls that had a blood glucose value of 90.03±6.59 mg/dL. In the study group, mean HbA1c was 7.95±1.99%. For the control group, the mean value of HbA1c was 5.65±0.32%. In the study group, GFR ranged between 47.70 and 118.90 mL/min./1.73 m^2. For the control group, GFR values were between 88.00 and 130.00 mL/min./1.73 m^2. In the analysis of blood cytology for the study group, there were changes in the smear type hypochromia, anisocytosis and poikilocyosis (20 patients – 66.66%). In terms of red cell morphology, changes were recorded anulocytes type, red cells in "mark to the target fired" (codocytes), bream (leptocytes), schizocytes, and red cells in "drop" (dacryocytes). We observed a high prevalence of the red cell morphology changes in diabetic patients compared with non-diabetic subjects. Our findings suggest the need of screening for routine hematological tests in type 2 diabetes mellitus.

Keywords: hematological alterations, diabetes mellitus, HbA1c.

Introduction

Diabetes mellitus brings together a group of metabolic diseases with multiple etiology, resulting from a deficiency in insulin secretion, insulin resistance or both of them, whose defining item so far is the glycemia value [1]. The entire world is currently facing a pandemic of diabetes mellitus and, according to the *International Diabetes Federation* data, the number of the patients diagnosed with this condition will increase from 194 000 000 in 2003 to 334 000 000 in 2025 [2, 3].

The impact of diabetes mellitus on the population is enormous due to the chronic complications that it can cause, the condition represents a main cause of cardiovascular disease, blindness, amputation, end-stage renal failure, hospitalization [4].

The importance of glycemic control has been demonstrated in numerous clinical trials, adequate glycemic control reducing certain specific complications of the disease [5–7]. The glycemic control can be assessed by self-monitoring blood glucose (*Self-Monitoring of Blood Glucose* – SMBG) or interstitial glucose and determining the level of glycated hemoglobin (HbA1c) [8]. Currently, HbA1c represents the golden standard procedure in assessing the glycemic control and since 2010, *The American Diabetes Association* recommended HbA1c in the diagnosis of diabetes if the value is ≥6.5%. It is highly recommended that the diagnostic test be performed *via* a method certified by the *National Standardization Program of HbA1c* or through a similar reference method proposed by *Diabetes Control and Complications Trial* [9].

Alteration of the structure, shape and function of the blood cells is a consequence of diabetes mellitus, therefore this disease should represent a research priority today.

In an issue of the *Indian Journal of Experimental Biology*, Sing & Shin published, in 2009, a review article entitled: "Changes in erythrocyte aggregation and deformability in diabetes mellitus", in which they report that "Erythrocytes remains in hyperglycemic environment throughout their life span and thus are subjected to a series of compositional changes which in turn affect their

flow properties through alteration of deformation at individual level and aggregation at collective level" [10]. Jones & Peterson reported hematological alterations in diabetes mellitus, such alteration of oxygen affinity in the diabetic erythrocyte concomitant with a decreased concentration of inorganic phosphorus [11], while Thomas (2007) noted that "Anemia is a common finding in patients with diabetes due to the high burden of chronic kidney disease in this population. Anemia is more prevalent and is found earlier in patients with diabetes than in those with kidney disease from other causes. Correction of anemia certainly improves performance and quality of life in diabetic patients. In the absence of additional data, treatment should be considered palliative, and any functional benefits must be matched against costs to the patient and the health system" [12].

The aim of our study was to estimate the prevalence of the red cell morphology changes in diabetic patients and their correlation with markers of glycemic control.

Patients and Methods

We conducted a retrospective study over a period of three months (May–July 2014) that included 30 patients with type 2 diabetes mellitus (T2DM) who were been followed-up at the Clinical Centre of Diabetes, Nutrition and Metabolic Diseases, University of Medicine and Pharmacy of Craiova, Romania. Criteria for inclusion in the study: patients diagnosed with T2DM, successively hospitalized in the clinic of diabetes, nutrition and metabolic diseases, aged between 18 and 80 years. Exclusion criteria: patients whose data were inaccessible or incomplete, previous treatments with medication interfering or influencing the level of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3), exogenous glucocorticoid, oral estrogen-replacement therapy, anti-estrogen therapy.

Patient demographics, relevant concomitant illnesses and medical history were recorded. Anthropometric, biochemical parameters (fasting plasma glucose – FPG, HbA1c, glomerular filtration rate – GFR) and morphology of blood smear were assessed. Blood pressure was recorded. The anthropometric measurement included weight, height, waist circumference (WC); waist circumference was taken at the midpoint between the lowest rib and the iliac crest. Blood pressure was measured with a mercury sphygmomanometer. The measurement protocol included three assays; the mean of all three measurements was used as systolic and diastolic blood pressure. Subjects were asked to fast for 12 hours before blood sampling, which was done between 8.00 and 9.00 a.m. The plasma glucose was measured enzymatically, HbA1c was determined by high performance liquid chromatography and GFR were calculated automatically (using link http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr).

Analysis of blood cytology

The staining technique used was May–Grünwald–Giemsa (MGG) technique. The microscopic examination was performed initially with the objective of 10×/20× to assess the staining and cellular distribution, achieving an estimate of the number of leukocytes and of the presence of abnormal cellular elements (blasts, erythroblasts), platelet aggregates, agglutinated erythrocytes. Subsequently, the smear was evaluated with an objective of 100× immersion, each cell type was evaluated for quantitative and qualitative abnormalities. Image acquisition was done after the examination of the preparations obtained with a 40× objective, using Image Pro Plus 6.0 software. There was used numerical grading (1+ to 4+) based on percentage of cells that differ in size or shape from normal erythrocytes (normal – 5%, slight – 5–10%, 1+ – 10–25%, 2+ – 25–50%, 3+ – 50–75%, 4+ >70%).

There was assessed and recorded the erythrocytes diameter. A custom Matlab (Mathworks) software was developed to quantify the size of the erythrocytes. The software scanned for "perfect" circles and exported their diameters. Touching and elliptic erythrocytes were not taken in consideration in order to have an accurate size estimation. Large objects (artifacts, white cells) were removed from the final estimation.

The results were compared with the same measurements in 30 subjects without diabetes mellitus. All the patients were informed about the study and expressed their written consent. Both the study protocol and their written consent were approved by the Institutional Ethics Committee.

Statistical analysis

Data are presented as mean±SD. Clinical characteristics were compared using the Student's *t*-test. Pearson's moment-product correlation coefficients were calculated to evaluate correlations between variables. Significance was defined at the 0.05 level of confidence. Calculations were performed using the Statistical Package for Social Sciences Software (SPSS) version 15. Anderson–Darling and Shapiro–Wilk tests were used to assess the distribution data.

Results

The patients of study group (15 women and 15 men) were aged 45 and 77 years, median age 60.93±8.50 years and had an evolution of diabetes between four months and 30 years. Eighteen patients of study group were from urban and 12 patients from rural areas. The subjects of control group (15 women and 15 men) were aged 40 and 79 years, median age 60.33±9.48 years. Eighteen subjects of study group were from urban and 12 subjects from rural areas. The groups were similar in terms of age and distribution by age of patients in the study group and of subjects of control group are shown in Figures 1 and 2.

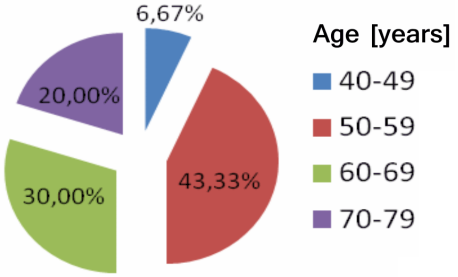

Figure 1 – ***Distribution by age of patients in the study group.***

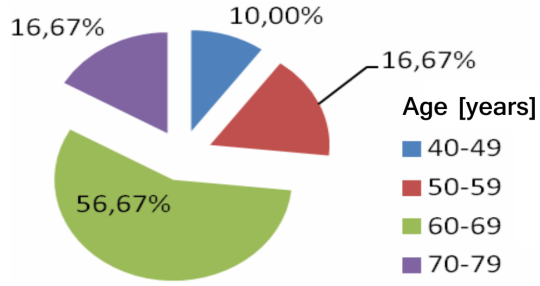

Figure 2 – ***Distribution by age of patients in the control group.***

There were statistically significant differences for the recorded parameters in patients of study group and control subjects. The main characteristics of patients with T2DM and control group recruited in the study are shown in Table 1.

Table 1 – ***Characteristics of patients with T2DM and control group recruited in study***

Recorded parameters	Characteristics of study group (*n*=30)	Characteristics of control group (*n*=30)	*P*
Age [years]	60.93±8.50	60.33±9.48	NS
FPG [mg/dL]	217.70±73.20	90.03±6.59	<0.05
HbA1c [%]	7.95±1.99	5.65±0.32	<0.05
GFR [mL/min./ 1.73 m^2]	88.43±16.56	105.50±1.86	<0.05

FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; GFR: Glomerular filtration rate; NS: Not significant. Comparison is significant at the p<0.05 level.

Regarding the study of FPG, in the study group were recorded averages of 217.70 mg/dL with a standard deviation of 73.20 compared with controls that had a blood glucose value of 90.03 mg/dL with a standard deviation of 6.59. Glucose distribution for the study group and the control group are shown in Figures 3 and 4.

In the study group, there were three (10%) cases with values of HbA1c between 4.5 and 5.7%, seven (23.33%) cases with values of HbA1c between 5.7 and 6.4%, and the vast majority of patients (20 patients – 66.67%) recorded values above 6.5%. Mean HbA1c was 7.95% with a standard deviation of 1.99. For the control group, the mean value of HbA1c was 5.65% with a standard deviation of 0.32. HbA1c distribution for the study group and the control group are shown in Figures 5 and 6.

In the study group, GFR ranged between 47.70 and 118.90 mL/min./1.73 m^2. For the control group, GFR values were between 88.00–130.00 mL/min./1.73 m^2. According to *National Kidney Foundation*, three (10%) patients of study group present zhronic kidney disease (CKD). The condition is defined by "Glomerular Filtration Rate (GFR) less than ≤60 mL/min./1.73 m^2 that is present for ≥3 months with or without evidence of kidney damage or evidence of kidney damage with or without decreased GFR that is present for ≥3 months as evidence by microalbuminuria, proteinuria, glomerular hematuria, pathological abnormalities, anatomical abnormalities" [13]. GFR distributions for the study and control groups are shown in Figures 7 and 8.

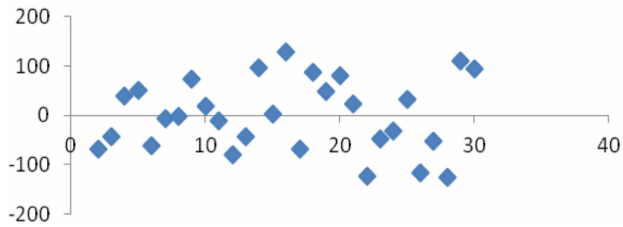

Figure 3 – ***Serum glucose distribution (from median) for the study group.***

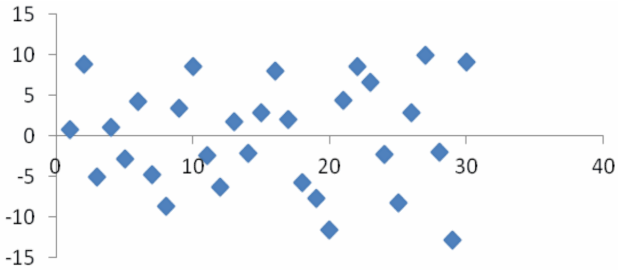

Figure 4 – ***Serum glucose distribution (from median) for the control group.***

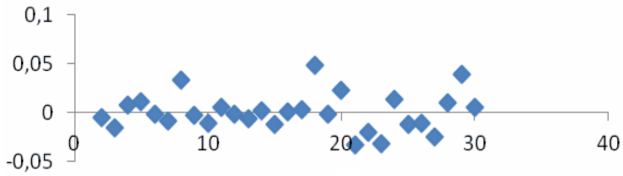

Figure 5 – ***HbA1c distribution (from median) for the study group.***

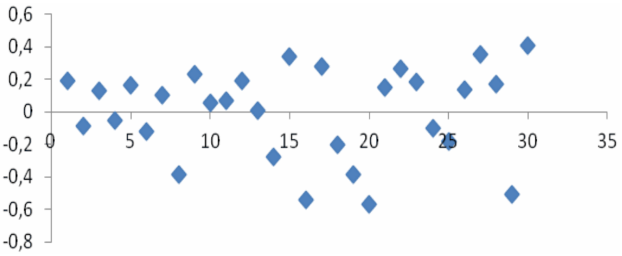

Figure 6 – ***HbA1c distribution (from median) for the control group.***

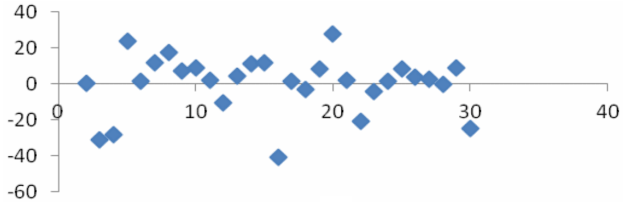

Figure 7 – ***GFR distribution (from median) for the study group.***

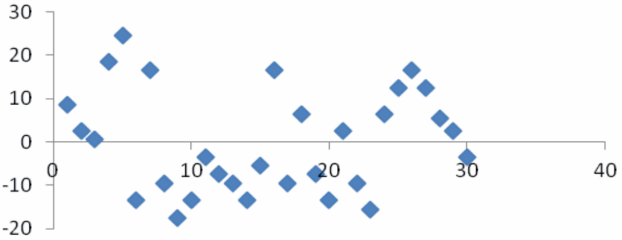

Figure 8 – ***GFR distribution (from median) for the control group.***

In the analysis of blood cytology for the study group, there were changes in the smear type hypochromia, anisocytosis and poikilocytosis. Normal blood erythrocytes turned red-orange smears in May–Grünwald–Giemsa staining (normochromic). The color was more intense at their periphery and gradually faded towards the center of the cell; thus, the center of erythrocytes (central pallor) occupied less than a third the diameter of the cell. In the present study, varying hypochromia degrees (from slight to severe quantified with 1+ quantified with 3+) were seen in 13 cases evaluated (43.33%). There was only hypochromia as unique morphological change of erythrocytes. Anisocytosis was the generic term used to describe an abnormally wide distribution of the sizes of the erythrocytes in the blood, such as macrocytes, which were larger than mature normal red blood cells, or microcyte (the presence of smaller red blood cells such as microcytes).

In physiological conditions, there was a moderate variation in erythrocyte diameter (the diameter ranged between 6.7–7.7 µm, with an average of 7.2 µm). Occasionally, erythrocytes might be present with a diameter less than or 4.75 µm and 9.5 µm high. Most of these cells were round but others were slightly oval.

In the present study, 10 (33.33%) cases presented a slight anisocytosis (1+) and five patients mild anisocytosis (2+), the erythrocyte smears were both small and large, still predominantly small ones.

Other morphological change observed in the red series was represented by poikilocytosis (10 patients – 33.33%). Poikilocytosis is a specific phenomenon, but occurs in many anemia, especially in severe anemia.

In the analysis of blood cytology for the study group, there were changes in the smear type hypochromia, anisocytosis and poikilocytosis (20 patients – 66.66%). Two patients recorded a change of the red cells, while 18 patients recorded combined changes.

Regarding the study of erythrocyte diameter, in the study group were recorded averages of 6.83 µm with a standard deviation of 0.66. Evaluation of erythrocyte diameter for the study group is presented in Figure 9 and changes in blood smear are presented in Table 2 and in Figures 10–13.

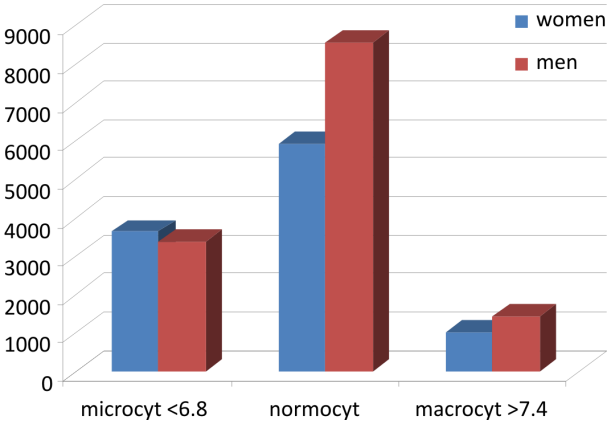

Figure 9 – ***Evaluation of erythrocyte diameter in the study group.***

Table 2 – ***Changes in blood smear in the study group***

Changes in blood smear in the study group	No. of cases	Degree – No. of cases		
		1+	*2+*	*3+*
Hypochromia	14	3	8	3
Anisocytosis	14	9	5	0
Poikilocytosis	10	8	1	1

In terms of red cell morphology, changes were recorded anulocytes type, red cells in "mark to the target fired" (codocytes), bream (leptocytes), schizocytes, and red cells in "drop" (dacryocytes) (Figures 14 and 15).

Among the control group, the prevalence of hypochromia was 6.66% (two subjects) and anisocytosis 3.33% (one subject).

Regarding the assessment of erythrocyte diameter, in the study group there were recorded averages of 7.01 µm with a standard deviation of 0.65 (Figures 16 and 17). The study showed no statistically significant differences in terms of the diameter of erythrocytes in the study group compared with the control group. Evaluation of erythrocyte diameter for the control group is presented in Figure 18.

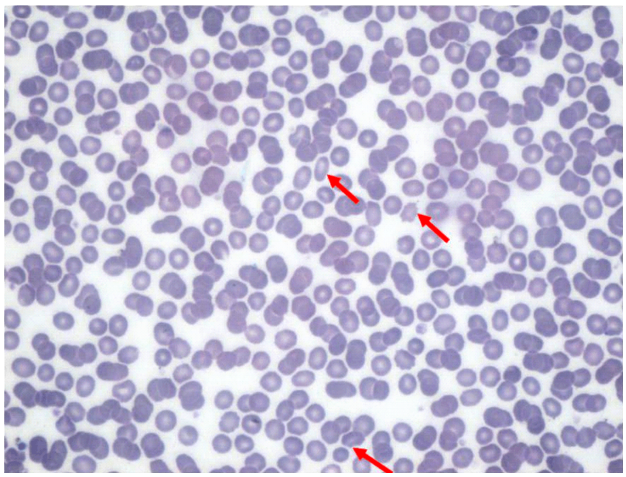

Figure 10 – ***Blood smear from a patient with diabetes: poikilocyosis (1+) – red arrows. MGG staining, ×400.***

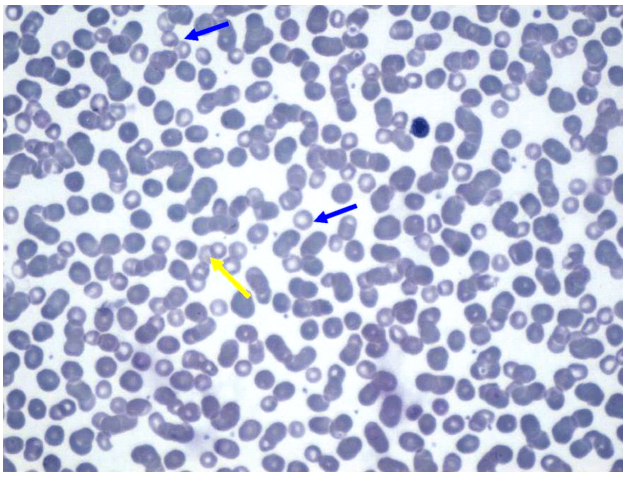

Figure 11 – ***Blood smear from a patient with diabetes: hypochromia (1+) – blue arrows, anisocytosis (1+) – yellow arrow. MGG staining, ×400.***

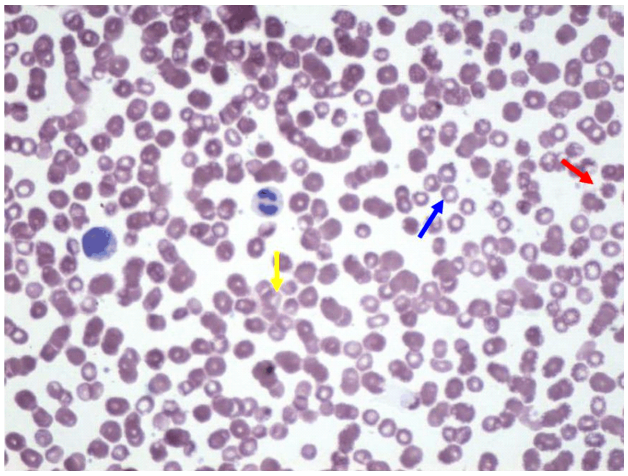

Figure 12 – *Blood smear from a patient with diabetes: hypochromia (2+) – blue arrow, anisocytosis (2+) – yellow arrow and poikilocyosis (1+) – red arrow. MGG staining, ×400.*

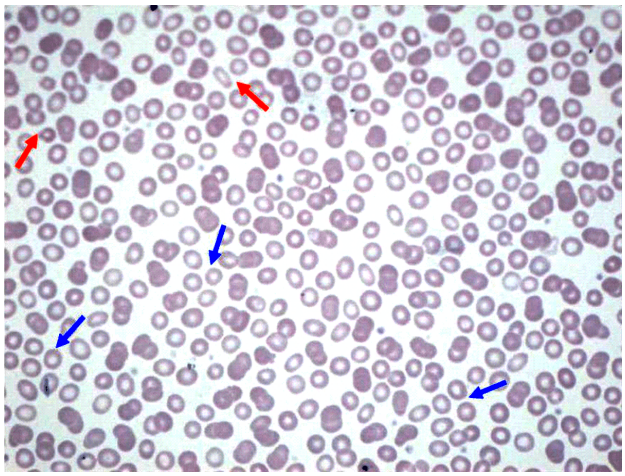

Figure 13 – *Blood smear from a patient with diabetes: hypochromia (3+) – groups of red blood cells at the tip of the blue arrows and poikilocyosis (2+) – oval-shaped red blood or cells drop (red arrows). MGG staining, ×400.*

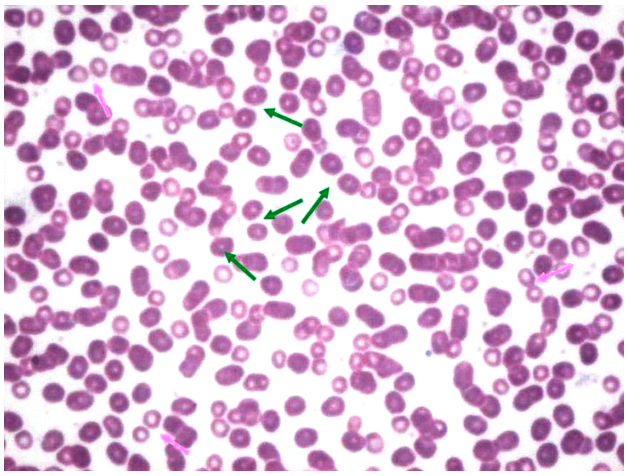

Figure 14 – *Blood smear from a patient with diabetes: hypochromia (3+), rare anulocytes – pink arrows, red blood cells in the target-shooting sign (codocytes) – green arrows; anisocytosis (2+). MGG staining, ×400.*

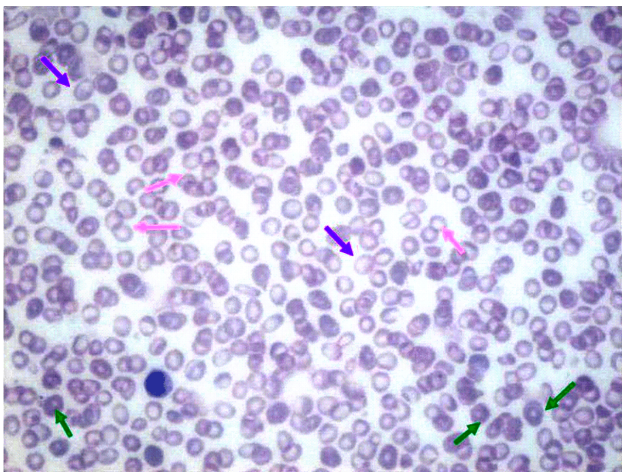

Figure 15 – *Blood smear from a patient with diabetes: hypochromia (3+), common anulocytes – pink arrows, rare codocytes – green arrows, anisocytosis (2+ – common macrocytes – purple arrows), poikilocyosis (3+ – common oval-shaped red blood, rare bream – leptocytes, rare schizocytes and rare red cell in "drop" – dacryocytes). MGG staining, ×400.*

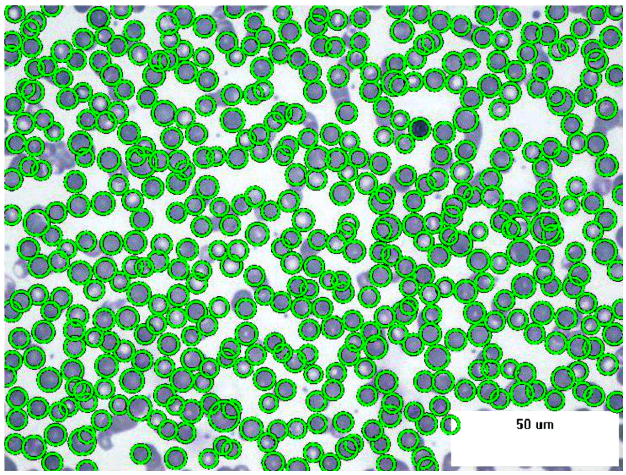

Figure 16 – *Determination of erythrocyte diameter of a blood smear from a patient with diabetes: hypochromia (1+), anisocytosis (1+). MGG staining, ×400.*

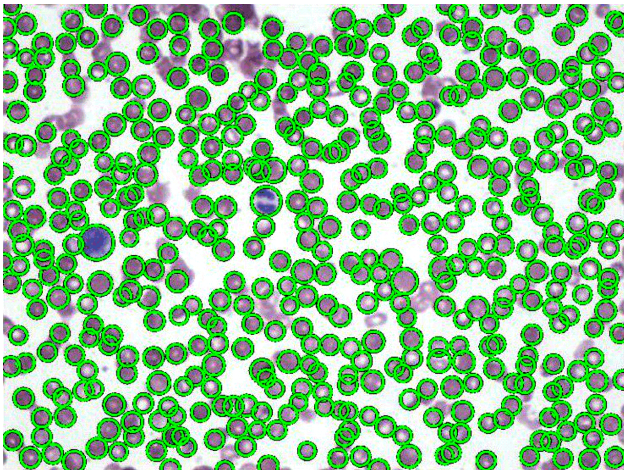

Figure 17 – *Determination of erythrocyte diameter of a blood smear from a patient with diabetes with hypochromia (2+), anisocytosis (2+) and poikilocyosis (1+). MGG staining, ×400.*

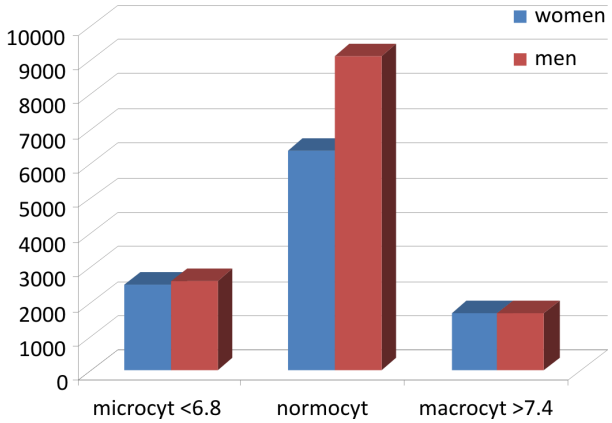

Figure 18 – *Evaluation of erythrocyte diameter in the control group.*

Discussion

Previous studies have shown that poor glycemic control causes alteration in various biochemical and hematological parameters [14–18].

The study aimed correlations between basal blood glucose, HbA1c, age, GFR and possible association with changes in blood smear for a group of 30 patients diagnosed with diabetes. The multiples regression analysis revealed a positive, significant correlation between the basal blood glucose and the HbA1c level in the study group (r=0.401, p=0.28) (Figure 19) and control group (r=0.375, p=0.041). No other correlations were found between parameters of metabolic control, age and GFR.

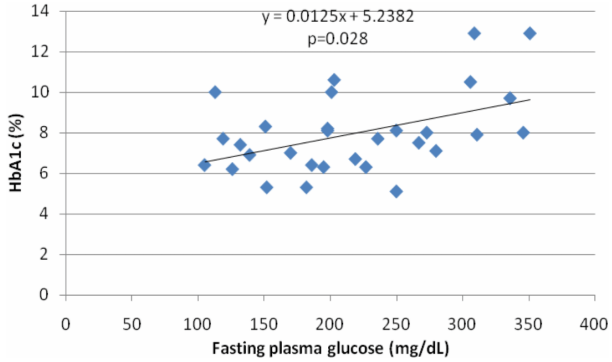

Figure 19 – *Correlation between the fasting plasma glucose and the HbA1c level in the study group.*

The prevalence of hypochromia in the study group was 46.66% (14 patients) and 6.66% (two subjects) in the control group. The term of hypochromia is a generic term in which the red blood cells are paler than normal, color determined by less than the normal amount of hemoglobin. The most common causes of hypochromia are iron deficiency and thalassemia. The diagnosis of iron deficiency is particularly challenging in patients with acute or chronic inflammatory affections. In an issue of *BioMed Research International* (formerly titled *Journal of Biomedicine and Biotechnology*), Urrechaga *et al.* published, in 2013, a review article entitled: "Biomarkers of hypochromia: the contemporary assessment of iron status and erythropoiesis", in which they report that "it has long been known that inflammation can mimic some aspects of iron deficiency by impairing the utilization of existing iron stores for red cell production and inducing an iron-sequestration syndrome and low serum iron" [19]. In 1998, Pickup & Crook proposed a hypothesis suggesting that chronic inflammation is associated with T2DM [20]. In the last years, it has been accepted that chronic subclinical inflammation is a part of the insulin resistance syndrome [21, 22] and that inflammation plays a significant role in the pathogenesis of diabetes [23, 24]. According to Weiss (2009), inflammatory cytokines may cause sequestration of iron in reticuloendothelial cells [25].

The prevalence of anisocytosis (variation in size) and poikilocytosis (variation in shape) in the study group was 46.66% (14 patients) and 30.30% (10 patients) and 3.33% (one subject) in control group. Data from other studies report that cytology of normal persons reveals small numbers of poikilocytes, usually less than 2%. From a diagnostic standpoint, poikilocytosis has no specificity, but the recognition of specific forms of poikilocytes often points to specific disorders [26]. No relevant data on prevalence of hypochromia, anisocytosis and poikilocytosis in diabetic patients were found in the literature.

A limitation of our study is represented by the relatively small number of patients enrolled. Future clinical and experimental studies should explore potential causal mechanisms linking hematological alterations and diabetes mellitus.

Conclusions

Our results lead to the conclusion that, for the selected patients, even though the distribution of the FPG values is significantly wider compared to the control group, most of the diabetic patients had a good therapeutic control as expressed by the level of the glycated hemoglobin. The results also show that the morphological changes of the red blood cells are highly present in the diabetic patients, compared to control; the patients were generally characterized by hypochromia, anisocytosis and poikilocytosis, but the frequency of these modifications was reduced in the control group, even the two groups were age-matched. These changes may have a direct impact on erythrocyte function and may contribute to the patient complex pathology. We conclude by suggesting that the morphological assays of red blood cells might give valuable information about the disease status of the patients with diabetes. Our results should also be completed with further ultrastructural analysis, which could be a very useful tool in evaluating the efficacy of treatment in diabetes patients as well as different associated risks, since the red blood cell status is crucial to the overall wellness of the diabetic patient.

Conflict of interests

The authors declare that they have no conflict of interests.

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