Original Article

Assessment of the Risk of Hypoxia During Pregnancy by Analyzing the Permeability of Erythrocyte Membranes

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Abstract

Among the total number of newborns, about 11% face hypoxia problems. It is hypoxia that becomes the main cause of premature birth, fetal and newborn mortality, as well as various pathologies. Fetal respiration passes through the placenta with the participation of the erythrocytes. Thus, fetal respiration is affected by the rate of passage of erythrocytes through small blood capillaries. Ultimately, the elasticity, area, and permeability of the erythrocyte membrane have a direct impact on the quality of fetal intrauterine respiration. This article discusses the main methods of studying the dynamic characteristics of erythrocytes and also analyzes the number of anion exchangers on the erythrocyte membrane. The experiment involved 4 pregnant women, two of whom have a risk of premature birth. These patients undergo tocolytic therapy, the purpose of which is to reduce the risk of premature birth. As a result of the study, the dynamics of changes in the ratio of dynamic strength to the elasticity of the erythrocyte membrane, the amount of band 3 protein, as well as the dynamics of the CO2-O2 exchange rate during tocolytic therapy of pregnant patients are determined.

Keywords: Fetal hypoxia, Risk of premature birth, Pregnancy, Tocolytic therapy, Erythrocyte

Introduction

Hypoxia is a lack of oxygen supply to the whole organism or its organs. Today, this pathology occupies a leading place in the structure of perinatal morbidity and mortality, reaching 68%. Also, fetal hypoxia occurs in 11% of cases of the total number of births. Even though hypoxia is a common problem, diagnosing the risk of developing pathology in early pregnancy has not yet been systematized.

It is important to note that fetal hypoxia during pregnancy has a significant correlation with preterm birth [1]. The most statistically reliable method to determine the likelihood of preterm birth is associated with monitoring the respiratory movements of the fetus [2]. Thus, the method of identifying fetal breathing problems in the early stages of pregnancy, while insufficient oxygen supply to the developing organism has not led to deviations in the process of embryogenesis, seems to be particularly relevant. With the timely detection of a fetal breathing problem, the effectiveness of tocolytic therapy (using saline magnesium sulfate) increases, which is widely used in clinical practice to reduce the risk of fetal hypoxia and preterm birth [3]. Fetal respiration passes through the placenta with the participation of maternal erythrocytes [4]. Erythrocytes are the main blood cells whose main function is the transport of O_2 from the lungs to tissues and the transport of CO_2 from tissues to the lungs. The static and dynamic characteristics of these cells, such as elasticity, area, and permeability of the erythrocyte membrane for HCO_3 ions, determine the rate of CO_2–O_2 exchange [5]. The elasticity of erythrocytes determines the rate of their passage through small blood capillaries, which affects the respiration of the fetus. The shape of erythrocytes also affects the passage of erythrocytes through blood capillaries [6]. However, despite the generally recognized important role of erythrocytes in providing the body with oxygen, there is still a lot of uncertainty about how changes in the morphological (shape) and functional (membrane transport) characteristics of erythrocytes affect the risk of fetal hypoxia during pregnancy.

Human erythrocytes in most cases have an axisymmetric discoid biconcave shape, which is called a discocyte, and

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unlike many other cells do not have a nucleus. Also, in normal blood, there are planocytes (with a flat surface) and other forms of erythrocytes - spike-shaped erythrocytes, or echinocytes, dome-shaped, or stomatocytes, and spherical, or spherocytes. The immediate part of the erythrocytes circulating in the blood is their young forms, called reticulocytes. Normally, they are from 1 to 5% of the number of all erythrocytes. The size of erythrocytes in normal blood also varies. Most have a diameter of 7-8 μm and are called normocytes. The rest of the erythrocytes are represented by microcytes (diameter <7 μm) and macrocytes (diameter >8 μm) [7].

The main function of erythrocytes is respiratory, which consists of the transport of oxygen and carbon dioxide, which requires the participation of a certain number of proteins [8]. Erythrocytes, being the most numerous formed elements of blood, affect its rheological properties. Their concentration is $4.5\text{-}5.5\cdot10^6$ μl^{-1} in men and $3.7\text{-}4.7\cdot10^6$ μl^{-1} in women [9]. The spatial configuration of erythrocytes (discocytes) has a larger surface area compared to spherical cells of the same volume. This contributes to the most efficient gas exchange between the cell and the extracellular environment. Moreover, this shape, as well as structural features of the membrane and cytoskeleton, provide greater plasticity for erythrocytes when they pass through narrow capillaries [10].

The erythrocyte has a developed membrane complex and a perfect receptor apparatus. The membrane is a permeable barrier with a high degree of selectivity, which ensures the maintenance of cellular homeostasis at various values of the chemical composition of the intra- and extracellular environment [11, 12]. The transport of substances across the membrane takes place depending on their chemical properties and stereometric form in various ways: by diffusion, by penetrating through lipid sites, or by interacting with carrier proteins built into the membrane. Approximately 60% of the mass of membrane proteins is accounted for by spectrin, glycophorin, and band 3 protein [13].

Spectrin is a peripheral membrane protein that is a long, thin, flexible fibril and is the main protein of the erythrocyte cytoskeleton. As a result of the interaction of spectrin with specific proteins, a flexible network structure is formed on the cytoplasmic surface of erythrocytes, which ensures the passage of cells through narrow vascular capillaries. This contributes to the reversible deformation of erythrocytes in the process of circulation through various capillaries. The value of the characteristic observed ultimate stretching of the membrane is from 2% to 4% of the surface area [14].

Ion permeability is predominantly due to transport proteins. Band 3 integral protein plays a key role in CO_2/O_2 metabolism and is a transmembrane glycoprotein whose polypeptide chain crosses the lipid bilayer many times. It occupies a quarter of the erythrocyte surface area (~10^6 molecules per cell) [15]. Due to the passive transmembrane transport of anions in 1:1 stoichiometry, the protein of band 3 represents the most powerful transport system of ions in the erythrocyte. The maximum frequency of transfer by one protein of such anions as Cl^-, HCO_3^- is about 10^4 cycles per second. The probability of protein conformation change without binding to anions is several orders of magnitude lower. Based on the geometry of transported molecules, the transfer rate is higher for monatomic (Cl^-) or linear molecules (HCO_3^-) [16].

A large number of different diseases lead to changes in the biophysical properties of erythrocytes. Therefore, to diagnose pathology and monitor the results of therapy used for detected pathology, methods for recording the characteristics of erythrocytes that are responsible for the considered pathological processes in the body are necessary.

There are several types of blood cell measurements based on the following principles:

- optical and electron microscopy;
- conductometric or impedance method;
- flow cytometry, light scattering method;
- spectrophotometric method;
- turbidity spectrum method (turbidimetric method).

Materials and Methods

To study the kinetics of isotonic hemolysis of erythrocytes, whole peripheral blood was taken by venipuncture into disposable plastic tubes with anticoagulant EDTA (ethylenediaminetetraacetic acid) and sodium citrate. Pregnant women from 25 to 35 years old took part as donors, and some of them had functional disorders of the body. We used an isotonic aqueous saline solution of ammonium chloride (salt concentration 150 mM) as a lysing solution. The measurements were carried out on a scanning flow cytometer, which makes it possible to measure the angular dependences of light scattering (indicatrix) of single blood cells.

Immediately before the test samples were placed in the sampler of the scanning flow cytometer, blood was added to the lysing solution in a volume ratio of 1:1000 (blood: buffer) to achieve the optimal measurement mode on the scanning flow cytometer in the cell concentration range in the sample (~10^6 cells/mL). For the subsequent calibration of the experimental data, a small amount of 4 μm and 2 μm polystyrene microspheres was added to the sample. Recording of the signal of the hemolysis process started at the moment of pipetting the sample to reduce the duration of the dead time (reaction time not recorded by the device). Then, in the real-time mode of the process of isotonic hemolysis, the cells from the sampler entered the registration area of a scanning flow cytometer, where the light scattering signals of lysing erythrocytes were directly measured. One experiment took from 7 to 20 minutes. All experiments were carried out at room temperature for five hours after blood sampling.

Results and Discussion

The experiments were carried out with venous blood samples from both healthy pregnant women and patients in the process of tocolytic therapy applied to them. The goal of this therapy is to reduce the risk of preterm birth. It is known that fetal hypoxia has a maximum correlation with preterm birth [17]. Therefore, registration of changes in dynamic characteristics (sphericity index, amount of band 3 protein, ratio of strength to elasticity of the erythrocyte membrane) provides monitoring of changes in the ability of maternal erythrocytes to carry out CO_2-O_2 exchange during therapy **(Table 1)**. **Table 2** reflects all the parameters of the ongoing research.

Table 1. Drugs and doses for tocolytic therapy

Day 1	Day 2	Day 3	Day 4
$MgSO_4$ 20-25% (20 ml)	$MgSO_4$ 25% (20 ml)	$MgSO_4$ 25% (20 ml)	$MgSO_4$ 25% (20 ml)
-	-	Ginipral (0.5 mg)	Ginipral (0.5 mg)

Table 2. Parameters of the studied patients

Patient	Pregnancy period, weeks	Observed pathology
Patient 1	31	Risk of preterm birth
Patient 2	35	Risk of preterm birth
Patient 3	36	-
Patient 4	32	-

Table 3 reflects the change in the ratio of dynamic strength to the elasticity of the erythrocyte membrane for the observed patients with pathology. The norm of this value and subsequent calculated values was calculated as the average of the characteristics obtained from the studied healthy patients. The measured parameters due to a small sample have an error of no more than 10%. The presence of characteristic changes in parameters during tocolytic therapy was noted when the error of linear interpolation of the data was smaller in the absolute value of the curve fitting parameters. You can also follow the dynamics of changes in the number of active protein [18, 19] band 3 on the membrane of a single erythrocyte **(Table 3)**.

Table 3. Dynamics of changes in the ratio of dynamic strength to the elasticity of the erythrocyte membrane and the amount of protein band 3 during tocolytic therapy

Patient	Day 1	Day 2	Day 3	Day 4	Norm
The ratio of dynamic strength to the elasticity of the erythrocyte membrane					
Patient 1	16±2	22±1	18.4±0.9	26±2	18.9
Patient 2	34±4	21±2	13.4±0.7	22±2	
The amount of protein band 3, 10^6					
Patient 1	3.2±0.4	6.2±0.8	4.9±0.4	7.2±0.7	5.8
Patient 2	4.2±1	3.9±0.3	3.9±0.2	5.9±0.9	

The graphs **(Figure 1)** clearly show the change in dynamic parameters depending on the day of therapy. It can be seen that the value of the amount of band 3 protein in the first patient increased and, accordingly, as a result of this change, the ratio that determines the elasticity of the erythrocyte and the ability to deform when passing through narrow capillaries changed. This is probably due to a change in the membrane structure due to the interaction of the Mg^{2+} ion with the protein responsible for the anion exchange. There is an assumption that the Mg^{2+} cation formed from magnesium sulfate used in tocolytic therapy can activate the protein due to a change in its spatial configuration. In the second patient, there is no tendency to increase the protein on the membrane and a change in the ratio of the dynamic strength to the elasticity of the erythrocyte membrane. This suggests that the mechanism of Mg^{2+} interaction with the band 3 protein is not fully understood at the moment, since it explains the presence of the interaction of the divalent cation with the erythrocyte membrane in the first patient and the apparent absence of a regular change in the effective number of the band 3 protein in the second patient, it is necessary to carry out further research.

If the first patient had an average increase in the amount of protein, the work of which determines the process of CO_2-O_2 metabolism, and, therefore, can significantly reduce the risk of developing fetal hypoxia during pregnancy, then in the second patient this parameter remained almost stable **(Figure 1)**.

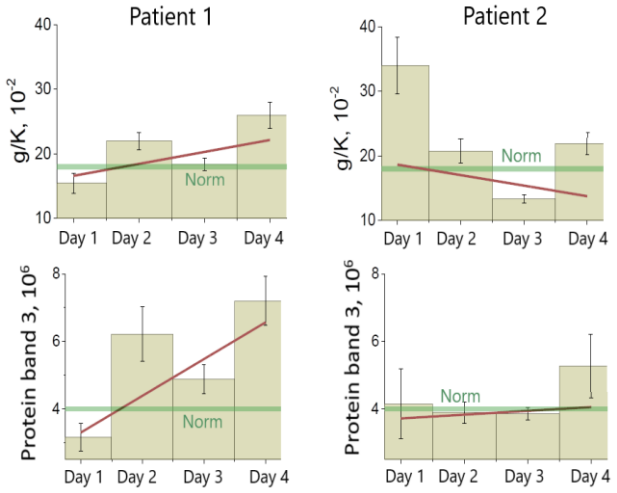

Figure 1. Dynamics of changes in the characteristics of erythrocytes (the amount of band 3 protein and the ratio of dynamic strength to elasticity of the erythrocyte membrane) during tocolytic therapy

Second, the total amount of effective band 3 protein in the test samples was analyzed. To determine the concentration of the anion exchanger, data on the concentration in erythrocyte populations obtained using a Hemolux hematology analyzer were used. **Table 4** shows the dynamics of changes in the concentration of band 3 protein (nM) against the background of tocolytic therapy.

Table 4. Dynamics of changes in the concentration of band 3 protein (nM) during tocolytic therapy

Patient	Day 1	Day 2	Day 3	Day 4	Norm
Patient 1	18	38	28	41	42
Patient 2	25	28	29	37	

Using the data **(Table 4)**, it is possible to build the dynamics of change in the concentration of band 3 protein from day to day as a result of tocolytic therapy. The values of this characteristic determine the ability to carry out the process of CO_2-O_2 exchange not on average by one erythrocyte but by the total population circulating in the body in the blood [20]. The above graphs **(Figure 2)** clearly show an increase in the number of active anion exchangers and, accordingly, an increase in the processes of exchange of 3 HCO_3^- for Cl^-, the amount of carbon dioxide removed.

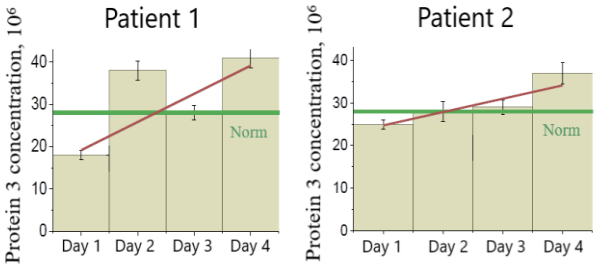

Figure 2. Dynamics of changes in the concentration of the band 3 protein during tocolytic therapy

The changes in protein concentration that occur can be represented by CO_2 consumption **(Table 5)**. And also compare the calculated removal of carbon dioxide with the consumption of oxygen. At the moment when the excretion rate is lower than consumption, oxygen is not absorbed by the cells and thus circulates in the blood without performing its vital functions.

Table 5. Changes in the rate of CO_2/O_2 metabolism in patients during tocolytic therapy

Patient	The studied indicator	Day 1	Day 2	Day 3	Day 4
Patient 1	Protein concentration of band 3, Nb3, mM	18	38	28	41
	Exchange rate, CO_2, M/h	1.4	3	2.2	3.2
Patient 2	Protein concentration of band 3, Nb3, mM	25	28	29	37
	Exchange rate, CO_2, M/h	2	2.2	2.3	3

As a result of the experiments, the sensitivity of the developed method to the registration of changes in the characteristics of erythrocytes during the tocolytic therapy applied to them was proved. The obtained changes in the characteristics of erythrocytes during therapy indicate a positive trend in patients, which is expressed in an increase in the rate of CO_2-O_2 metabolism, and therefore in a decrease in the risk of fetal hypoxia.

Conclusion

In the course of this work, the theoretical model of isotonic hemolysis was modified, which does not require taking into account the distribution functions for the parameters of erythrocytes for each patient, designed to determine the characteristic times of lysis and processing of the experiment. The method of recording the dynamic characteristics of erythrocytes made it possible to reveal changes in the rate of CO_2–O_2 metabolism in the body during tocolytic therapy. The obtained experimental data recorded a positive trend in the process of anionic HCO_3/Cl exchange in the examined patients during tocolytic therapy.

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ETHICS STATEMENT: The experiment was carried out with patients who signed an agreement for volunteer participation in the experiment. All raw data are available upon request from the corresponding author.

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