Hematopathology Dr. Khalid Abdulkhadim Altaii
4th Year Lecture 1

Jaundice

Definition

Jaundice (Kernicterus) is the yellowish discoloration or pigmentation
of the skin , white of the eyes , other mucous membranes and body fluids
, caused by hyperbilirubinemia (increased levels of bilirubin in blood).
This hyperbilirubinemia subsequently causes increased levels of bilirubin
in extracellular fluid. The concentration of bilirubin in plasma is normally
below 1.2 mg/dl, Jaundice is typically seen when bilirubin concentration
in plasma exceeds 2mg/dl , i.e when a pathological process interferes
with the normal functioning of the metabolism and excretion of bilirubin .
It is often seen in liver diseases , such as hepatitis or liver cancer. It may
also indicate obstruction of biliary tract, for example by gallstones or
pancreatic cancer. Jaundice may also arise from increased break down of
red blood cells or from inherited changes in bilirubin metabolism.

Bilirubin and bilirubin cycle

Jaundice itself is not a disease , but rather it a sign of one of many possible underlying pathological processes that occur at some point along
the normal physiological pathway of the metabolism of bilirubin in blood.
When red blood cells complete their life span of approximately 120 days ,
or when they are damaged. As each red blood cell traverses through the
reticuloendothelial system (mainly the spleen), it's cell membrane ruptures when this membrane is fragile enough to allow this. Cellular
components , including hemoglobin , are released into the blood stream.

The hemoglobin is phagocytosed by macrophages , and split into it's
heme and globin portions. The globin portion degraded into amino acids
and plays no role in jaundice. The breakdown of heme from the red cells
liberates iron for re-circulation via plasma transferrin to bone marrow
erythroblasts , and protoporphyrin which is broken down to bilirubin by
two reactions , the first reaction is catalyzed by heme oxygenase enzyme
and results in biliverdin (green color pigment) , and the second step is the
reduction of biliverdin to a yellow color pigment called bilirubin by
biliverdin reductase enzyme. This bilirubin is unconjugated ," free" , or
"indirect" bilirubin.
The unconjugated bilirubin , which is insoluble in water, then travels to
the liver through blood stream bound to plasma albumin. Once it arrives
to the liver , it is conjugated with glucoronic acid to form bilirubin
diglucuronide or just "conjugated bilirubin" to become water soluble.
The conjugated bilirubin is excreted from the liver into the biliary duct as
part of bile , and thus secreted out into small intestine (duodenum) to aid

in the digestion of fats. Intestinal bacteria convert the bilirubin into
urobilinogen. From here , urobilinogen can take two pathways. It can
either be further converted to stercobilinogen , which is then oxidized to
stercobilin and passed out in the faeces , or it can be reabsorbed by the
intestinal cells , transported in the blood to the kidneys , and passed out in
the urine as the oxidized product urobilin. Stercobilin and urobilin are the

products responsible for the coloration of faeces and urine respectively.

Types of pathological jaundice

Hematopathology Dr. Khalid Abdulkhadim Altaii

4th Year Lecture 1

Jaundice is classified into three categories , depending on which part of
the physiological mechanism is affected:

Category	Definition
Pre-hepatic	The pathology is occurs prior to the liver
Hepatic	The pathology is located within liver
Post-hepatic	The pathology is located after the conjugation of bilirubin in the liver

1. **Pre-hepatic jaundice (hemolytic jaundice) :**

It is caused by anything which causes an increased rate of hemolysis
(break down of red blood cells). Unconjugated bilirubin comes from the
breakdown of the heme part found in red blood cell's hemoglobin. The
increased breakdown of erythrocytes leads to an increase in the amount of
unconjugated bilirubin present in the blood and deposition of this
unconjugated bilirubin into various tissues can lead to a jaundiced
appearance. Malaria can also cause jaundice in this manner. Certain
genetic diseases , such as sickle cell anemia , spherocytosis , thalassemia
and G.6.P.D deficiency can lead to hemolytic jaundice. Commonly ,
kidney diseases such as hemolytic uremic disease may also cause
pre-hepatic jaundice. Defects in bilirubin mechanism also cause jaundice
as in Gilbert's syndrome , which is a mild inherited condition associated
with decreased bilirubin conjugation due to a decrease in enzyme activity
and Crigler-Najjar syndrome , which is also an inherited condition that
may lead to severe unconjugated hyperbilirubinemia.

In jaundice secondary to hemolysis , the increased production of bilirubin

leads to the increased production of urine urobilinogen.

Lab. findings include:

Urine: no bilirubin present , urobilinogen > units

Serum: increased unconjugated bilirubin

Kernicterus is associated with increased unconjugated bilirubin : neonates
are especially vulnerable to this due to increase permeability of the blood
brain barrier.

Enzyme pattern is not affected

2. **Hepatic jaundice (hepatocellular):**

It can be caused by acute or chronic hepatitis , hepatotoxicity , cirrhosis
, drug-induced hepatitis and alcoholic liver disease. Cell necrosis reduces
the liver's ability to metabolize and excrete bilirubin leading to a buildup
of unconjugated bilirubin in the blood. Other causes include primary
biliary cirrhosis leading to an increase in plasma conjugated bilirubin
because there is impairment of excretion of conjugated bilirubin into the

bile. The blood contains abnormally raised amount of conjugated bilirubin and bile salts which are excreted in the urine.

Lab. findings depend on the cause of jaundice:

Serum total , unconjugated and conjugated bilirubins are increased

Urine: conjugated bilirubin present , urobilinogen is decreased.

Kernictirus is not associated with increased conjugated bilirubin

Plasma proteins show characteristic changes

Plasma albumin level is low but globulins are raised due to an increased
formation of antibodies.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)
are increased

3. **Post-hepatic jaundice:**

Also called **obstructive jaundice** , is caused by any interruption to the
drainage of bile in the biliary system. The most common causes are

gallstones in the common bile duct and pancreatic cancer in the head of
pancreas , causing obstructive jaundice. Other causes include strictures of
the common bile duct such as ductal carcinoma , pancreatitis and a group
of parasites known as liver flukes.
In case of complete obstruction of the bile duct , no urobilinogen is found
in the urine , since bilirubin has no access to the intestine and it is in the
intestine that bilirubin gets converted to urobilinogen to be later released
into the general circulation. In this case, presence of bilirubin (conjugated) in the urine without urine-urobilinogen suggests obstructive
jaundice , either intra-hepatic or post-hepatic.
The presence of pale stools and dark urine suggests an obstructive or
post-hepatic cause as normal faeces get their color from bile pigments.
Serum alkaline phosphatase (ALP) is increased in post-hepatic jaundice.

Table of differential tests among types of jaundice

Function test	**Pre-hepatic**	**Hepatic**	**Post-hepatic**
Total bilirubin	Normal/Increased	Increased	Increased
Conjugated bilirubin	Normal	Increased	Increased
Unconjugated bilirubin	Increased	Normal/Increased	Normal
Urine colour	Normal	Dark	Dark
Urine urobilinogen	Increased	Normal	Normal
Stool colour	Normal	Normal	Pale
Alkaline phosphatase	Normal	Normal	Increased
ALT & AST	Normal	Increased	Normal

Complications of jaundice:

Complications include sepsis especially cholangitis , biliary cirrhosis ,
pancreatitis , coagulopathy , renal and liver failure**.**

Neonatal physiologic jaundice

It is a yellowing of the skin and other tissues of a newborn infant. A bilirubin level of more than 5 mg/dl manifests clinical jaundice in neonates. Neonatal jaundice is usually harmless and often seen in infants
around the second day after birth , lasting until day 8 in normal births , or
around day 14 in premature births. Serum bilirubin normally drops to a
low level without any intervention required. Most infants develop visible
jaundice due to elevation of unconjugated bilirubin concentration , which
accumulates due to increased red cell destruction and inability of underdeveloped liver to metabolize it. In extreme cases , a brain damaging condition known as kernicterus can occur , leading to significant lifelong disability due to the passage of bilirubin through the
infant's blood-brain barrier.

A bili light (phototherapy) is often the tool used for early treatment , which often consists of exposing the baby to intensive phototherapy
(ultraviolet light) to decrease bilirubin concentration. Phototherapy works
through a process of isomerization that changes non-water soluble bilirubin into water soluble , which can be excreted from the kidneys.

Bilirubin is also lowered through bowel movements and urination , so
regular and proper feedings are especially important.

Sunbathing is effective treatment , and has the advantage of ultra-violet-B
, which promotes Vitamin D production.

Exchange transfusions depend on the health status and age of the newborn. It should , however be used for any newborn with a total serum
bilirubin of greater than 25 mg/dl.

Pathological jaundice of neonates

Any of the following features characterizes pathological jaundice:

1. Clinical jaundice appearing in the first 24 hours.
2. Increases in the level of total bilirubin by more than 0.5 mg/dl per
hour or 5 mg/dl per 24 hours.
3. Total bilirubin more than 19.5 mg/dl.
4. Direct (conjugated) bilirubin more than 2 mg/dl.

Causes of pathological neonatal jaundice:

Many other causes can lead to pathological jaundice in newborns such
as sepsis , hypothyroidism , Gilbert-Najjar syndrome , hepatitis B , galactosemia , biliary atresia , hereditary hemolytic anemias , breast milk
feeding and hemolytic disease of newborn (HDN).

Hematopathology Dr. Khalid Abdulkhadim Altaii
4th Year Lecture 1

Breast feeding jaundice

Caused by insufficient breast milk intake , resulting in inadequate quantities of bowel movements to remove bilirubin from the body. This
can be improved by frequent breastfeeding sessions of sufficient duration
to stimulate adequate milk production

.

Hematopathology Dr. Khalid A. Hadi Dept. of Medical Lab. Technology 3id Year Lecture 5

Hemoglobinopathies

Sickle Cell Anemias

Hemoglobinopathies are genetic defects that result in abnormal structure of one of the globin chains of the hemoglobin molecule. Most of the hemoglobinopathies are not clinically apparent, others produce asymptomatic abnormal hematologic laboratory findings, while a very few produce serious disease. The most well known examples of hemoglobinopathies are sickle cell anemias and thalassemias.

Sickle Cell Anemias

It is an inherited disorder that leads to the production of hemoglobin S (HbS) , an abnormal hemoglobin variant. Hemoglobin S production arises from an altered (mutated) gene. A person who has one normal gene copy and one altered copy (heterogenous) will produce about 20 to 40% hemoglobin S , but he will produce enough hemoglobin A (the normal hemoglobin) , that he does not generally experience any health problems.

This single altered copy is called sickle cell trait , and it can be passed on to a person's children.
When a person has two copies of the altered gene (homozygous) , he produces no normal hemoglobin A , but rather 80 to 100% hemoglobin S and he will have sickle cell anemia.
In sickle cell patients , the abnormality is due to substitution of the amino acid valine for glutamic acid in position 6 of the β chain. This will cause the hemoglobin to become insoluble and form crystals when exposed to low oxygen tension , so the red cells become crescent-shaped or sickle-shaped which makes them rigid , pointed and sticky.
Sickle cell genetic alteration or mutation is found predominantly in people of African ancestry , but may be found in Mediterranean area , South and Central America , the Middle East , India and the Caribbean.

Clinical features of sickle cell anemia

Sickle cell disease is extremely variable. Some patients have very few difficulties , and the disease is discovered accidentally , while other patients have multiple complications.
Signs and symptoms of sickle cell anemia include pallor , jaundice , fever , chest pain , and shortness of breath. Leg ulcers can also occur in sickle cell anemia in addition to inflammation of the fingers (dactylitis).
One of the more frequent complications is called ""sickle cell painful crisis"" and consists of pain in the bones , particularly the long bones. The painful crisis or episode is promoted by certain factors , such as decreased

oxygen , infection , dehydration , high altitudes , fever and cold. This pain appears to arise from blocking of blood vessels , and may cause damage to them by sickle cells , which stick to those vessels (vasoocclusion). Other complications of the disease may include organ damage such as spleen , kidneys and eyes.

Laboratory diagnosis of sickle cell anemia

Like other types of hemolytic anemias , there will be severe decrease in hemoglobin and red cell count. Increased unconjugated serum bilirubin with reticulocytosis.

Blood film may show sickle red cells , target cells and Howell Jolly bodies.

The sickle cell test may show the sickling of the red cells after their incubation with a reducing agent , but this test can not tell us the type of the disease i.e {sickle cell trait(SA) or disease(SS)}.

The specific test which can confirm the diagnosis is the hemoglobin electrophoresis , a test that measures the types of hemoglobin and detects the presence of abnormal HbS among all other hemoglobins , with the presence of 5-15% HbF.

Hemoglobin C (HbC) Disease

It is also an abnormal hemoglobin with substitution of lysine for glutamic acid in the position 6 of the β globin chain . this mutated form reduces the normal plasticity of red cells. In patients who are heterozygous for the mutation , about 28-44% of the total hemoglobin is HbC and no anemia develops. In homozygous patients , nearly all the hemoglobin is in the HbC form , resulting in normocytic hemolytic anemia.

Hematology Asst. proof Dr. Khalid .A. HADI
3id Year Lecture

Hemoglobinopathies

Thalassemias

Normal hemoglobins

HbA : 2αchains + 2β chains … 97% of normal adult Hb

HbA_2 : 2αchains + 2δ chains … 1-3.5% of normal adult Hb

HbF : 2αchains + 2γ chains … <1.5% of normal adult Hb , but 70% of fetal and neonate Hb

Thalassemias are a group of congenital (inherited) , autosomal recessive disorders in which there is a defect in the synthesis of one or more of the globin subunits of hemoglobin. There are four genes that code for alpha globin chains and two genes that code for the beta globin chains.

The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α-thalassemia , production of the α globin chain is affected , while in β-thalassemia , production of the β globin chain is affected.
β globin chains are encoded by a single gene on chromosome 11 , while α globin chains are encoded by two closely linked genes on chromosome 16. Thus , in normal person with two copies of each chromosome , there are two loci encoding the β globin chain , and four loci encoding the α globin chain. Deletion of one of the α loci has a high prevalence in the people of African-American or Asian descent , making them more likely to develop α thalassemias. The α-thalassemias are common in African-Americans , but also in Greeks and Italians.

Beta (β) Thalassemias

They are inherited autosomal recessive disorders , present mainly in Mediterranean basin and Asians. There are three types of β-thalassemias:

1. Beta β thalassemia minor or (beta thalassemia trait)

It occurs when the patient inherits a single abnormal gene (heterozygous). It is usually asymptomatic with occasional palpable spleen.

Diagnosis of β-thalassemia minor

Blood film shows hypochromic , microcytic anemia. Target cells and poikilocytosis may be seen. How do you differentiate it from iron deficiency anemia?

TIBC is normal , and serum ferritin is normal.

Hb electrophoresis reveals increase in HbA_2 and slight increase in HbF.

2. Beta thalassemia major (Cooley's anemia)

Occurs when the patient has two abnormal (homozygous) genes , and no β chain is synthesized , so there is no HbA ,but all the Hb is HbF and HbA_2.

Because the β chain is not synthesized in the hemoglobin of the red blood cells , the α chain occurs in excessive amounts , so they precipitate in the red cells leading to their destruction , therefore , a severe anemia will occur , which is blood transfusion dependent since childhood.

Clinical features of β-thalassemia major

Severe anemia since childhood and infancy , jaundice , hepato-splenomegaly , growth retardation , and bone deformities (mongoloid facies and skeletal abnormalities)**.**

Laboratory diagnosis of β-thalassemia major

1. There is a severe hypochromic , microcytic anemia with raised reticulocyte , target cells and basophilic stippling in the blood film.
2. Hb electrophoresis reveals absence or almost complete absence of HbA , with almost all the Hb circulating is HbF. The HbA_2 percent is normal or slightly raised.

3. beta thalassemia intermedia

It is a condition that intermediates between the major and the minor forms. Affected individuals can often manage a normal life but may need occasional transfusions e.g at time of illness or pregnancy , depending on the severity of their anemia.

Alpha (α)Thalassemias

These groups of anemias are caused by defects in α genes. We have 4 genes or 4 grades of severity depending on the number of defective genes:

1. (αα / α -) : One defective gene. Asymptomatic carrier , and there are no clinical symptoms (silent carrier).
2. (αα / - -) : Two defective genes (α-thalassemia trait) , it is also asymptomatic , but may have mild hypochromic microcytic anemis. (similar to β-thalassemia minor).

3. (α - /- -) : Three defective genes (HbH disease). Presents in adults due to excess β chain production. It causes hemolytic anemia similar to β-thalassemia major. Hb electrophoresis shows HbH of 4 beta chains.
4. (- - / - -) : Four defective genes (Hb Barts). It is incompatible with life leading to Hydropis fetalis , with severe anemia hepatosplenomegaly and oedema.

Iron Deficiency Anemia (IDA)

Iron deficiency anemia is the most common cause of anemia in every country of the world , affecting about 500 million people worldwide. This is because the body has a limited ability to absorb iron and excess loss of iron as a result of hemorrhage is frequent.
Iron deficiency anemia is the most important cause of a microcytic hypochromic anemia , in which the two red cell indices MCV (mean cell volume) and MCH (mean cell hemoglobin) are reduced and the blood film shows microcytic and hypochromic cells. This appearance of cells is caused by a defect in hemoglobin synthesis.

Iron absorption and metabolism

Iron is absorbed in small intestine (duodenum & upper jejunum). Iron is found in two forms , the haem iron which forms (90%) and non-haem iron which forms (10%).
Haem form is present in red meat and liver and is absorbed rapidly , while non-haem iron is present in vegetables and cereals and needs to be converted into ferrous state.
Following the oral intake of iron in the ferric state (Fe^{+3}) , stomach secretions reduce the iron to the ferrous state (Fe^{+2}).
When iron is absorbed , it is transferred to bone marrow for the synthesis of hemoglobin and to a lesser extent for the synthesis of myoglobin (in the muscles) and cytochromes (iron-containing enzymes).
When iron is absorbed , it is carried in blood bound to transferring , a glycoprotein which is formed in the liver.
When the erythrocytes are destroyed , iron is stored in the RES as ferritin and hemosiderin.
The average adult has 3.5-5 grams of total iron. Normal iron loss is very small , amounting to less than 1 mg/day. Iron is lost from the body through exfoliation of intestinal epithelial , skin cells , bile and through urinary excretion.

The daily intake of iron is 10 mg and only 10% is absorbed , so daily requirements for an adult male is 1 mg/day , and that is doubled in females because of menses.
During pregnancy , iron requirement is 3 mg/day. In children , daily requirement for iron is 1.5 mg because of growth requirements.

About 70% of iron is present in hemoglobin and 500-1000 mg in the stores.

Causes of iron deficiency anemia

1. Nutritional deficiency: in which insufficient amount of iron is consumed to meet the normal and daily demand (e.g poor diet and imbalanced vegetarian diet) , therefore it occurs among low social class people.

2. Faulty or incomplete iron absorption: e.g achlorhydria in certain disorders or following gastric resection , chronic diarrhea associated with celiac disese , sprue , Crohn disease , resection of small bowel and gastrectomy.

3. Increased demand of iron: e.g pregnancy , growth years of children and in prematurity.

4. Excessive blood loss: e.g acute and chronic hemorrhages (peptic ulcers , carcinoma of stomach , colon or rectum , heavy menstruation in females , hematuria , bleeding hemorrhoids and worm infestation by the hook worm *Ancylostoma duodenale*.

Clinical features of iron deficiency anemia

When iron deficiency is developing , the reticuloendothelial stores (ferritin and hemosiderin) become completely depleted before anemia occurs. As the condition develops , the patient may show the general symptoms and signs of anemia. The general features of IDA include: weaknes , lassitude , pallor , dizziness , tinnitus , impairment of work performance , glossitis , angular stomitis , dysphagia as a result of pharyngeal webs , flat-brittle nails which then become spoon-shaped (koilonychia) and pica (dietary cravings) when the patient ingests strange non-nutritive items like soil , chalk , ice or papers.

Laboratory finding in IDA

The Hb and PCV are decreased , and the red cell indices (MCV , MCH , & MCHC) are also decreased , but the RDW is increased.

Blood film examination will show hypochromic and microcytic red cells with anisocytosis. Cigar shaped and target red blood cells may also be seen.

Reticulocyte count is normal or decreased , but increased when the patient takes iron as treatment.

The platelet count is normal , but it may be increased following acute blood loss.
Serum iron is low while TIBC (Total iron binding capacity) is increased , because TIBC measures transferring.
Serum ferritin is decreased , because it measures iron stores.
Bone marrow examination (biopsy) is rarely needed , but when done it reveals depleted iron stores with erythroid hyperplasia.

Macrocytosis

Megaloblastic anemia

Megaloblastic anemia is the commonest cause of macrocytosis , in which the red cells are larger because they can not produce DNA quickly enough to divide at the right time as they grow , and thus grow too large before division. The erythroblast in the bone marrow shows a characteristic abnormality, and therefore the maturation of the nucleus being delayed relative to that of the cytoplasm. This is often due to the deficiency of vitamin B_{12} and / or folic acid , which are needed to produce DNA.

Clinical features of megaloblastic anemia

The onset is usually insidious with a gradually progressive symptoms and signs of anemia. The patient may be mildly jaundiced (lemon yellow colour of the skin) , glossitis , angular stomatitis , purpura , wide spread melanin pigmentation and loss of weight may also present.
Severe megaloblastic anemia may cause a progressive neuropathy affecting the peripheral sensory nerves and the spinal cord. The neuropathy is symmetrical and affects the lower limbs more than the upper. The patient suffers from tingling and numbness in the feet with difficulty in walking. This condition is called subacute combined degeneration of the spinal cord (SCDSC).
Folate or vitamin B_{12} deficiency in the mother predisposes to neural tube defect (NTD) or spina bifida in the fetus.

Causes of megaloblastic anemia

1. Nutritional : dietary Vit. B_{12} or folate deficiency , or vegans (vegetarians) in case of Vit. B_{12} deficiency.
2. Malabsorption : e.g celiac disease , tropical sprue , or Crohn's disease , partial or total gastrectomy , and gastric carcinoma.
3. increased demand : as in pregnancy , infants and growth requirements.
4. Abnormal metabolism of Vit. B_{12} or folate.
5. Defects in DNA synthesis: and this defect may be due to :

a- Congenital causes

b- Acute severe pancreatitis and fish tape worm infestation (*Diphyllobotherium latum*).

c- Therapy with cytochemical drugs (hydroxyurea or methotextrate) or with certain antiviral and antibacterial drugs.

Vitamin B_{12} (Cobalamin) metabolism

Vitamin B_{12} is synthesized in nature by microorganisms and by internal production from intestinal bacteria. It is found in foods of animal origin , such as liver , fish , and dairy products. Daily requirement of vitamin B_{12}

is 1-2 mg/day. The total stores in the liver and in all body is 1-2 gm , which is enough for 2-3 years.

Vitamin B_{12} is released from protein complexes in food and is combined with a glycoprotein called intrinsic factor (IF) , which is synthesized by the gastric parietal cells , and the complex is then attached to ileal surface receptors. Vitamin B_{12} is absorbed into portal blood where it is attached to a plasma binding protein called (Transcobalamin II) , which delivers it to bone marrow and other tissues.

Folic acid (Pterol glutamic acid) metabolism

Folic acid is found in green vegetables , milk and wheat. So deficiency due to decreased uptake is rare , but tissue stores can be depleted in few weeks. Folate is absorbed in the duodenum and jejunum and conjugated in the presence of vitamin B_{12} , which is very important in the DNA synthesis.

The daily requirement of folate is 100μg , while the tissue stores is about 200 mg , which is enough for few weeks.

Laboratory diagnosis of megaloblastic anemia

Hb concentration and RBC counts are below normal , while MCV is high > 100FL.

Reticulocytes are normal or slightly decreased.

Leukocytes and platelets are moderately reduced.

Blood film shows macrocytes with ovalocytes. Anisocytosis and poikilocytosis are also seen.

Presence of hypersegmented neutrophils in the blood films of megaloblastic anemia.

Blood biochemistry may show increased unconjugated bilirubin and LDH enzyme.

Decreased serum B_{12} concentration in case of vitamin B_{12} deficiency , and decreased serum folate in case of folic acid deficiency.

Bone marrow reveals megaloblastic hyperplasia (hypercellular megaloblasts).

Causes of macrocytosis other than megaloblastic anemia

1. Pernicious anemia
2. Liver diseases
3. Reticulocytosis
4. Some aplastic and refractory anemias
5. Myelodysplastic and myeloproliferative syndromes
6. Alcoholism
7. Hypothyroidism 8. Neonatal