CLINICAL PRACTICE

The assessment of erythrocyte deformability by filtration rate

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Abstract

The aim of the study was to monitor the erythrocyte deformability as one of important factors securing the appropriate tissues perfusion in healthy subjects and diabetic patients with insulin dependent diabetes mellitus. Erythrocyte deformability was determined by the method of filtration and centrifugation, and the erythrocyte filtrability was calculated as a percentage of filtered erythrocytes out of the number of erythrocytes counted before centrifugation. Diluted blood suspensions were filtered by centrifugation through membrane filters with pores of 5 μm in diameter. The speed and duration of centrifugation of 1400 rpm and 5 min respectively were selected as the best ones for filtration due to the lowest value of the coefficient of variance.

The values of the arithmetic mean and standard deviation of erythrocyte filtrability were 72.2±7.9 % in normal subjects. In the group of diabetic patients with the long history of insulin therapy the values amounted to 69.1±4.4 %. In diabetic patients, the average value of blood glucose was 11.7 mmol.L^{-1}, and the glycated haemoglobin concentration reached 9.04 %. From the viewpoint of reference values, these facts indicate good compensation of diabetes mellitus. Other haematological values, namely erythrocyte count, haematocrit value, haemoglobin concentration and mean cell volume were within normal reference ranges. No difference between the groups of healthy subjects and diabetic patients was found. ***(Fig. 4, Ref. 18.)***

Key words: erythrocyte deformability, erythrocyte filtration, diabetes mellitus.

Erythrocyte deformability (ED) is one of important physiological factors securing the appropriate tissues perfusion. To realize this function, erythrocytes have to deform their shapes by reducing their stationary diameter of 7–8 μm to diameters as small as 1.5 μm in order to be able to traverse the narrow capillary beds and splenic sinusoids. The deformability of normal human erythrocytes is the consequence of their low cytoplasmic viscosity, their excess of membrane surface in relation to cell volume and their viscoelastic properties of cell membrane. The erythrocyte flexibility is determined by the combination of these factors. Loss of deformability may arise from impairment of any of these properties or their combinations (1).

Erythrocyte deformability changes play an important role in the pathophysiology of some diseases, e.g. haematologic diseases (2, 3), diabetes mellitus (4, 5, 6), ischaemic heart disease (7, 8), stroke and hypertension (9), as well as in coincidence with hard exercise (10, 11). Because of the diagnostic importance of ED determination, several methods have been developed enabling to measure it.

There are various methods and instruments available for ED measuring: methods, in which erythrocyte suspensions are sheared in geometric systems (2, 6), e.g. viscometry and laser diffraction ellipsometry (10, 12, 13) and methods in which erythrocytes are made to traverse narrow channels – micropipettes (14) or filter pores (7, 15). To study the erythrocyte microrheology, the method of cation-osmotic haemolysis has been developed (16) and used in many experimental and clinical studies (17, 18).

Erythrocyte filtration is a technique widely used in clinical assessment of ED. In this work the method of erythrocyte filtration was selected for its low costs and quickness of procedure.

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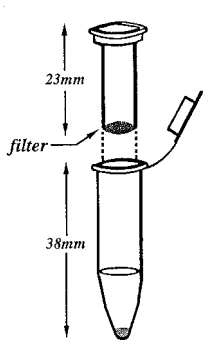

Fig. 1. An ultrafree membrane filter.

Materials and methods

Two groups of students were examined. The first consisted of 7 healthy subjects, non-diabetics, aged 20 to 23 and the second one included 16 patients with insulin dependent diabetes mellitus (IDDM) aged 21 to 31. They had been treated by insulin for 14 years on the average.

The haematologic analysis and ED determination were made from venous blood samples taken into sterile syringes with potassium-EDTA as anticoagulant. Blood suspensions for erythrocyte filtration were prepared by diluting 20 μL of anticoagulated blood with 10 mL of diluting solution RR I (Bachem, Slovakia), which contains boric acid and sodium chloride. The dilution of whole blood in suspension was 1:500. An aliquot of 100 μL of each erythrocyte suspension was taken to 10 ml of diluting solution to determine the erythrocyte count (RBC count), haematocrit value, and mean cell volume by the semiautomated cell counter Picoscale 5 (Medicor, Hungary). Another 200 μL aliquot of each suspension was pipetted into the centrifugal tube (Fig. 1) equipped with membrane filter with average pores of 5 μm in diameter (Ultrafree MC, Millipore, Japan). The tubes with blood suspensions were centrifuged by microcentrifuge Micro 20 (Hettich, Deutschland) at 1400 rpm for 5 minutes. After centrifugation, in the course of which the erythrocytes were passing through narrow pores of filter, each supernatant was well mixed to diffuse filtered erythrocytes uniformly. The filtrated suspensions were appropriately diluted and counted by the cell counter to determine the haematological values described above. The haemoglobin concentration was measured by the cyanmethaemoglobin method and reticulocytes were counted microscopically. All measurements were carried out at the laboratory temperature of 22–24 °C and performed no later than four hours after venepuncture.

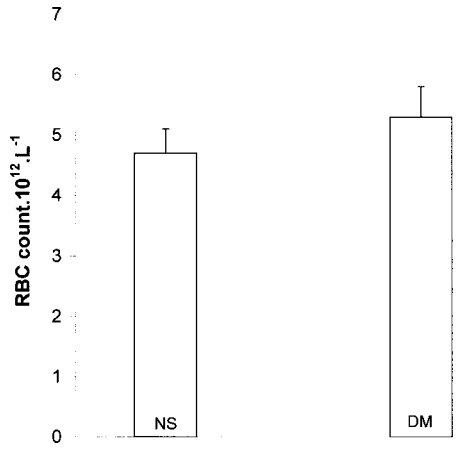

Fig. 2. Red blood cell count in normal subjects (NS) and patients with diabetes mellitus (DM).

ED assessment was calculated on the basis of filtration rate:

$$\text{filtration rate} = \frac{\text{RBC count after centrifugation}}{\text{RBC count before centrifugation}} \text{ x100 (\%)}$$

Means and standard deviations of filtration measurements were used to calculate the coefficient of variation (CV):

$$\text{CV} = \frac{\text{standard deviation}}{\text{mean}} \text{ x100}$$

To find the best conditions for filtration, the means and variation coefficients were studied under different centrifugation conditions with eigth aliquots. The tubes were centrifuged at 1200 rpm/5 min, 1200 rpm/10 min, 1300 rpm/5 min and 1400 rpm/5 min. The mean with the standard deviation of filtration rate and the coefficient of variation were calculated for each centrifugation condition. The lowest CV 7.1 was obtained under centrifugation of 1400 rpm/5 min. Due to this result, all experiments were performed under the same conditions.

The experimental data were statistically tested by non-parametric Kolmogorov–Smirnov test.

Results

In the group of normal subjects, the average erythrocyte count was $4.7\pm0.4.10^{12}12.L^{-1}$ (Fig. 2). After the erythrocytes filtration, the filtration rate 72.2±7.9 % was calculated (Fig. 3). Other haematologic values were assessed: mean cell volume was $82.7\pm6.4.10^{-15}$L (Fig. 4), haemoglobin concentration 153.5±16 $g.L^{-1}$ and haematocrit value 39.1±4.0 % were in the range of physiological values. Reticulocytes count presents 0.90±0.2 % of the total erythrocyte count in normal subjects.

In the group of diabetic patients the average glucose value was 11.7 $mmol.L^{-1}$ and glycated haemoglobin concentration was 9.04 %. This fact reffers to good compensation of diabetes from the viewpoint of physiological values. In this group, the average value of erythrocyte count was higher $5.3\pm0.5.10^{12}.L^{-1}$ ($p=0.06$) against the control group (Fig. 2). The obtained erythrocyte filtration value of 69.1±4.4 % (Fig. 3) was lower by 4.3 % in comparison with the group of normal subjects. Other haematological variables that were assessed as average values – mean cell volume $87.8\pm3.5.10^{-15}$L (Fig. 4), haemoglobin concentration 172.0±13 $g.L^{-1}$ and haematocrit value

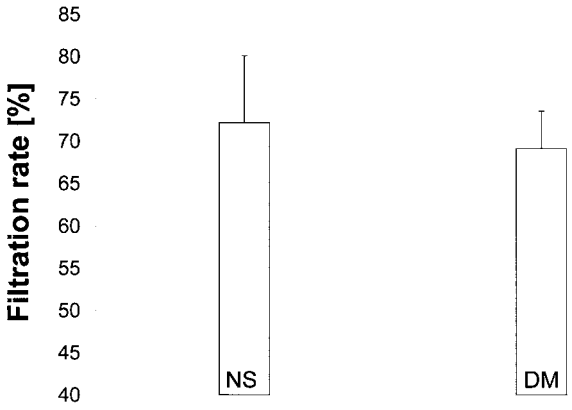

Fig. 3. Filtration rate in normal subjects (NS) and patients with diabetes mellitus (DM).

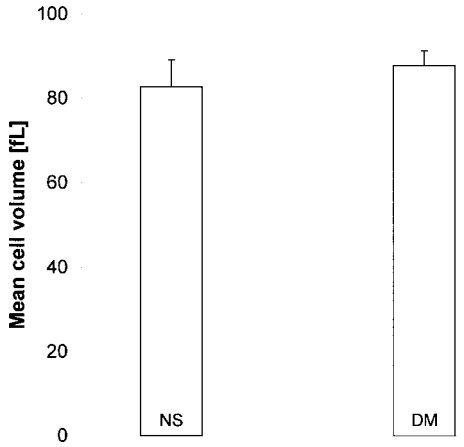

Fig. 4. Mean cell volume in normal subjects (NS) and patients with diabetes mellitus (DM).

46.7±3.1 %. Reticulocyte count from the total erythrocyte count being 0.90±0.2 % was the same as in the group of normal subjects.

Discussion

The presented filtration method for ED determination was tested under different centrifugation conditions. The best condition found for centrifugation was 1400 rpm/5 min, as it yielded the lowest value of variation coefficient of 7.1. Membranal filters with pores of 5 μm in diameter were used according to the recommendations of International Committee for Standardization in Haematology, that proposes the pores to range between 3–5 μm in diameter (1).

Our results of erythrocyte filtration rate correspond with the literary data (4, 5). It was found out that significantly lower erythrocyte filtration rate in patients with IDDM was not significantly lower in comparison to the control group. This fact can be explained by the protective effect of long-term insulin therapy and good compensation of diabetes mellitus. Linderkamp et al (6) tested the influence of insulin therapy on ED. In children with IDDM before the initiation of insulin treatment, ED was decreased by 28, after 5 to 8 years of insulin treatment ED improved and decreased by 17 when compared with healthy children. This outcome refers to the fact that long-term insulin therapy has a positive effect on ED. Our results also refer to the favourable effect of long-term insulin therapy. Erythrocyte deformability value is probably influenced by the assessed higher values of mean cell volume, haemoglobin concentration and haematocrit value in the group of diabetic patients.

The application of the filtration method for the assessment of ED will contribute to the specification of diagnostic and therapeutic procedures in clinical practice.

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