University of Al-Ameed
College of Pharmacy

physiology

Hemoglobin Determination

Asist. lec. Doaa aljeml

Objectives

Purpose of this test ?

As part of a complete blood count (CBC), during a health checkup, or when a healthcare practitioner suspects that you have a condition such as anemia or polycythemia.

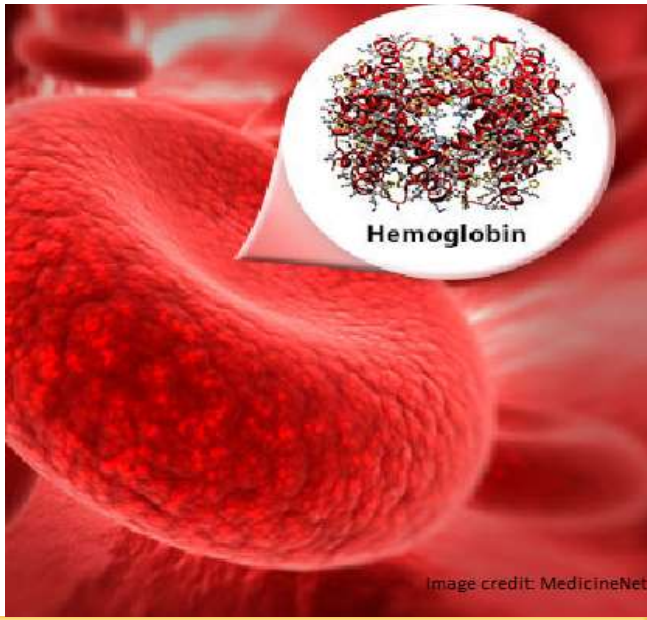
By the end of this lab, you should have useful information about the structure of Hb, its functions and types, methods for determination Hb value, and clinical importance of Hb .

Hemoglobin

Hemoglobin is an important component of red blood cells (RBCs). is the iron-containing oxygen transporter (metalloprotein) in the red blood cells of humans. It gives the blood its red color.

- Function of hemoglobin :

It is responsible for carrying oxygen to different parts of the body and carbon dioxide for excretion out of the lungs. ▪

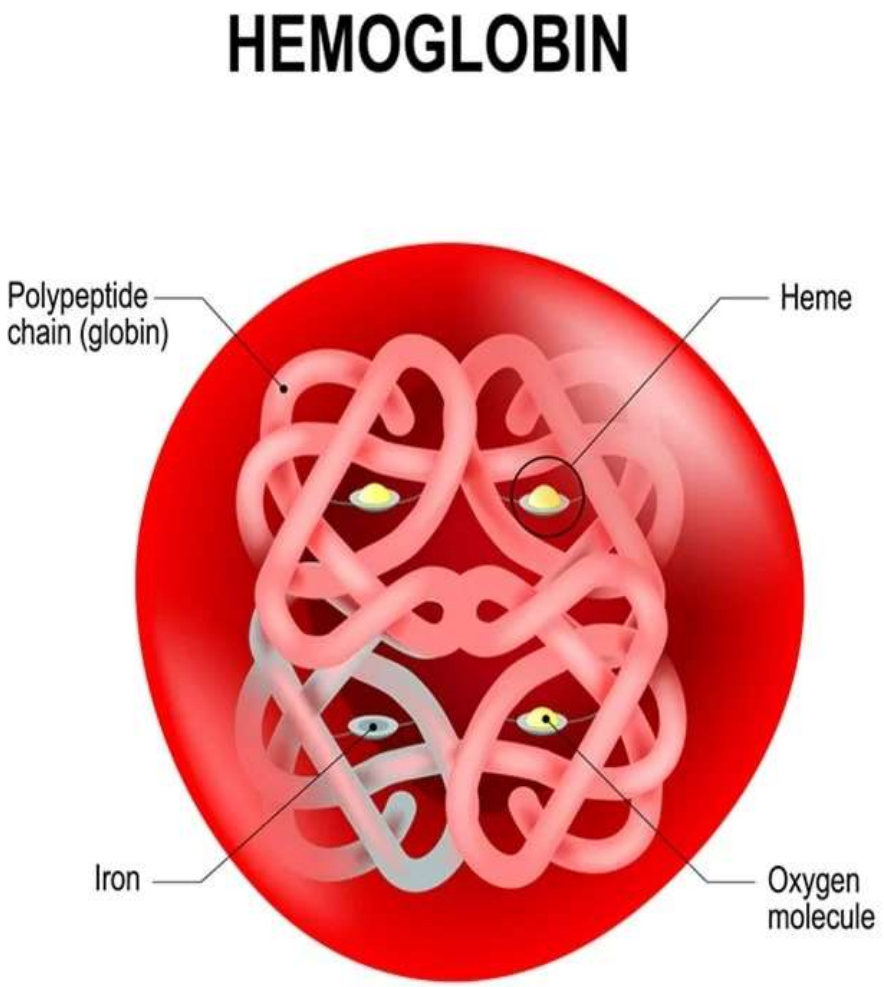


This protein (hemoglobin) is also responsible in keeping red blood cells in their normal disc shape

Structure

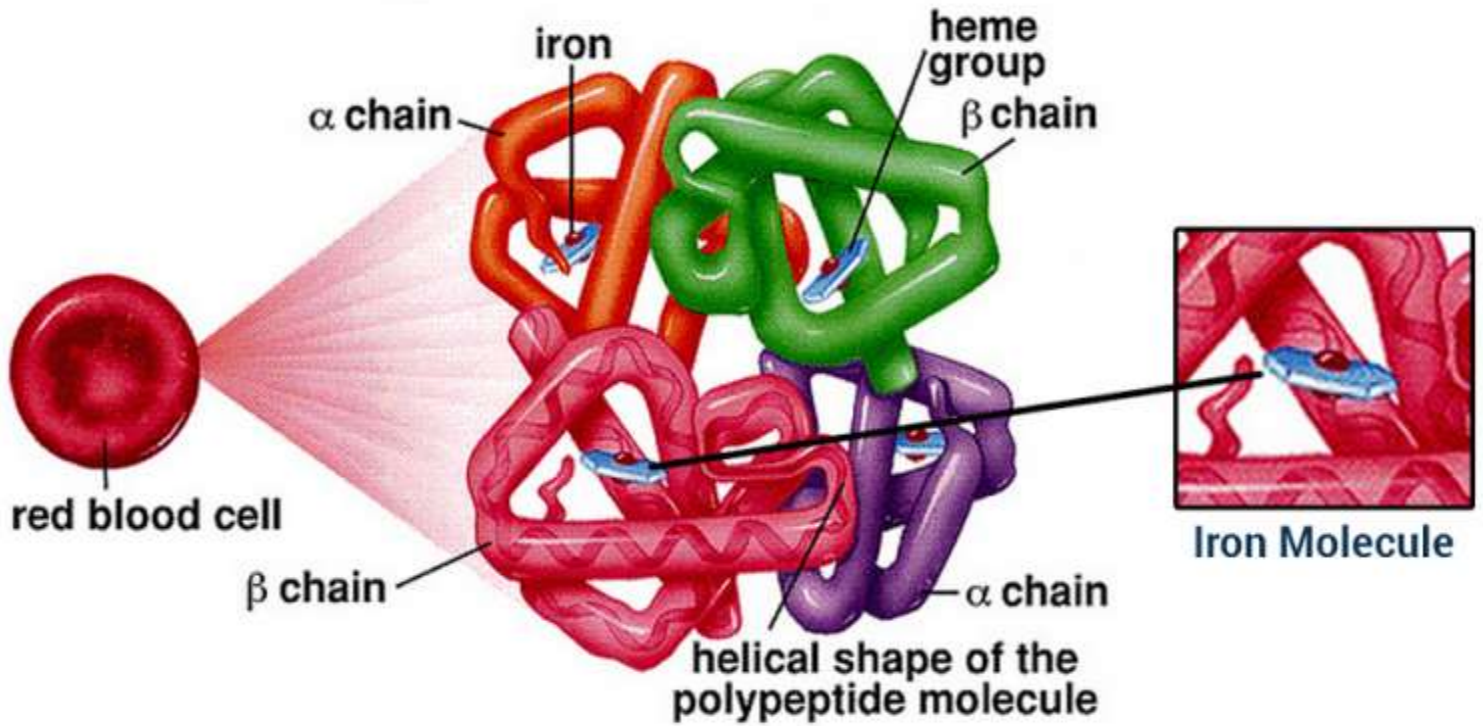
Hemoglobin molecule is a tetramer consisting of two pairs of similar polypeptide chains called globin chains.

To each of the four chains heme is attached which is a complex of iron in ferrous form and protoporphyrin. It gives red color to the blood.



Structure

The major (96%) type of hemoglobin present in adults is called HbA and it has 2 alpha globin chains & 2 beta globin chains (α2β2)



Normal hemoglobin types include:

- **Hemoglobin A1 (Hb A1 or Hb A):** makes up about 95%-98% of hemoglobin found in adults; it contains two alpha (α) chains and two beta (β) protein chains.
- **Hemoglobin A2 (Hb A_2):** makes up about 2%-3% of hemoglobin found in adults; it has two alpha (α) and two delta (δ) protein chains.
- **Hemoglobin F (Hb F, fetal hemoglobin):** makes up to 1%-2% of hemoglobin found in adults; it has two alpha (α) and two gamma (γ) protein chains. It is the primary hemoglobin produced by the fetus during pregnancy; its production usually falls shortly after birth and reaches adult level within 1-2 years.

Forms of Hemoglobin

- 1 Oxyhemoglobin
- • Hb combined with O2. Each of the 4 iron atoms in Hb molecule can
- bind reversibly to one O2 molecule. The iron stays in the ferrous state,
- so that the reaction is oxygenation not oxidation.
- 2 Carbaminohemoglobin
- • Hb combined with CO2.

Forms of Hemoglobin

3 Carboxyhemoglobin

Hb combined with CO. A concentration of about 0.5% carboxyhemoglobin is produced by the normal degradation of Hb. Slightly increased level can be found in the smoker's blood and due to environmental pollution.

Forms of Hemoglobin

4-Methemoglobin

A type of Hb in which the iron in the heme group is in ferric (fe+3) state , not ferrous (fe+2) of normal Hb . Some oxidation of Hb to MetHb occurs normally but an enzyme called MetHb reductase in RBC converts MetHb back to Hb. The congenital absence of that enzyme is one cause of hereditary Methemoglobinemia.

5 Sulfhemoglobin

- Hb containing sulfar and is unable to transport O2. It is usually formed by certain oxidizing drugs.

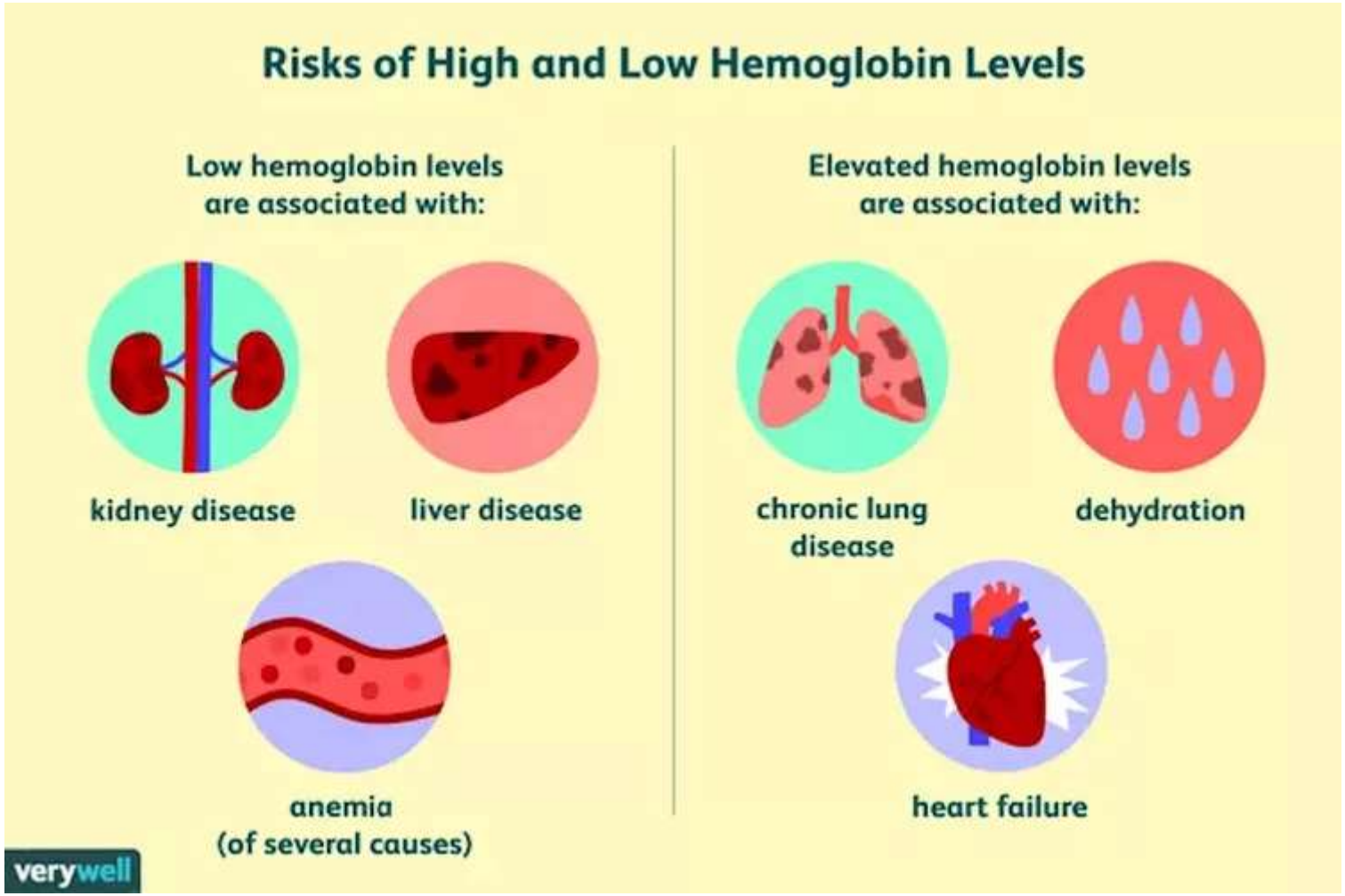
Normal Values:

Adult males: 14–18 g/dL .

Adult females: 12–16 g/dL.

Newborn 21 g/dL.

Risk


Risks of High and Low Hemoglobin Levels
Low hemoglobin levels are associated with:
kidney disease
liver disease
anemia (of several causes)
Elevated hemoglobin levels are associated with:
chronic lung disease
dehydration
heart failure
verywell

Medical conditions:

Anemia :-is a decrease of hemoglobin concentration.

Polycythemia:-is an increase of hemoglobin concentration.

Conditions that decrease hemoglobin concentration

Physiological	Pathological
- Pregnancy - Children - Women	- Different types of anemia. - After severe hemorrhage. - Hemolysis due to transfusion of incompatible blood, reactions to chemicals and drugs, bacteremia, and artificial heart valves. - Variety of systemic diseases e.g., leukemia, lymphoma, kidney disease, cirrhosis, hyperthyroidism, and systemic lupus erythematosus (SLE).

Conditions that increase hemoglobin concentration

Physiological	Pathological
- High altitudes. - Newborns and infants - Excessive sweating (hemoconcentration)	- Haemoconcentration states of blood (due to loss of body fluid), e.g., severe burns, severe diarrhea, and vomiting. - Polycythaemia vera. - Chronic obstructive pulmonary disease (COPD) - Congestive heart failure (CHF).

Apparatus and chemicals used for measuring hemoglobin:

- Sahli's tube which is having red and yellow scales on two sides.

Red scale is percentage scale and yellow scale is gram percentage or g/100ml scale.

- Heamometer which is having two standards.
- Sahli's pipette.
- Error percentage is 3% why ???

Apparatus and chemicals used for measuring hemoglobin:

- Chemical:

- Hydrochloric acid (HCL) N/10
- Distilled water

Procedure

The haemoglobin tube was filled with N/10 hydrochloric acid (HCL) up to 2 gm marking.

This graduated tube was placed in Sahli's Hemoglobinometer (Comparator with Brown glass).

1-Blood sample obtained from capillary or venous blood was drawn in Sahli's pipette up to 20µl mark .

2- add in haemoglobin tube containing N/10 HCL. The blood and acid are mixed with glass stirrer and allowed to stand for 5 minutes for acid haematin formation.

Procedure

3-Drop by drop distilled water was added to dilute the acid haematin compound color till it matches with the standard color plates of the comparator. Results were read as gm/dl presented on the haemoglobin tube.

Thank You