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Evaluating the Likelihood of Hypoxia in Pregnant Women through Analysis of Erythrocyte Membrane Permeability

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ABSTRACT

Approximately 11% of newborns experience hypoxia, which is a primary factor contributing to premature births, neonatal and fetal mortality, and various health complications. Fetal oxygen exchange occurs through the placenta, facilitated by erythrocytes. Consequently, fetal respiration is affected by the efficiency of erythrocyte passage through small capillaries. The elasticity, surface area, and permeability of the erythrocyte membrane directly affect the effectiveness of intrauterine respiration. This study examines various techniques for assessing the dynamic properties of erythrocyte and examines the number of anion exchange proteins present on erythrocyte membranes. This study included 4 pregnant women, 2 of whom were at risk of premature labor and received tocolytic therapy to mitigate this risk. The findings of this study show changes in the dynamic strength-to-elasticity ratio of erythrocyte membranes, variations in the levels of band 3 protein, and alterations in the CO2-O2 exchange rate during the tocolytic treatment of the pregnant participants.

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

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Introduction

Hypoxia refers to insufficient oxygen delivery to tissues or organs, and it stands as a leading cause of perinatal morbidity and mortality, accounting for up to 68% of cases. In total, approximately 11% of newborns experience fetal hypoxia, which significantly contributes to premature birth, stillbirth, and a range of health complications. Despite its prevalence, there is a lack of a standardized system for identifying the risk of hypoxia early in pregnancy.

Fetal hypoxia has a notable association with preterm labor [1]. One of the most reliable indicators for predicting preterm birth is the observation of fetal respiratory movements [2]. Early detection of breathing abnormalities in the fetus, before oxygen deprivation causes disruptions to embryogenesis, is especially critical. When detected early, treatments like tocolytic therapy (e.g., magnesium sulfate) show better success in preventing hypoxia and reducing the risk of premature delivery [3].

Fetal respiration depends on the placenta's function, aided by maternal erythrocytes [4], which are vital for oxygen transport from the lungs to body tissues and the removal of carbon dioxide. The characteristics of erythrocytes, such as their membrane permeability to bicarbonate ions, elasticity, and surface area, directly influence the rate

of oxygen-carbon dioxide exchange [5]. The flexibility of erythrocytes enables them to navigate through small capillaries, influencing fetal respiratory function. Additionally, the shape of erythrocytes impacts their ability to pass through capillary networks [6]. Despite the recognized role of erythrocytes in oxygen supply, how changes in their morphological and functional properties affect the risk of fetal hypoxia remains unclear.

Erythrocytes typically assume a biconcave disc shape, called discocytes, and lack nuclei, unlike many other cell types. Variations in erythrocyte shape, including planocytes, echinocytes, stomatocytes, and spherocytes, also exist. Additionally, reticulocytes, the immature forms of erythrocytes, make up 1-5% of the total erythrocyte count. Erythrocytes range in size, with most having a diameter of 7-8 μm (normocytes), but smaller microcytes (< 7 μm) and larger macrocytes (> 8 μm) are also present in the blood [7].

The primary role of erythrocytes is to transport oxygen and carbon dioxide, processes that require the involvement of specific proteins [8]. As the most abundant blood cells, erythrocytes influence blood rheology, with concentrations typically ranging from 4.5 to 5.5 million/μl in men and 3.7 to 4.7 million/μl in women [9]. The discocyte shape increases surface area, promoting efficient gas exchange with the surrounding environment. Additionally, this shape, coupled with the membrane and cytoskeletal structure, allows erythrocytes to maintain their flexibility as they travel through narrow capillaries [10].

The erythrocyte membrane contains a complex structure with a highly specialized receptor system. This membrane acts as a selectively permeable barrier, maintaining cellular homeostasis across varying intra- and extracellular chemical compositions [11, 12]. Transport of substances through the membrane occurs via diffusion, lipid interactions, or carrier proteins embedded within the membrane. Spectrin, glycophorin, and band 3 protein together account for about 60% of the mass of membrane proteins [13].

Spectrin is a peripheral membrane protein known for its long, thin, and flexible fibrillar structure. It is a major component of the erythrocyte cytoskeleton. When spectrin interacts with other proteins, it forms a flexible network on the inner surface of erythrocytes that aids in their passage through narrow blood capillaries. This structure enables erythrocytes to deform reversibly as they circulate through these small vessels. The ultimate membrane stretching, observed under stress, ranges from 2% to 4% of the surface area of the erythrocyte [14].

Ion permeability in erythrocytes is primarily mediated by transport proteins. Band 3, an integral membrane protein, plays a critical role in the metabolism of CO2 and O2. This glycoprotein spans the lipid bilayer multiple times, occupying about a quarter of the erythrocyte's surface area (~106 molecules per cell) [15]. Band 3 facilitates passive transmembrane transport of anions like chloride (Cl-) and bicarbonate (HCO3-) in a 1:1 ratio, forming a highly efficient ion transport system within the erythrocyte. The transfer rate for each anion by a single Band 3 protein can reach approximately 10,000 cycles per second. In contrast, the likelihood of conformational changes in the protein without binding to anions is much lower. The transfer rate is notably higher for smaller, monatomic ions (Cl-) or linear molecules (HCO3-) due to their geometry [16].

Various diseases can lead to alterations in the biophysical properties of erythrocytes. Consequently, it is essential to have methods for assessing erythrocyte characteristics to diagnose underlying pathologies and monitor the effects of treatments. Several techniques for measuring these characteristics include:

- Optical and electron microscopy
- Conductometric or impedance methods
- Flow cytometry with light scattering
- Spectrophotometric analysis
- Turbidimetric methods, using the turbidity spectrum.

This study examines various techniques for assessing erythrocyte dynamic properties and explores the number of anion exchange proteins present on erythrocyte membranes.

Materials and Methods

To analyze the kinetics of isotonic hemolysis in erythrocytes, blood samples were drawn from pregnant women aged 25 to 35 years, some with body dysfunctions, into disposable plastic tubes containing anticoagulants EDTA and sodium citrate. The study utilized an isotonic ammonium chloride solution (150 mM) as a lysing agent. A scanning flow cytometer was used for measurement, allowing the detection of light scattering patterns (indicatrix) from individual erythrocytes.

Before testing, blood samples were combined with the lysing solution at a ratio of 1:1000 (blood to buffer), ensuring optimal measurement conditions in the scanning flow cytometer within a concentration range of approximately 106 cells/mL. To calibrate the experimental data, polystyrene microspheres of 4 μm and 2 μm were added. Hemolysis tracking began immediately after pipetting to minimize dead time, and real-time light scattering signals of lysing cells were recorded as they passed through the registration area of the cytometer. Each experiment lasted between 7 and 20 minutes, and the testing was conducted at room temperature within five hours after blood collection.

Results and Discussion

Blood samples from both healthy pregnant women and those receiving tocolytic therapy were analyzed. Tocolytic therapy, designed to mitigate the risk of preterm birth, is closely linked to fetal hypoxia, which is a key factor in preterm births [17]. By tracking dynamic characteristics like the sphericity index, band 3 protein levels, and the balance between membrane strength and elasticity, the study monitors how maternal erythrocytes perform in CO_2-O_2 exchange during therapy **(Table 1)**. **Table 2** provides a detailed overview of all the research parameters assessed.

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 shows the variation in the ratio between the dynamic strength and the elasticity of the erythrocyte membrane for patients with pathology. The reference value for this ratio and subsequent calculations was determined by averaging the characteristics of the healthy control group. Due to the limited sample size, the margin of error in the measurements was kept under 10%. Distinct changes in the parameters during tocolytic therapy were observed when the linear interpolation error of the data was smaller than the absolute fitting parameters of the curve. The table also tracks the changes in the number of active band 3 proteins on the erythrocyte membrane **(Table 3)** [18, 19].

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 data visualized in **Figure 1** highlights variations in dynamic parameters throughout therapy. In the case of the first patient, a noticeable increase in band 3 protein levels was observed, which subsequently led to changes in the ratio that governs the erythrocyte membrane's elasticity and its ability to navigate through narrow capillaries. This shift could likely be attributed to alterations in the membrane's structure as a result of Mg^{2+} ions interacting with the protein involved in anion exchange. It is hypothesized that magnesium sulfate, used in tocolytic treatment,

may activate band 3 protein by modifying its three-dimensional configuration. In contrast, the second patient did not show any similar increase in membrane-bound protein or the dynamic strength-to-elasticity ratio of the erythrocyte. This suggests that the interaction between Mg^{2+} and band 3 protein is not fully understood, and the observed variations in the first patient versus the second need further exploration. While the first patient displayed an average increase in the amount of protein responsible for CO_2 and O_2 metabolism, thereby potentially lowering the risk of fetal hypoxia, the second patient's data remained nearly unchanged as shown in **Figure 1**.

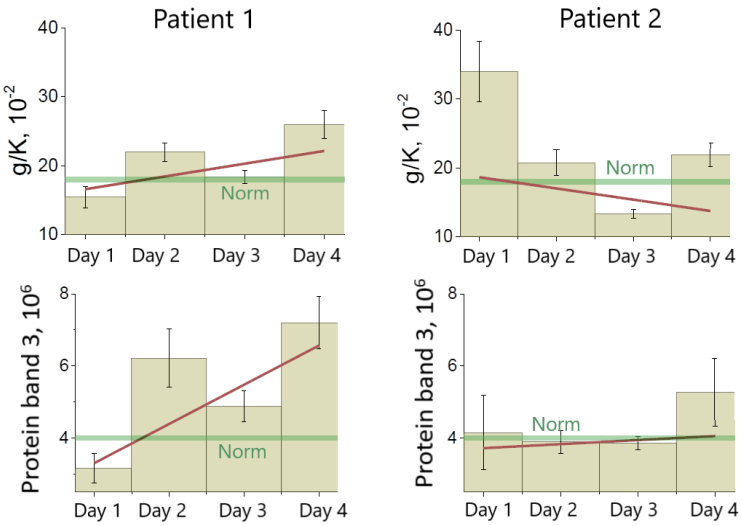

Figure 1. Dynamics of changes in the characteristics of erythrocytes (the amount of band 3 protein and the ratio of dynamic strength to elasticity of the erythrocyte membrane) during tocolytic therapy

Next, the overall quantity of active band 3 protein in the samples was evaluated. To assess the concentration of the anion exchanger, information on erythrocyte populations gathered via the Hemolux hematology analyzer was utilized. The dynamics of band 3 protein concentration (nM) during tocolytic therapy are presented in **Table 4**.

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	

The data from **Table 4** allow for tracking the changes in band 3 protein concentration throughout the days of tocolytic therapy. This concentration reflects the erythrocyte population's overall ability to facilitate CO_2-O_2 exchange, rather than focusing on individual cells [20]. The corresponding graphs **(Figure 2)** show a noticeable rise in the number of active anion exchangers, leading to an increased exchange of HCO3- for Cl-, and consequently, a greater amount of carbon dioxide being eliminated.

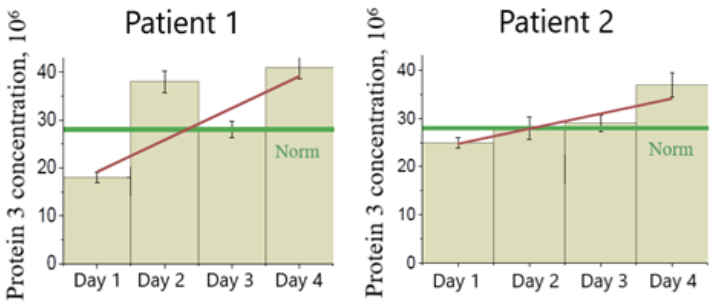

Figure 2. Dynamics of changes in the concentration of the band 3 protein during tocolytic therapy

The fluctuations in protein levels can be depicted by measuring CO2 uptake **(Table 5)**. Furthermore, a comparison between the CO2 removal rate and oxygen consumption is also possible. If the excretion rate of CO2 falls below its consumption, oxygen fails to enter the cells and remains in circulation, unable to perform its critical 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

The experiments demonstrated that the developed method is sensitive to detecting alterations in erythrocyte characteristics during tocolytic therapy. The observed changes in erythrocyte properties throughout the therapy suggest a favorable trend in the patients, reflected in an enhanced CO2-O2 metabolic rate, which consequently reduces the risk of fetal hypoxia.

Conclusion

The theoretical model for isotonic hemolysis was updated during this research, removing the necessity to consider the distribution functions of erythrocyte parameters for each patient, aiming to establish the key times for lysis and data processing. By utilizing the method to monitor the dynamic characteristics of erythrocytes, the study was able to identify changes in CO_2–O_2 metabolism rates during tocolytic treatment. The results demonstrated a positive trend in the HCO3/Cl anion exchange process among the patients undergoing the therapy.

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Conflict of Interest: None

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Ethics Statement: The research was conducted with participants who gave voluntary informed consent. Raw data are available upon request from the corresponding author.

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