MEASUREMENT OF OSMOTIC RESISTANCE OF NORMAL AND PATHOLOGICAL HUMAN RED BLOOD CELLS

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Summary: The changes in the osmotic resistance of red blood cells (RBC) due to various pathological conditions like exposure to heat, anemia as well as the effect of storage and anticoagulants have been investigated. The results have shown that exposure to a temperature of 50°C makes the red blood cells fragile to osmotic pressures. Also, red blood cells from the patients suffering from anemia exhibited decreased osmotic resistance. Storage up to 9 days showed a marginal increase in the osmotic fragility of the RBC and beyond that, there was no significant effect up to 15 days. Also, exposure to anticoagulants like citrate, ammonium oxalate, and EDTA increased the osmotic fragility of the cells. The present study shows that the various traumas investigated will affect the mechanical properties of the red blood cell membrane.

Key words: osmotic fragility anticoagulants thermal trauma storage anemia

INTRODUCTION

Red blood cells play a vital role in the carriage of oxygen, carbon dioxide and acids, which makes them indispensable for the survival of every cell and organ of the body. Red blood cells are in the shape of biconcave discoids in the unstressed state, and packed with a respiratory pigment called hemoglobin which is enclosed by a multilayered complex membrane made up of lipid liquid bilayer and protein network which provides structural rigidity to the cell. In the normal conditions, red blood cells offer little or no resistance to shape changes and hence maximum flexibility for the efficient flow of the blood in the circulation, especially in the narrow vessels. Exposure to various physical and chemical traumas such as heat, cold, hypoxia, anemia and febrile diseases etc. have been observed to produce imbalance in the cell membrane may cause hemolysis leading to the destruction of the erythrocytes (4,6,9,11,12).
The present study was undertaken to understand the effect of various factors (such as thermal trauma, anticoagulants, anemia and storage) on the red blood cell membrane using the osmotic fragility method. This study may be both clinically as well as physiologically useful in the diagnosis of various pathological disorders of the red blood cells.

MATERIAL AND METHODS

Measurement of osmotic fragility:

Whole human blood 20-30 ml was collected in the anticoagulant (i.e. heparin) from normal male volunteers through regular blood bank procedure. A standard stock solution of saline containing buffer was prepared to prevent the effect of pH on the normal osmotic fragility of red blood cells (11) and from this stock solution various diluted hypotonic saline solutions were prepared for the fragility test by using method of Parpart et al. (11). Osmotic fragility of red cells were determined by transferring 4 ml of each of hypotonic saline solutions to centrifuging tubes 0.02 ml of blood were added to each containing solutions of different osmolarity and mixed gently. The samples were then incubated at room temperature for 45 min subsequently centrifugation of tubes was done at 2000 r.p.m. for 10 min. Supernatant cell-free hemoglobin solution was then transferred carefully for determining percentage hemolysis in a photoelectric colorimeter at the wave length 540 \( \mu m \). Calibration for the percentage hemolysis was done with the same group of sample of blood by mixing 4 ml of 0.1% saline solution with 0.02 ml of blood. This reading given the value for 100 percent hemolysis and distilled water was used as blank. Results for each blood sample was plotted with saline concentration along the abscissa and the percentage hemolysis as the ordinate. The identical osmotic fragility procedure was used to measure percentage hemolysis in RBC with various traumas like anticoagulants (i.e. Citrate, Oxalate and EDTA), thermal trauma (50°C), and effect of storage.

Preparation of Blood with various Anticoagulants:

Fresh 30 to 40 ml of human blood were collected and blood sample was immediately transferred into different 5 ml sterilized glass vials containing citrate, oxalate and EDTA anticoagulants for studying the effect of anticoagulants on osmotic fragility of the red blood cells. The samples were allowed to stay at room temperature for few minutes before use in the experiments.
Preparation of Heated Red Blood Cells:

Anticoagulated whole blood (20-30 ml) were heated in the constant shaking and heating water bath by gradually increasing the temperature up to 50°C and kept for 1 min. The temperature of the blood cells was monitored continuously and never allowed to exceed 50°C. The cells were then gradually cooled to room temperature.

Preparation of Aged Blood:

30 ml whole blood in heparin as anticoagulant stored at 4°C were used for the study of the effect of length of storage on the red blood cells.

RESULTS

Since the results for the heparinized blood agreed closely with defibrinated blood (6), heparinized blood was used as the standard control anticoagulant for the study.

Both the thermally treated (50°C) and chronic hemolytic anaemic red blood cells showed significant reduction in the membrane osmotic resistance of the red blood cells. Changes can be appreciated from the values tabulated in Table I, wherein the percentage hemolysis in the various hemolysing concentration of saline solution are listed.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Blood sample type</th>
<th>Percent hemolysis in NaCl-Po₄ solution of concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>1.</td>
<td>Fresh Normal (Heparin)</td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>Thermal Trauma (50°C)</td>
<td>49</td>
</tr>
<tr>
<td>3.</td>
<td>Anaemia</td>
<td>73</td>
</tr>
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</table>

The effect of various anticoagulants on the osmotic fragility of the RBC is plotted in Fig. 1. It is observed from the plots that the various anticoagulants make the RBC more fragile, although the extent depends on the type of additive used.
Table II shows the effect of length of storage on the osmotic fragility of the RBC. To facilitate quantitative analysis, percents hemolysis in hypotonic solutions of fixed NaCl-Po4 concentrations have been plotted as a function of number of storage days (Fig. 2). Although the trend is not very pronounced, the results indicate an increase in the osmotic fragility during initial period of storage upto 9 days. Subsequently no significant effect is observed upto 15 days of storage.

![Fig. 1: Effect of anticoagulants on the osmotic resistance of red blood cells.](image)

![Fig. 2: Effect of storage on red blood cells osmotic fragility at different NaCl-Po4 concentrations.](image)
TABLE II: Effect of storage on osmotic fragility of the Red Blood Cells.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age of blood sample</th>
<th>Percent hemolysis in NaCl-Po₄ solution of concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>1.</td>
<td>0 day</td>
<td>13</td>
</tr>
<tr>
<td>2.</td>
<td>3rd day</td>
<td>23</td>
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<tr>
<td>3.</td>
<td>6th day</td>
<td>49</td>
</tr>
<tr>
<td>4.</td>
<td>9th day</td>
<td>61</td>
</tr>
<tr>
<td>5.</td>
<td>12th day</td>
<td>49</td>
</tr>
<tr>
<td>6.</td>
<td>15th day</td>
<td>44</td>
</tr>
</tbody>
</table>

DISCUSSION

Research during the past decade has firmly established the concept that the human red cells should be highly deformable in the circulation where it is subject to repeated and extensive stresses while passing through channels and openings considerably smaller than their own diameter. This property of deformability is dependent on interrelated discoid shape having a high ratio of surface area to volume and intrinsic membrane flexibility.

Cell membrane of the red cells which is a composite structure made up of lipid bilayer provides structural rigidity to the RBC (3, 10). Various factors seem to affect the shear rigidity of the red cell. The earlier investigations of Seshadri et al. (13) have shown that heating to 50°C causes loss in the deformability of red blood cells even through no noticeable change in the external size and shape of the cells had taken place. This was established by making rheological measurements in narrow tube. The present study also confirms this fact since an increase in the osmotic fragility of red blood cells can be attributed to loss in the mechanical strength of the RBC membrane. Shear rigidity of the surface of the red cells has been observed to be lost in severe burns and during exposure to high temperatures (1). Karle et al. (5) observed that rheological properties of the red blood cells get changed due to rise in temperature inducing a decrease in the plasticity of the cells, thus decreasing the ability of red blood cells to pass through the narrow passages in microcirculation. The present study also showed that in the heparinized human blood heated up to 50°C for 1 min, red blood cells exhibit less resistance to osmotic pressure.

A decrease in the osmotic resistance with various anticoagulants which has been observed in our study can be explained by the simple observation that ratio of volume to surface area (sphericity) of red blood cells increase in the presence of various chemical substances (7,8). Out of the various anticoagulants investigated, EDTA appears to have the maximum effect on the red blood cells. EDTA is a commonly used anticoagulant.
but its effect on the red blood cells has not been studied by other workers and hence no plausible explanation can be offered for this paradox.

Another phenomenon that has come to light in the present study is the decrease in the osmotic resistance of the red blood cells during the first 9 days of storage of blood at low temperature. But after 9 days, fragility becomes more or less constant up to the 15th day. It has been also observed that storage of red cells at usual temperature shows a slight increase in the hemolysis in first week but thereafter, fragility remains constant (6.9). In the present investigation decreased osmotic resistance has been observed for the blood of chronic haemolytic jaundice patients. It has been reported that increased magnitude of hemolytic crisis may be precurser of various febrile states (2,12).

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REFERENCES