

Al-Rasheed University College Pharmacy Department Physiology & Pathology Lab. 1st Semester



Blood Grouping (ABO system)

The surfaces of erythrocytes have molecules called **antigens** and in the plasma there are proteins called **antibodies**. Antibodies are very specific, meaning that each antibody can combine only with a certain antigen. When the antibodies in the plasma bind to the antigen on the surface of the erythrocytes, they form molecular bridges that connect the erythrocytes together. As a result, **agglutination or clamping** of the cells occurs. The combination of antibodies with antigens also can initiate reaction that cause hemolysis or rupture of the erythrocytes. **The debris formed from the ruptured erythrocytes can trigger clotting within small blood vessels**. **As a result of these changes, tissue damage and death may occur. Although many blood groups are recognized, the ABO and Rh blood groups are among the most important**.



Figure (1): Categories of ABO Blood Grouping

A and B antibodies are not found in the blood until about 2 months after birth.

A **donor** is a person who gives blood, and a **recipient** is a person who receives blood. Usually a donor can give blood to recipient if they are both have the same blood type.



Figure (2): Transfusion Reaction (Agglutination) between Antigen and its Specific Antibody

Historically, **people with type O blood have been called universal donors because they usually can give blood to the other ABO blood types without causing an ABO transfusion reaction.** Their erythrocytes have no ABO surface antigens and therefore do not react with the recipient A or B antibodies.

However, **it could be noted that the term universal donor is misleading**. There are 2 circumstances in which transfusion of type 0 blood can produce transfusion reaction. **First**, mismatching blood groups other than the ABO blood group can cause a transfusion reaction. To reduce the likelihood of a transfusion reaction, all the blood groups must be correctly matched. **Second**, antibodies in the blood of the donor can react with antigens in the blood of the recipient. For example, type 0 blood has type A and B antibodies. If type 0 blood is transfused into a person with type A blood, the A antibodies (in the type O blood) react with the A antigen (in the type A blood). Usually such reactions are not serious because the antibodies in the donor blood are diluted in the blood of the recipient, and few reactions take place. Now, because type O blood can cause transfusion reaction with other blood groups; it is only considered in life threatening conditions.



Rh Blood Grouping:

Another important blood group is the Rh blood group, so named because it was first studied in the rhesus monkey. People are Rh+ if they have certain Rh antigens on the surface of their erythrocytes, and they are Rh- if they do not have these Rh antigens. The ABO blood type and Rh blood type usually are designated together. For example, a person designated as A+ is type A blood group and Rh+.

Antibodies against the Rh antigen do not develop unless an Rh-person is exposed to Rh+ blood. This can occur through a transfusion or by the transfer of blood across the placenta to mother from her fetus. When an Rh- person receives a transfusion of Rh+ blood, the recipient becomes sensitized to the Rh antigen and produces Rh antibodies. If the Rh- person unfortunate enough to receive a second transfusion of Rh+ blood after becoming sensitized, transfusion reaction results.

The Rh Blood Grouping and Pregnancy:

Rh incompatibility can pose a major problem in some pregnancies, especially when the mother is Rh- and the fetus is Rh+. If fetal blood leaks through the placenta and mixes with the mother blood, the mother becomes sensitized to the Rh antigen. The mother produces Rh antibodies that cross the placenta and cause agglutination and hemolysis of fetal erythrocytes. **This disorder called hemolytic disease of the newborn (HDN) or erythroblastosis fetalis and it can be fatal to the fetus.** In the first pregnancy there is often no problem. The leakage of fetal blood is usually the result of a tear in the placenta that takes place either late in the pregnancy or during delivery. Thus there is not enough time for the mother to produce enough Rh antibodies to harm the fetus. In later pregnancies, however, there can be a problem because the mother has been sensitized to the Rh antigen. Consequently, if the fetus is Rh+ and if any fetal blood leaks into the mother blood, she rapidly produces large amounts of Rh antibodies and HDN develops.

Prevention of HDN is often possible if the Rh-woman is given an injection of a specific type of antibody preparation called anti-Rho (D) immune globulin (RhoGAM) immediately after each delivery or abortion. The injection contains antibodies against Rh antigens. The injected antibodies bind to the Rh antigens of any fetal erythrocytes that may have entered the mother's blood. This treatment inactivates the fetal Rh antigens and prevents sensitization of the mother. If HDN develops, treatment consists of slowly removing the blood of the fetus or newborn and replacing it with Rh- blood. Exposure of the newborn skin to fluorescent light is also used **because it helps to break down bilirubin in the blood as the blood flows through the skin. High levels of bilirubin are toxic to the nervous system and can cause destruction of brain tissue.**



Figure (3): Hemolytic Disease of the Newborn

Acute Kidney Shutdown after Transfusion Reaction:

One of the most lethal effects of transfusion reactions is kidney failure, which can begin within a few minutes to few hours and continue until the person dies of renal failure. **The kidney shutdown seems to result from three causes: -**

- 1. Powerful renal vasoconstriction,
- 2. circulatory shock,
- 3. Renal tubular blockage from excessive levels of hemoglobin released from the hemolyzed RBCs.

If the shutdown is complete and fails to resolve, the patient dies within a week to 12 days, unless treated with an artificial kidney.

Principle of the Experiment:

The RBCs contain a series of antigens known as agglutinogens (antigen) on their cell membrane while the plasma contains antibodies known as agglutinins (antibody). The test antibodies react with the antigens of the blood sample to form agglutination. The slide is then observed for the presence or absence of agglutination.

Aim of the experiment:

To find out a person's exact blood grouping by the presence or absence of agglutination in the test blood sample

Procedure:

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- 1. Obtain 3 clean microscope slides. Using a glass marking pencil, mark each one with A, B and D
- 2. Lance your finger to obtain blood. Place one drop of blood on A and B slides while place 2 drops of blood on D slide
- 3. Add one drop of anti-A to the A slide. Add one drop of anti-B to the B slide and one drop of anti-D to the D slide. Mix the antiserum and blood on each slide with a toothpick, using a different toothpick for each side. Spread each mixture over an area of about three quarters in diameter. Make certain you do not mix the anti-A and anti-B antisera.
- 4. Observe the slide for any agglutination of red cells after 2 min. of mixing. Explain the antigen – antibody basis for these reactions. The strength of the agglutination reaction is not the same for every person; in some cases it may be necessary to observe the cells under the microscope to ascertain if agglutination has actually taken place, especially in the observation of Rh antigen because it is usually weaker than the other ABO antigens.



Figure (4): Agglutination Reaction in Different Blood Groups

Summary:

- 1. The antigens are found on the surface of RBCs and they are also called agglutinogens, while the antibodies are found in the plasma of blood and they are also called agglutinins.
- 2. The reaction between an antigen and its specific antibody is called agglutination.

A antigen (RBC surface) + A antibody (plasma) = agglutination

B antigen (RBC surface) + B antibody (plasma) = agglutination

- 3. The major blood groups are A, B, AB and O and they are named so due to the presence of the respective antigens on the surface of RBCs
- 4. Each blood group carries the opposite antibody and never the same like:
 Type A carries B antibodies
 Type B carries A antibodies
 Type AB carries no antibodies
 Type O carries both A and B antibodies
- 5. A Donor refers to the person who gives blood, while a Recipient refers to the person who receives blood
- 6. People with type O blood group have been called universal donors, while people with type AB blood group have been called universal recipients
- 7. The term universal donor is misleading and only should be considered in life threatening conditions because:
 - The presence of subgroups of blood groupings other than the ABO blood group that can cause transfusion reaction
 - The antibodies present in the plasma of type O blood will react with the antigens of the other blood groups, but such reaction is not serious because the antibodies of the donor (type O) are diluted in the blood of the recipient (other groups)

- 8. Rh blood group was discovered during studies on the rhesus monkey. A person with Rh-negative blood does not produce antibodies unless he/she receives blood from a person with Rh-positive
- 9. If a person with Rh-negative blood receives blood from Rh-positive then:
 - First time: there will be sensitization and production of antibodies to the Rh antigen
 - Second time: more antibodies will be produced and transfusion reaction will occur
- 10. HDN usually occurs in the second child rather than the first, especially if the mother with Rh-negative did not receive the RhOGAM injection after the first delivery (usually the injection should be given within 48-72 hr after the first delivery)
- 11. The ABO blood type and Rh blood type usually are designated together. For example, a person designated as A+ is type A blood group and Rh+.

Home Work:

- 1. What is the outcome if O+ blood is transferred to O- blood multiple times?
- 2. What will happen if O+ blood is transferred to AB- blood?
- 3. What is the outcome if plasma of O- type is transferred to person with B+ type of blood?
- 4. How does HDN occur? And how it can be prevented?
- 5. What is the outcome if O- blood is transferred to A+ blood?