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Estimation of erythrocyte population state by the spherical index distribution

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Abstract

The densities of cell distributions by spherical index (SI) in erythrocyte populations from healthy adults and donors with endocrine pathologies were determined via the developed method. The investigation shows that this characteristic varies for different donors, thereby reflecting the erythrocyte population state of an individual donor. Individual distribution curves obtained from healthy donors are close to Gaussian and are characterized by smooth curve plot with one maximum. Cells distribution by SI in donors with endocrine pathologies has a polymodal character. Our research shows that the developed method for determining erythrocyte distribution density by SI is a sensitive and informative test for quantitative evaluation of an erythrocyte population state. Moreover, this characteristic has clear physical and physiological significance, because an erythrocyte shape is strongly conditioned by the cell age and influences the ability to pass through microcapillaries in blood circulation.

Keywords: Erythrocytes; Spherical index; Endocrine pathologies

1. Introduction

Since the erythrocytes of each individual have unique properties, it is quite a fruitful endeavor to study erythrocytes as a cell population. The heterogeneity of an erythrocyte population contains significant information about the state of the blood system as a whole. A number of diseases cause a variation in the distribution of cell properties (age, shape, size, activity of cell ferments, etc.), which deviate from the normal value. It is essential to characterise a cell population not only by the mean values of its characteristics, but also by the distribution in the population, as it is a more informative approach which adequately describes the population. Advantages of such an approach are quite obvious, but it is accompanied by significant methodological difficulties, which require a conversion from the general consideration of mean values to the analysis of separate cells. This analysis is labour-intensive and cannot be widely used in research and especially in clinical routine.

2. Materials and methods

2.1. Materials

Investigations were performed on blood samples collected from donors at Hospital of Institute for Endocrine Pathologies of Academy of Medical Sciences of Ukraine (Kharkiv), where the presence or absence of endocrine pathologies were diagnosed.

2.2. Theoretical basis of method

A method for determination of the probability density of erythrocyte distribution by spherical index has been developed. It is based on the physical and mathematical model for the membrane transport occurring in cells placed into the hypotonic solution of non-penetrating substance and uses the small-angle light scattering method for obtaining experimental curves of osmotic fragility. The osmotic fragility

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for determination of the probability density of erythrocyte distribution in population by SI allows the calculation not only for the mean value of SI, but for the whole spectrum of this parameter in the population.

Fig. 1 presents the erythrocyte distribution by SI at 37 °C in the populations of three adult donors. Experiments show that this characteristic varies for different donors reflecting the erythrocyte population state of the individual donor. The erythrocyte distributions obtained for individual healthy adults as a rule approach to Gaussian distribution and have a smooth curve with one maximum. The cells with SI in the range from1.38 to 1.58 form the maximum percentage. The quantitative values obtained for the centres of the SI distributions correlate to an erythrocyte volume and membrane surface area. According to Ref. [3], erythrocyte surface area ranges from 120 to 155 μm^2, and volume from 90 to 93 μm^3. Consequently, the SIs of most erythrocytes are in the range of 1.22–1.57. The averaged curve for eight donors has a maximum SI of 1.48. Interestingly, the majority of the curves for individual healthy donors have maximum rates at the same SI.

For patients with endocrine pathologies, both with hyper- and hypothyroidism, erythrocyte distributions by SI have bi- or three-modal character. Besides the main maximum, the distribution curves have one or two additional maximums. In the case of hyperthyroidism among the cells with non-standard SI, the higher percentage (25%) is composed of cells with lower SI = 1.07–1.22, and only 10% of the cells have SI = 1.73–1.9 (Fig. 2).

For patients with hypothyroidism, erythrocyte distribution curves mostly have three-modal character (Fig. 3) with a higher portion (~ 30%) of the flattened erythrocytes (SI = 1.65–2.46) compared to spherical erythrocytes (3%, SI = 1.015–1.3). In addition, the SI of flattened erythrocytes considerably exceeds that of young discocytes in control samples (Fig. 1), which have a small percentage of cells with SI higher than 1.9.

Different hormones synthesized in human organism have a different spectrum of action. Some of them specifically regulate only the function of distinct target organs. Action of other hormones is directed to regulate distinct ways of metabolism. Among such metabolic differentiation, the polymorphous action of thyroid hormones is distinguished. They influence a number of metabolic pathways and almost all structural levels: the functions of all cells and subcellular structures, the activity of most important enzymes. They control the most important biochemical reactions of proteins, carbohydrates, lipids and water–salt balance [4].

The effect of thyroid hormones in organism is accomplished both by genome way (via synthesis of various substances) and non-genome way (direct action on cell function processes) [4]. In particular, synthesis of the fatty acids could be disordered [5]. The activity of Na,K-ATPase is also regulated by its synthesis [6]. At the same time, one can suppose that thyroid hormones control the Ca-ATPase activity in erythrocytes by a non-genome way [4,7,8]. The

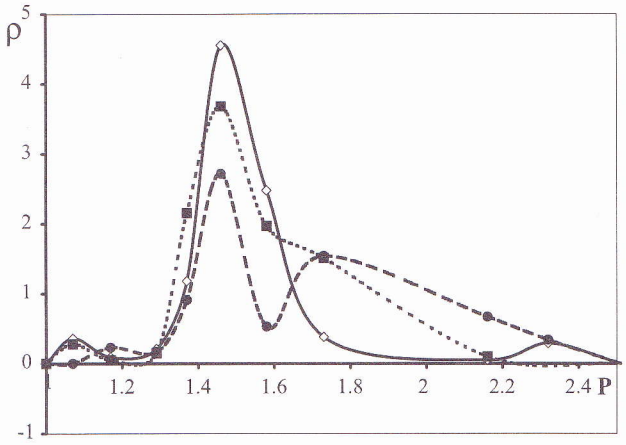

Fig. 3. Density of erythrocyte distribution by spherical index in the individual populations of three patients with hypothyroidism (T = 37 °C).

Ca-ATPase activity in erythrocytes occurs at hyperthyroidism and decreases at hypothyroidism [9]. Another non-genome action of iodine thyroids lies in the influence on cytoskeleton via intracellular regulation of disulphide isomerase, which plays an important role in de novo synthesis of proteins in endoplasmic reticulum [4]. A report was also made about T_3 and pyruvate kinase interaction in human erythrocytes with a decrease both in ATP production during glycolysis and pyruvate accessibility for other cell reactions, e.g., citric acid cycle [10]. Thus, thyroid hormones influence various processes in the human organism, and, in particular, could change the cell membrane permeability and mechanical properties, as well as the ability to maintain cell homeostasis [4,11].

Taking into account the general character of the action of thyroid hormones on an organism, cells and cell membranes, it is difficult to distinguish the exact causes of the observed effects. However, it is possible to suppose that the developed method for measuring the probability density of erythrocyte distribution via spherical index is a sensitive and a good reproducible test for quantitative estimation of the erythrocyte population state of different donors with various pathologies. Moreover, this characteristic has clear physical and physiological significance, because erythrocyte shape is strongly conditioned by age and influences the ability to pass through microcapillaries in blood circulation.

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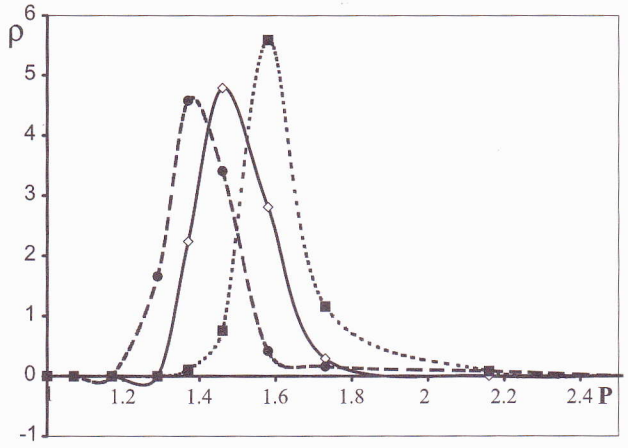


Fig. 1. Density of erythrocyte distribution by spherical index in the populations of three adult donors (T=37 °C).

was determined in the NaCl solutions with concentrations from 0.15 to 0.05 mol/l.

The spherical index (SI) is defined as a relationship of erythrocyte volume to the volume of the sphere with the same surface area $P_0 = V_{sp}/V_0$:

$$P_0 = \frac{S_0^{3/2}}{6\sqrt{\pi}V_0}$$

This geometrical parameter quantitatively characterises the deviation of erythrocyte shape from the spherical one (P_0=1 for the spherical cell). It rises with the deviation of the cell shape from the spherical one. There is no deformation in the erythrocyte membrane, or there can be insignificant bending deformation under physiological conditions [1]. Under these conditions, a normocyte has a volume significantly less than the maximum possible volume (which is spherical one) at a given membrane surface area S_0. Such a shape provides a free pass for erythrocytes through thin capillaries. In this case, an erythrocyte membrane has shift deformations at a constant surface area of the cell. The shape of aging erythrocytes approaches the spherical one, while SI approaches 1. The possibility of such cells to undergo the deformation without destruction decreases.

When a cell is placed in a solution with the osmotic pressure of non-penetrating substance π^{out}, less than the osmotic pressure of physiological solution π_0, then its volume V_0 increases under the influence of osmotic forces up to the volume

$$V_\infty = V_0(\alpha + (1-\alpha)\pi_0/\pi^{out}),$$

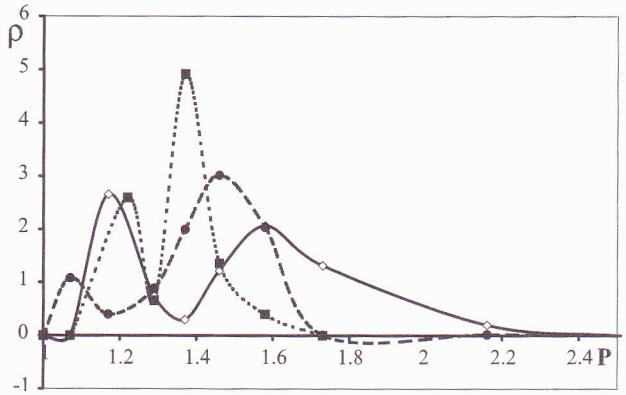
where α represents the intracellular volumetric content of osmotically inactive substances. Decrease in the osmotic pressure of the extracellular non-penetrating substance will cause the increase in the erythrocyte volume until it becomes a sphere. An erythrocyte membrane surface area is generally accepted to be constant and equal to the initial one during swelling when the erythrocyte shape changes from the initial to the spherical one. If the extracellular osmotic pressure continues to cause cell swelling and cell volume begins to exceed the volume of a sphere with the surface area of S_0 ($V_\infty > V_{sp}$), then the erythrocyte membrane undergoes an isotropic tension and, after a certain period of time, there forms a pore of a macroscopic size (i.e., its size significantly exceeds the distance between the molecules in the membrane) [2]. Therefore, after cells contact sodium chloride hypotonic solution, all those with SI less than 1or $P_0 < \alpha + (1-\alpha/\pi^{out})$ will hemolyse.

The fraction of erythrocytes with initial SI lower than $y_\infty = \alpha + (1-\alpha)/h\hat{\pi}^{out}$ can be determined experimentally from the fraction of hemolysed cells h at the osmotic pressure of the external solution π^{out}.

Converting osmotic fragility curves to dependencies $h = h(y)$, one can obtain the initial distribution of cells by spherical index in an erythrocyte population. It represents the cell fraction with SI, which exceeds an appropriate argument value. An appropriate density of cell distribution by SI in an erythrocyte population could be found by differentiation of this function on the argument.

3. Results and discussion

The maximum value of the relative erythrocyte volume (V/V_0) before hemolysis ranges from 1.5 to 1.9 according to different authors. In Ref. [1], individual erythrocytes were directly observed under a microscope. During osmotic hemolysis an erythrocyte becomes a sphere with the maximum volume 1.5- to 1.6-fold higher than a discocyte volume in isotonic media. The fact of the variety of the maximum volume observed can be explained by the erythrocyte distribution by SI in erythrocyte populations. The values of the maximum volume presented, correspond to the erythrocyte SIs at the initial state (before swelling in hypotonic solution). But in order to obtain the actual mean value of this parameter, it should be measured for a great number of cells—which is practically inconvenient for observation under a microscope. The developed method



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