HEMOGLOBIN LEVELS TESTING

A. Definition of Hemoglobin

Hemoglobin is an iron-containing oxygen-carrying metalprotein in red cells in the blood of mammals and other animals. The hemoglobin molecule consists of globin, an apoprotein and four heme groups, an organic molecule with one iron atom.

Hemoglobin has an affinity for oxygen and with oxygen it forms oxyhemoglobin in red blood cells. Through this function, oxygen is carried from the lungs to the tissues. Hemoglobin is the oxygen-carrying compound in red blood cells. Hemoglobin can be measured chemically and the amount of Hb/100 ml of blood can be used as an index of the oxygen-carrying capacity of the blood.

Hemoglobin consists of a protein-pigment complex containing iron. The complex is red in color and is present in erythrocytes. A hemoglobin molecule has four haeme groups containing ferrous iron and four globin chains. Hemoglobin is a protein compound with Fe called conjugated protein. As a core Fe and with the framework of protoporphyrin and globin (tetra phirin) cause red blood color because of this Fe.

Eryt Hb combines with carbon dioxide to form carboxyhemoglobin and is dark red in color. Arterial blood contains oxygen and venous blood contains carbon dioxide. Hemoglobin is a spherical molecule consisting of 4 subunits. Each subunit contains a heme moiety conjugated to a polypeptide. Heme is an iron-containing porphyrin derivative. These polypeptides are collectively referred to as the globin portion of the hemoglobin molecule.

B. Hemoglobin Level (Hb)

Hemoglobin level is a measure of the respiratory pigment in red blood cells. The amount of hemoglobin in normal blood is about 15 grams per 100 ml of blood and this amount is usually called “100 percent”. It is difficult to determine the normal limits for hemoglobin values for a person because hemoglobin levels vary between ethnic groups. However, WHO has set limits for normal hemoglobin levels based on age and sex.

Table 1. Limits of Hemoglobin Levels

Kelompok Umur	Batas Nilai Hemoglobin (gr/dl)
Anak 6 bulan - 6 tahun	11,0
Anak 6 tahun - 14 tahun	12,0
Pria dewasa	13,0
Ibu hamil	11,0
Wanita dewasa	12,0

C. Structure of Hemoglobin

At the center of the molecule consists of a heterocyclic ring known as a porphyrin that holds one iron atom, this iron atom is the site/local oxygen bond. Porphyrins containing iron are called heme. The name hemoglobin is a combination of heme and globin, globin as a generic term for a globular protein. There are several heme-containing proteins and hemoglobin is the best known and most studied.

In adult humans, hemoglobin is a tetramer (containing 4 submit proteins), consisting of two non-covalently bound alpha and beta subunits each. The sub units are structurally similar and are about the same size.

Each sub unit has a molecular weight of approximately 16,000 Daltons, bringing the total molecular weight of the tetramer to 64,000 Daltons. Each subunit of hemoglobin contains one heme, so overall hemoglobin has a capacity of four oxygen molecules.

D. Use Hemoglobin (Hb)

Hemoglobin in the blood carries oxygen from the lungs to all body tissues and brings back carbon dioxide from all cells to the lungs to be removed from the body. Myoglobin acts as an oxygen reservoir: it receives, stores and releases oxygen in muscle cells. Approximately 80% of the body's iron is in hemoglobin.

The uses of hemoglobin include:

1. Regulates the exchange of oxygen with carbon dioxide in body tissues.
2. Taking oxygen from the lungs and then carried to all body tissues to be used as fuel.
3. Carries carbon dioxide from body tissues as a result of metabolism to the lungs for disposal.

To find out whether a person is deficient in blood or not, it can be known by measuring hemoglobin levels. A decrease in hemoglobin levels from normal means a lack of blood which is called anemia.

E. Factors Affecting Hemoglobin Levels

Some of the factors that affect hemoglobin levels are:

1. Adequacy of Iron in the Body

Iron is needed for the production of hemoglobin, so iron deficiency anemia will result in the formation of smaller red blood cells and a lower hemoglobin content. Iron is also an essential micronutrient in producing hemoglobin which functions to deliver oxygen from the lungs to body tissues, to be excreted into the respiratory air, cytochromes, and other components of the respiratory enzyme system such as cytochrome oxidase, catalase, and peroxidase. Iron plays a role in the synthesis of hemoglobin in red blood cells and myoglobin in muscle cells. The content of ± 0.004% body weight (60-70%) is found in hemoglobin which is stored as ferritin in the liver, hemosiderin in the spleen and bone marrow.

Approximately 4% of iron in the body exists as myoglobin and iron compounds as oxidative enzymes such as cytochromes and flavoproteins. Although the number is very small, it has a very important role. Myoglobin participates in the transport of oxygen across cell membranes into muscle cells. Cytochromes, flavoproteins, and other iron-containing mitochondrial compounds play an important role in the oxidation process to produce Adenosine Tri Phosphate (ATP), which is a high-energy molecule. So if the body has iron deficiency anemia, there will be a decrease in the ability to work. In school children, it has an impact on increasing school absenteeism and decreasing learning achievement.

The recommended iron adequacy is the minimum amount of iron that comes from food that can provide enough iron for every healthy individual in 95% of the population, so as to avoid the possibility of iron deficiency anemia.

2. Iron Metabolism in the Body

Iron contained in the body of a healthy adult amounted to more than 4 grams. The iron is in red blood cells or hemoglobin (more than 2.5 g), myoglobin (150 mg), cytochrome phorphyrin, liver, spleen bone marrow (> 200-1500 mg). There are two parts of iron in the body, namely the functional part that is used for metabolic purposes and the part that is a reserve. Hemoglobin, myoglobin, cytochromes, and heme and nonheme enzymes are the functional forms of iron and are between 25-55 mg/kg body weight. Meanwhile, iron reserves when needed for physiological functions and the amount is 5-25 mg/kg body weight. Ferritin and hemosiderin are reserve forms of iron that are normally found in the liver, spleen and bone marrow. Iron metabolism in the body consists of the processes of absorption, transport, utilization, storage and excretion.

F. Method of Examination of Hemoglobin (Hb) Levels

Among the methods most often used in the laboratory and the simplest is the Sahli method, and the more sophisticated is the cyanmethemoglobin method.

In Sahli's method, hemoglobin is hydrolyzed with HCl to ferroheme globin. Ferroheme by oxygen in the air is oxidized to ferriheme which will immediately react with Cl ions to form ferriheme chloride which is also called hematin or brown hemin. This formed color is compared with the standard color (with the naked eye only). To facilitate comparison, the standard color was kept constant, which changed the color of the hemin formed. Hemin color changes are made by dilution in such a way that the color is the same as the standard color. Because the comparison is with the naked eye, subjectivity is very influential. In addition to the eye factor, other factors, such as sharpness, irradiation and so on can affect the reading results. However, for inspections in areas that do not yet have sophisticated equipment or inspections in the field, the Sahli method is still adequate and if the examination is trained late, the results can be relied upon.

A more sophisticated method is the cyanmethemoglobin method. In this method, hemoglobin is oxidized by potassium ferrocyanide to methemoglobin which then reacts with cyanide ions to form cyan-methemoglobin which is red in color. The color intensity is read with a photometer and compared with a standard. Because those who compare electronic devices, the results are more objective.

HEMOGLOBIN EXAMINATION WORKSHEET (Hb)

1. **Practical Purpose**

   Students are able to understand about hemoglobin and are able to measure hemoglobin levels in the blood.

2. **Practical Tools**

   Standard Sahli Hemometer.

   Pipette HB 20 l.

   Drop pipette.

   Stirring rod.

   Haemometer Diluent Tube

3. **Material**

   Hcl 0,1 N

   Aquadest

4. **How to Check Hb Sahli**

   a. Put 5 drops of 0.1N HCl into the hemometer diluent tube.
   b. Suck blood (capillary, EDTA/Oxalate) with an Hb pipette up to the 20µl mark line. Remove blood adhering to the outside of the pipette tip.
   c. Record the time and immediately drain the blood from the pipette into the bottom of the diluent tube containing 0.1 N HCl earlier. Do not let air bubbles occur.
   d. Lift the pipette slightly, then suck the clear 0.1 N Hcl into the pipette 2-3 times to clean the blood that is still left in the pipette.
   e. Mix the contents of the tube so that the blood and HCl are combined; mixed color to dark brown.
   f. Add aquadest drop by drop, stirring with a stirring rod. Comparison of the mixed color with the standard color should be achieved within 3-5 minutes after mixing the blood and HCl. At the time of equalizing the color of the tube is rotated until the dividing line is not visible.
   g. Read Hb levels in grams/100 ml of blood.

5. **Nilai Normal**

Adult male 13 g %	Child : 6 mth-6 yr 11 g%
Non-pregnant women 12 g %	6 yrs-14 yrs 12 g %
Pregnant women 11 g %	

6. Hemoglobin Examination Result

Proband's name		
Age		
Gender		
Checkup result		
Status	Normal/Not Normal	Normal/Not Normal

7. Discussion

a. What is Hemoglobin?

b. What are the results of the examination of Hb levels in this practicum proband? Is it normal? What is the normal value for Hb levels in the proband?

c. What are the causes of abnormal Hb levels? Explain!

PRACTICE

ERYTROCYTE SEDIMENTATION RATE EXAMINATION

Definition of ESR

Sedimentation rate is the rate of sedimentation of erythrocytes from a blood sample that is examined in a certain tool which is expressed in mm/hour. ESR is often also termed in foreign languages BBS (*Blood Bezenking Snelheid*), BSR (*Blood Sedimentation Rate*), ESR (*Erythrocyte Sedimentation Rate*) and in Indonesian it is KPD (Blood Sedimentation Rate).

The process of blood deposition occurs in 3 stages, namely the rouleaux formation stage, the deposition stage and the compaction stage. In the laboratory, the methods to check the ESR that are often used are the Wintrobe method and the Westergren method.

In the Wintrobe method, the reference value for women is 0-20 mm/hour and for men 0-10 mm/hour, while in the Westergren method the reference value for women is 0-15 mm/hour and for men 0-10 mm/hour.

Factors that can affect the erythrocyte sedimentation rate (ESR) are erythrocyte factors, plasma factors and engineering factors. The number of erythrocytes/ul blood that is less than normal, erythrocyte size that is larger than normal and erythrocytes that are easy to agglutinate will cause a fast ESR. Rouleaux formation depends on the plasma protein composition. Increased levels of fibrinogen and globulins facilitate the formation of roleaux so that the ESR is fast, while high levels of albumin cause the ESR to be slow.

ESR phases:

1. The first phase (rouleaux formation phase)

   In this phase, the rouleaux formation occurs, where the erythrocytes begin to unite with each other. The time required is from a few minutes to 30 minutes. The presence of macromolecules with high concentrations in plasma, can reduce the mutual repulsion between erythrocyte cells, and cause erythrocytes to be more easily attached to one another, thus facilitating the formation of rouleaux. Rouleaux are clumps of erythrocytes that occur not due to antibodies or convalent bonds, but due to mutual attraction between cell surfaces. When the ratio of globulin to albumin is increased or the fibrinogen level is very high, rouleaux formation is facilitated until the ESR increases.
2. Second phase (rapid deposition phase)

   This phase is also calESR the maximum deposition phase, because there has been aggregation or the formation of rouleaux or in other words the erythrocyte particles

become larger with a smaller surface so that their deposition becomes faster. The rate of deposition in this phase is constant. The time is 30 minutes to 120 minutes.

3. Third phase (slow depositional/solidification phase)

   This phase occurs very slowly deposition of erythrocytes. Under normal circumstances it takes half an hour to an hour to reach the third phase. This erythrocyte deposition is referred to as the erythrocyte sedimentation rate and is expressed in mm/1 hour.

Factors Affecting ESR

1. Erythrocyte factor

   The most important factor that determines the rate of erythrocyte deposition is the size or mass of the sediment particles. In some diseases with impaired plasma fibrinogen and globulin, which can cause changes in the erythrocyte surface and an increase in ESR, the ESR is inversely related to plasma viscosity.

2. Plasma factor

   Some plasma proteins have a positive charge and cause the surface charge of the erythrocytes to be neutral, this causes the erythrocyte repulsion force to decrease and accelerates the occurrence of erythrocyte aggregation or deposition. Several acute phase proteins contribute to aggregation.

3. Technical and mechanical factors

   The most important factor in checking the ESR is that the tube must be completely perpendicular, changing and causing an error of 30%. In addition, during inspection the tube rack should not vibrate or move. The length of the inner diameter of the ESR tube also affects the inspection results.

Factors that enhance ESR

1. The number of erythrocytes is less than normal

   The size of the erythrocytes is larger than the normal size, so it's easier or faster forming a rouleaux, so the ESRs can increase.

2. Increased levels of fibrinogen in the blood will accelerate the formation of rouleaux, so the ESR can increase.
3. Test tube shaking/vibrating will accelerate deposition, ESR may increase.
4. The temperature during inspection is higher than the ideal temperature (>20ºC) will accelerate the precipitation, so that the ESR can increase.

Factors that lower ESR

Severe leukocytosis, polycythemia, protein abnormalities (hyper-viscosity), technical factors (dilution problems, blood clotting, short ESR tube, vibration on examination).

ESR is found to be increased during the inflammatory process/acute inflammation, acute and chronic infections, tissue damage (necrosis), collagen disease, rheumatoid, malignancy, and physiological stress conditions (e.g. pregnancy).

A fast erythrocyte sedimentation rate (ESR) indicates an active lesion, an increase in ESR) compared to before indicating an extensive process, while a decreased erythrocyte sedimentation rate (ESR) compared to before indicates an improvement. In addition to pathological conditions, rapid ESR can also be found in physiological conditions such as during menstruation, pregnancy after the third month and in the elderly.

ESR Inspection with the Westergren Method

1. Anticoagulants

   In determining the ESR, non-clotting blood is required, so anticoagulants are usually used. The anticoagulant used is 3.8% Na citrate.

2. ESR Inspection Principle

   Blood mixed with anticoagulants was put into a Westergren tube and allowed to stand at room temperature and in an upright position for one minute, then the erythrocytes would settle to the bottom of the tube and the plasma left behind.

3. ESR Measurement

   There are two methods used in measuring ESRs, namely macro and micro. On a macro basis, the crista method (Hellige volmer) and the landau method. Both methods are not very popular in Indonesia. The Westergren method got a higher value, this was because the Westergren pipette was almost twice the length of the Wintrobe pipette.

   The reading of the Westergren method is seen by the length of the plasma column above the erythrocyte pole by paying attention to several things, namely the color of the plasma above the erythrocytes, the clarity of the plasma for example becoming cloudy due to hyperlipemia, the layer of leukocytes in the erythrocyte column will increase by leukocytosis and leukemia, sharp boundaries between blood and plasma which is not sharp by anisocytosis

   It is very important to place the pipette or ESR tube in an upright position, a small difference from the vertical line can have a big impact on the ESR results.

ESR Check Error

1. The presence of clots in the blood can cause the ESR to be incorrect.
2. Air bubbles in the tube causing an error.
3. ESR tube tilt.

EDICATION RATE PRACTICUM WORKSHEET

1. **Purpose**

   Students can find out how to determine the erythrocyte sedimentation rate using the westergreen method on probandus blood.

2. **Tools**

   - Westergreen or Wintrobe pipettes and their pipettes
   - 10 ml test tube
   - Westergreen Shelf
   - push ball

3. **Material**

   - Venous blood with certain anticoagulants.
   - Na Citrate 3.8%

4. **How to Check the Erythrocyte Sedimentation Rate with Westergren**

   - Suck in a syringe (inject) 0.4 ml of 3.8% sodium citrate solution.
   - Perform a venipuncture with the syringe and aspirate 1.6 ml of blood to obtain 2 ml of the mixture.
   - Put the mixture into the tube and mix well.
   - Suck the blood into the Westergreen pipette to the 0 mm mark then leave the pipette in an upright position on the Westergreen rack for 60 minutes.
   - Read the plasma layer height in millimeters and report that number as an ESR.

5. **Normal Value**

   - Male = up to 10 mm/hour I
   - Female = up to 15 mm/hour I

6. **Results of the Erythrocyte Sedimentation Rate**

Probandus name		
Age		
Gender		
Checkup result		
Status	Normal/Not Normal	Normal/Not Normal

7. **Discussion**

- What is meant by erythrocyte sedimentation rate?

- What are the results of the examination of ESR levels in this practicum proband? Is it normal? What is the normal value of the ESR examination at the proband?

- What are the causes of abnormal ESR levels? Explain how the mechanism!