Osmotic fragility of red blood cells test

Definition : is a test used to measure erythrocyte resistance to hemolysis that occurs when a sample of red blood cells (RBC) are subjected to physical stress (osmotic stress) by being exposed to varying levels of dilution of a saline solution (hypotonic solution), the sooner hemolysis occurs when osmotic fragility of RBC is greater. An osmotic fragility test is performed to diagnose two hereditary conditions thalassemia and hereditary spherocytosis, both of these genetic condition associated with RBC membrane abnormalities can cause hemolytic anemia.

Normal RBC with a biconcave shape, when RBC are placed in isotonic solution (physiological solution) 0.9% of NaCl that keep the normal shape of RBC. While being placed in hypotonic solution (<0.9% NaCl) water enters the RBC and causes swelling and expanding their volume and eventual burst (lysis) allowing hemoglobin (Hb) to exit, however in hypertonic solution (>0.9% NaCl) water exit from RBC and causes shrink.

Normal rang : hemolysis onset at 0.45 - o.5 % NaCl and complete at o.3 - 0.35 % NaCl. The older RBC(more sensitive) are hemolysized in hypotonic solution with concentration (0.40% -0.74%), while the young RBC (more resistance) are hemolysized in hypotonic solution with concentration (0.28% - 0.48%).

Factors affecting on the osmotic fragility test

The primary factor affecting the osmotic fragility test is the shape of the red cell which inturn depends on the 1- functional state of the RBC Cell membrane (permeability)

2- Surface -area to volume ratio.

Some diseases linked to Increase osmotic fragility of RBC may be due to decreased surface –area to volume ratio, RBC are more susceptible to lysis, include spherocytosis, hemolytic anemia, and older red cell are also more fragile. Spherocytosis is a disorder characterized by defective RBC membrane and decreased surface –area to volume ratio, the RBC takes in fluid it becomes more round (spherocytic) therefore follows that the spherocyte has the smallest

surface area for its volum and has increased fragility, ruptures the most quickly in less hypotonic solutions than normal RBC.

While other diseases linked to decrease osmotic fragility may be due to Increased surface –area to volume ratio: RBC are more resistant to hemolysis and has decreased fragility, the cell is capable of absorbing the more fluid before rupture .

As thalassemia, sickle cell anemia, iron deficiency anemia, liver disease and any condition associated with the presence of target cell has largest surface area amount of membrane for its size,

Functional state cell membrane

The aged erythrocyte will be hemolyzed easily hence the more healthy cell membrane the less fragile cell such as reticulocyte, polycythemia and splenectomy.

Interfering factors:

-Presence of hemolytic organisms in sample

-Severe anemia or other conditions with fewer RBC available for testing

-Recent blood transfusion

-Old sample

-The pH of blood –saline mixture is important and should be(7.4)

Material and method:

whole blood**,** normal saline (NaCl 0.9%), distilled water, test tubes and tube with anticoagulant, anticoagulated blood is used only heparin , in order to avoid adding more salts to the blood such as oxalate, EDTA or citrate.

Procedure:

- The test should be carried out within 2hrs of collection with blood stored at room temperature or within 6 hrs if the blood has been kept at 4ºC

- In the test used 11 test tubes, deliver 5ml of distilled water to the 1 tube (control), add varying concentrations of buffered sodium chloride solution put in 10 test tubes, 5ml of buffered sodium chloride solution to the 11 tube (control).
- Fresh blood (50 µl) being added to series of solutions, mixed blood and leave the suspensions for 30 min at room temperature. These samples undergo centrifugation for 5 min at 3000 rpm and absorbance reading at 540 nm by using a spectrophotometer to calculate the percentage of hemolysis for each solution

% of hemolysis = O.D of sample / O .D of tube 11 X 100

the results of the test may then be curve with the percentage hemolysis on the vertical axis and NaCl concentration on the horizontal axis

Tubes	1	2	3	4	5	6	7	8	9	10	11
D.W	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	0
NaCl Salt	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5

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