23-24

جامعة الفراهيدي – كلية الصيدلة

Disorders of Carbohydrates Metabolism

Clinical Chemistry for 5th-year Pharmacy Students

1

ا.م.د. رائد ضياء هاشم

Disorders of Carbohydrates Metabolism

Introduction

Carbohydrates, including sugar and starch, are widely distributed in plants and animals. They perform multiple functions, such as being structural components as in RNA and DNA (ribose and deoxyribose sugars) and providing a source of energy (glucose).

The main two types of sugars are:

A- Monosaccharides:

Also called simple sugars, they are soluble in water, common examples:

1. Fructose ……. 2. Glucose ……. 3. Galactose

B- Disaccharides:

Composed of 2 monosaccharides joined together, soluble in water, must be broken down to monosaccharides before they can be absorbed within the digestive system, common examples:

1. sucrose: glucose + fructose
2. lactose: glucose + galactose
3. maltose: glucose + glucose

Oligosaccharides consist of 3-9 monosaccharides joined together, only partially digestible in the digestive system, present in some types of plants (onion, soya beans), useful for healthy digestion.

Polysaccharides consist of polymers of chains of mono and disaccharides all joined together, tasteless, insoluble in cold water, and the main groups of polysaccharides are:

1- starch (in plants)
2- glycogen (in animals)

In summary, carbohydrates can be classified into:

1. monosaccharides: include glucose, fructose and galactose.
2. disaccharides: include sucrose, maltose, and lactose
3. oligosaccharides: contain 3-9 simple sugars (monosaccharide)
4. polysaccharides: these are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides.

Concerning glucose, the sources of blood glucose are:

(1) the breakdown of carbohydrates in the diet (grains, starchy vegetables, and legumes) or body stores (glycogen).

(2) endogenous synthesis from protein or the glycerol components of triglycerides.

When energy intake exceeds expenditure, the excess is converted to fat and glycogen for storage in adipose tissue and liver or muscle, respectively. When energy expenditure exceeds caloric intake, endogenous glucose formation occurs from the breakdown of carbohydrate stores and noncarbohydrate sources (e.g., amino acids, lactate, and glycerol).

Insulin, glucagon, and epinephrine maintain the glucose concentration in the blood within a fairly narrow interval under diverse conditions (feeding, fasting, or severe exercise).
Diabetes mellitus is the most commonly encountered of carbohydrate metabolism with hundreds of millions of people affected worldwide, 50% of whom are unaware of their illness. On the other extreme, hypoglycemia is much less common, especially in those on no insulin treatment.

Biochemical importance of carbohydrates

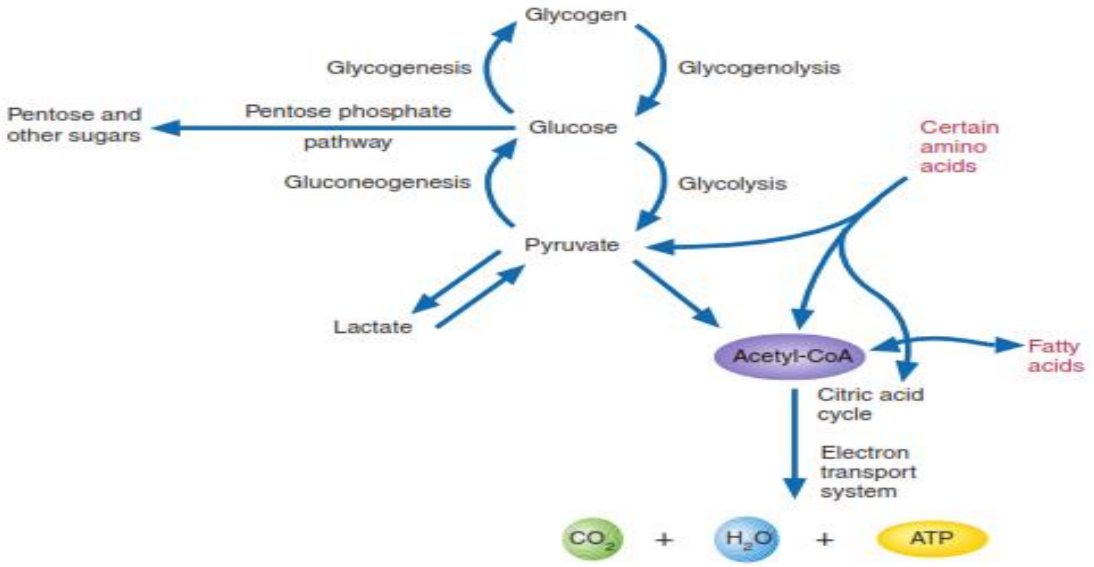
1. Carbohydrates are important constituents of the cell structure in the form of glycolipid, glycoproteins, heparin, cellulose, starch and glycogen.
2. Carbohydrates serve as an important source and store of energy.
3. Carbohydrates play an important role in the metabolism of amino acids and fatty acids.
4. Lactose promotes the growth of desirable bacteria in the small intestine. It also increases calcium absorption.
5. They protect friction surfaces such as blood vessels, trachea, etc. against mechanical damage.
6. It plays an important role in maintaining osmotic and ionic regulation of the body.
7. It works as an intracellular cementing material.
8. It spares protein.
9. Heparin is a carbohydrate, which works as an anticoagulant in the body.

Metabolism of glucose

Glucose is the primary energy source for the human body. The first step of carbohydrate digestion occurs in the mouth by the action of salivary amylase on glycogen and starch but its action is strongly inhibited by the acidity of the stomach. The alkaline pancreatic secretion will allow pancreatic amylase to complete the digestion mainly to maltose which will be further hydrolyzed in addition to sucrose and lactose into glucose, galactose and fructose by intestinal disaccharidases to be absorbed. Monosaccharides are actively absorbed with fructose being absorbed more slowly than glucose and galactose.

After absorption, the metabolism of glucose proceeds according to the body's requirements. This metabolism results in:
(1) Energy production by conversion to carbon dioxide and water (by glycolysis and citric acid cycle).
(2) Storage as glycogen in the liver or triglycerides in adipose tissue (glycogenesis).
(3) Conversion to ketoacids, amino acids, or protein (by pentose monophosphate pathway).

The pentose phosphate pathway, also known as the hexose monophosphate shunt, is an alternative pathway for glucose metabolism that generates the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), which is used in maintaining the integrity of the red blood cell membrane.



Role of different hormones in carbohydrate metabolism

The carbohydrate metabolism is regulated & the normal blood sugar level is maintained by a balance between the actions of **insulin, glucocorticoids, growth hormones, adrenalin & thyroid hormones.**

A- Insulin

1- Insulin increases the utilization of glucose in energy production & lipogenesis, decreases glucose formation from glycogen as well as non-carbohydrates & indirectly enhances carbohydrate storage in tissues (glycogenesis).

2- It increases glucose uptake from the extracellular fluid by muscles, adipocytes, mammary glands, lens & many other extrahepatic tissues.

3- It enhances glycolysis in muscles, liver & other tissues.

4- Insulin also inhibits the production of glucose gluconeogenesis from fats and amino acids, partly by inhibiting lipolysis and proteolysis.

B- Glucagon

1- Glucagon is stimulated by a fall in blood sugar level; it is antagonistic to insulin & increases blood sugar and decreases liver glycogen.

2- It increases glycogenolysis in the liver by activating glycogen phosphorylase.

3- It decreases hepatic glycogenesis & thus reduces the removal of blood glucose by the liver.

In non-diabetic individuals, insulin is released from pancreas in different patterns:

- Around 50% of the total insulin is secreted during the basal periods to prevent excessive lipolysis, proteolysis, and glycogenolysis.
- The other fraction of insulin is secreted in response to meals (postprandial), and it occurs in 2 phases:

1- the 1st phase begins within the first 2 minutes of ingestion and persists for 10-15 minutes. It is associated with a sharp increase in insulin release form the stores in the beta cells of pancreas.

2- the 2nd phase follows the 1st one and continues till normalizing blood glucose level usually within 60-120 minutes.

In patients with type 2 DM, the second phase is preserved but the 1st one is lost.

In patients with type 1 DM, there is a minimal or no insulin response.

Insulin also directly increases the transport of amino acids, potassium and phosphate into cells, especially muscle; these processes are independent of glucose transport.

In the longer term, insulin regulates growth and development.

The half-life of insulin in the circulation is between 4 and 5 minutes with total daily secretion of about 40 U.

C- Adrenaline

1- Adrenaline or epinephrine has glycogenolytic action as it increases blood glucose by enhancing hepatic glycogenolysis.

2-It has gluconeogenic action as it increases hepatic gluconeogenesis.

3- It reduces the utilization of blood glucose by increasing adipose tissue lipolysis.

D- Glucocorticoids

1- Adrenal Glucocorticoids tend to raise blood sugar. They help to maintain hepatic glycogen during fasting, glucocorticoids act as antagonists to insulin.

2-It increases gluconeogenesis in the liver by inducing the synthesis of key gluconeogenesis enzymes.

3- They decrease amino acid incorporation into protein by increasing protein catabolism in extrahepatic tissues.

E- Growth hormones

Growth hormone (GH) is antagonistic to insulin in most of its effects on carbohydrate metabolism.

1- It increases hepatic gluconeogenesis & mobilizes fatty acids from adipocytes for utilization.

2- GH reduces insulin sensitivity & thereby decreases the hypoglycemic effects of insulin.

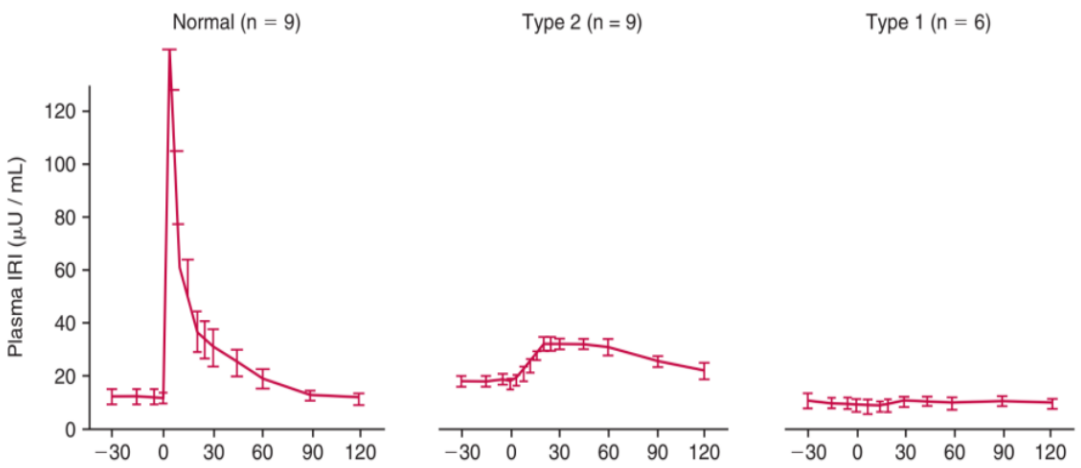
3- It can also increase muscle & cardiac glycogen levels probably by reducing glycolysis. G

F- Thyroid hormones

1- Thyroid hormones raise blood sugar; reduce glucose tolerance & increase glucose utilization.

2- Increase hepatic glycogenolysis.

Insulin secretagogues	Insulin inhibitors
Glucose	hypoglycemia,
incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)	somatostatin (produced in the pancreatic δ-cells),
cholecystokinin, peptide YY	drugs (e.g., α-adrenergic agonists, β-adrenergic blockers, diazoxide, phenytoin, phenothiazines



Insulin secretion in response to glucose

Overview of disorders of carbohydrate metabolism

Disorders of carbohydrate metabolism can be classified into two major groups:
1- genetic: usually rare, a major example of inborn errors of metabolism.
2- acquired: relatively common, the most important examples include diabetes mellitus and its complications (diabetic ketoacidosis, hyperosmolar coma) and hypoglycemia.

Diabetes mellitus and related complications

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism characterized by hyperglycemia caused by an absolute or relative deficiency of insulin.
It is a relatively common medical problem, affecting 1–2% of western populations. It is estimated that around 400 million people currently have diabetes, 80% of whom live in developing countries. In China, it was suggested that up to 11% of the population are diabetic and, more alarming, 50% of the rest of the population are prediabetics. Worldwide diabetes caused at least $612 billion in health expenditures and an estimated 4.9 million deaths in 2014.4 Acute and chronic complications make diabetes the fourth most common cause of death in the developed world.
DM results in chronic hyperglycemia, usually accompanied by glycosuria and many other biochemical abnormalities, expressed as a wide range of clinical presentations ranging from asymptomatic patients with relatively mild biochemical abnormalities to patients admitted to hospitals with severe metabolic decompensation of rapid onset that has led to coma.
Lack of insulin affects the metabolism of carbohydrates, protein and fat, and can cause significant disturbance of water and electrolyte homeostasis; death may result from acute metabolic decompensation. Long-term complications may develop, including retinopathy, neuropathy and nephropathy. It is a major risk factor for cardiovascular disease.

Diabetes may be a secondary consequence of other diseases. For example, in diseases of the pancreas, such as pancreatitis or hemochromatosis, there is a reduction in insulin secretion. In some endocrine disorders, such as acromegaly or Cushing's syndrome, there is an antagonism of insulin action by abnormal secretion of hormones with opposing activity. Several drugs adversely affect glucose tolerance.
Examples of causes of secondary DM or hyperglycemia are listed in the following table:

Category of cause	Examples
Drugs	Estrogen-containing oral contraceptives, Corticosteroids, salbutamol, thiazide diuretics
Endocrine disorders	Acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, prolactinoma, thyrotoxicosis
Insulin receptor abnormalities	Autoimmune insulin receptor antibodies, congenital lipodystrophy
Pancreatic disease	Chronic pancreatitis, hemochromatosis, pancreatectomy

Types of primary DM

Most cases of diabetes are not associated with other conditions but are primary.

Types of primary DM are:

1- Type 1 diabetes mellitus

Type 1 diabetes usually presents acutely over days or a few weeks in young non-obese subjects but can occur at any age and represents 5-10% of the total cases of DM.

In addition to polyuria, thirst and glycosuria, there is often marked weight loss and ketoacidosis. Insulin is required for its treatment. Type 1 diabetes is an autoimmune condition with genetic and environmental precipitating factors in its pathogenesis. It is eventually associated with a complete absence of insulin.

Islet-cell antibodies that react with the β-cells of the pancreas have been demonstrated in serum from over 90% of patients with newly diagnosed type 1 diabetes but some patients have no evidence of autoimmunity and are classified as type 1 idiopathic.

The peak incidence occurs in childhood and adolescence. Approximately 75% acquire the disease before the age of 18 but Age at presentation is not a criterion for classification.

There is a well-recognized association between type 1 diabetes and other autoimmune endocrinopathies, such as hypothyroidism and Addison's disease, and also with pernicious anemia.

Biomarkers of β-cell autoimmunity are circulating antibodies, which might be detected in the serum years before the onset of hyperglycemia. These antibodies include:

1- Islet cell cytoplasmic antibodies (ICAs) presents in 75 to 85% of patients with type 1 DM.
2- Insulin autoantibodies (IAAs) are present in more than 90% of children who develop type 1 diabetes before age 5.
3- Antibodies to the 65 kDa isoform of glutamic acid decarboxylase (GAD) present in ≈60% of patients.
4- Insulinoma-associated antigens (IA-2A and IA-2βA), detected in more than 50% of patients.
5- Zinc transporter ZnT8 was identified recently as a major autoantigen in type 1 diabetes, ZnT8 in 60 to 80% of patients.

2- Type 2 diabetes mellitus

Usually occurs in older (>40 years) patients who are obese; many patients have clearly had the condition for some time (even years) before diagnosis. It accounts for 90% of cases of diabetes.

Type 2 diabetes is rare in younger patients but is increasing with the increased prevalence of obesity in this age group. Among children in Japan, type 2 diabetes is now more common than type 1. Measurable levels of insulin are present, and the metabolic defect appears to lie either in defective insulin secretion or in insulin resistance. In fact, Insulin concentrations may be normal, decreased, or even increased. Weight loss alone is associated with improvement in glycemic control even before starting diet therapy, oral antihyperglycemic drugs or insulin. In general, insulin administration is not required for the prevention of ketosis, as these patients are relatively resistant to its development. However, insulin may be needed to correct the abnormalities of blood glucose. There is a strong genetic element to this disorder as shown from the strong family history in a high percentage of patients.

3- Gestational diabetes

Gestational diabetes is a term describing carbohydrate intolerance of variable severity that is either first recognized or has its onset during pregnancy. It can, therefore, include patients

with previously unrecognized type 1 or type 2 diabetes. In the United States, gestational diabetes mellitus (GDM) occurs in 6% to 8% of pregnancies.
In these patients and in established diabetic patients who become pregnant, poor blood glucose control is associated with a higher incidence of intrauterine death and fetal malformation and urgent treatment is needed.
The best strategy for screening and diagnosing gestational diabetes remains controversial.
In early pregnancy, an important aim of screening is to identify previously undiagnosed type 2 diabetes, and women with risk factors such as a high body mass index, a family history of diabetes, or previous gestational diabetes should have HbA1c or fasting blood glucose measured. If results diagnostic of diabetes are obtained they should be regarded as having pre-existing diabetes. Women with intermediate results should be assessed for the need for home glucose monitoring.
All women with risk factors should undergo an oral glucose tolerance test at 24–28 weeks.
When GTT is used, the criteria for the diagnosis of gestational diabetes differ from the criteria applied for non-pregnant subjects, these criteria are:
1- a fasting plasma glucose of 92 mg/dl or more.
2- a one-hour value of 180 mg/dl or more.
3- a two-hour value of 153 mg/dl or more.
It is important to mention that random or fasting glucose measurement is not recommended for screening because of poor specificity.
Women with GDM are at significantly increased risk for the subsequent development of type 2 diabetes mellitus, which occurs in 6 to 62%. At 6 to 12 weeks postpartum, all patients who had GDM should be evaluated for diabetes using nonpregnant OGTT criteria. If diabetes is not present, patients should be reevaluated for diabetes at least every 3 years.

4- Neonatal diabetes

Neonatal diabetes is diabetes diagnosed within the first 6 months of life and maybe transient or permanent. Up to 60% of patients with permanent neonatal diabetes have a mutation in one of the potassium channel genes, which results in failure of insulin production.

5- Maturity onset diabetes of the young (MODY)

This autosomal dominant disorder is rare and characterized by:
- presentation occurs in adolescents or young adults before the age of 25 years.
- represent around 1% of all cases of DM.
- frequently misdiagnosed as type 1 or type 2 DM.
- many subtypes of MODY are present, with different severity, and sometimes require no treatment.
- the more severe subtype is usually responsive to sulphonylureas.
- insulin therapy may be required later in life.

6- Latent autoimmune diabetes in adults (LADA)

Latent autoimmune diabetes in adults (LADA) is a disorder in which, despite the presence of islet antibodies at diagnosis of diabetes, the progression of autoimmune β-cell failure is slow.

LADA patients are therefore not insulin-requiring, at least during the first 6 months after diagnosis of diabetes.
LADA is characterized by the following:
- LADA is the most prevalent form of adult-onset autoimmune diabetes and probably the most prevalent form of autoimmune diabetes in general.
- LADA shares genetic features with both type 1 and type 2 diabetes.
- Phenotypically, LADA patients are often misdiagnosed as having type 2 diabetes.
- LADA patients generally have worse HbA1c levels than type 2 diabetes patients.
- Clinically, LADA patients tend to have a lower mean age at diabetes onset, lower body mass index and more frequent need for insulin treatment than patients with type 2 diabetes.

Diagnosis

The diagnosis of DM is based on the measurement of A1C level, fasting or random blood glucose level, or oral glucose tolerance testing. Urine glucose measurements are inadequate for diagnosing diabetes. They potentially yield false-positive results in subjects with a low renal threshold for glucose, and in a patient, with diabetes, they may yield false-negative results if the patient is fasting.
The reference range of plasma glucose is:
- FBS: 70 – 100 mg/dl
- 2-hour postprandial plasma sugar: < 140 mg/dl (including GTT)

According to the WHO criteria, DM is diagnosed when one or more of the following features are present:
1- a random venous plasma [glucose] of 200 mg/dl or more.
2- a fasting plasma [glucose] of 126 mg/dl or more.
A single result is sufficient in the presence of typical hyperglycemic symptoms of thirst and polyuria. In their absence, a venous plasma [glucose] in the diabetic range should be detected on at least two separate occasions on different days.
3- HbA1c of more than 6.5%.
4- plasma sugar 200 mg/dl or more on 2 hours of GTT (or postprandial).
According to previous criteria, a third group will appear other than diabetic or non-diabetic groups, this group is known as impaired fasting glucose when FBS lies between 100-125 mg/dl, or impaired glucose tolerance when 2 hours' postprandial plasma sugar lies between 140-200. This group has a higher risk of developing DM.

Monitoring the treatment of diabetic patients

There is now excellent evidence that in both type 1 and type 2 diabetes, the incidence of long-term complications such as retinopathy can be reduced by achieving tight control.
This level of control requires meticulous monitoring of glycemic control.
Monitoring treatment of DM can be done by:

1- Home blood glucose monitoring

In a patient already diagnosed with DM, a FBS of less than 140 mg/dl and a 2-hour' postprandial blood sugar of less than 160 mg/dl is considered acceptable and the DM is considered controlled.

2- HbA1c

This test is also used to monitor treatment for someone with DM. It helps to evaluate how well the patient's glucose levels have been controlled by treatment over the last 2-3 months. For monitoring purposes, an HbA1c of less than 7% indicates good glucose control and a lower risk of diabetic complications for the majority of diabetics.

Advantages and disadvantages of assays for glucose and HbA1c		
	Glucose	**HbA1c**
Patient preparation prior to collection of blood	Stringent requirements if measured for diagnostic purposes.	None.
Processing of blood	Stringent requirements for rapid processing, separation and storage of plasma or serum minimally at 4°C.	Avoid conditions for more than 12hr at temperatures >23C. Otherwise, keep at 4C (stability minimally 1 week).
Measurement	Widely available	Not readily available worldwide
Standardization	Standardized to reference method procedures.	Standardized to reference method procedures.
Routine calibration	Adequate.	Adequate.
Interferences: Illness	Severe illness may increase glucose concentration.	Severe illness may shorten red-cell life and artifactually reduce HbA1c values.
Hemoglobinopathies	Little problem unless the patient is ill.	May interfere with measurement in some assays.
Hemoglobinopathy traits	No problems.	Most assays are not affected.
Affordability	Affordable in most low and middle-income country settings.	Unaffordable in most low and middle-income country settings.

Some of the factors that influence hba1c and its measurement	
1. Erythropoiesis Increased HbA1c: iron, vitamin B12 deficiency, decreased erythropoiesis. Decreased HbA1c: administration of erythropoietin, iron, vitamin B12, reticulocytosis, chronic liver disease.	**4. Erythrocyte destruction** Increased HbA1c: increased erythrocyte life span: Splenectomy. Decreased A1c: decreased erythrocyte life span: hemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.
2. Altered Hemoglobin Genetic or chemical alterations in hemoglobin: hemoglobinopathies, HbF, and methemoglobin, may increase or decrease HbA1c.	**5. Assays** Increased HbA1c: hyperbilirubinemia, carbamylated hemoglobin, alcoholism, large doses of aspirin, chronic opiate use.
3. Glycation Increased HbA1c: alcoholism, chronic renal failure, decreased intra-erythrocyte pH. Decreased HbA1c: aspirin, vitamin C and E, certain hemoglobinopathies, increased intra-erythrocyte pH. Variable HbA1c: genetic determinants.	**Variable HbA1c**: hemoglobinopathies. **Decreased HbA1c**: hypertriglyceridemia

3- Microalbumin

Urinary 'microalbumin' is a term that refers to urinary albumin loss that is greater than normal, but which remains below the threshold of detection by the urinalysis dipstick tests widely used for detecting the presence of urinary protein.

The presence of microalbuminuria has been shown to signal an eventual progression to diabetic nephropathy.

4- Fructosamine

The measurement of plasma fructosamine concentrations may be used to assess glucose control over a shorter time course than that of HbA1c (about 2–4 weeks), but the assay has methodological limitations. Fructosamine reflects glucose bound to plasma proteins, predominantly albumin, which has a plasma half-life of about 20 days but is problematic in patients with hypoalbuminemia, for example, due to severe proteinuria. This assay may sometimes be useful in pregnancy and also if hemoglobin variants, for example, HbS or HbC, exist that may interfere with certain HbA1c assays.

Metabolic complications of diabetes mellitus

Patients with diabetes can develop severe metabolic derangements potentially leading to coma and even death.

Acute metabolic complications of DM can be classified as follows:

1- hyperglycemia, with or without ketoacidosis
2- lactic acidosis, with or without hyperglycemia
3- hypoglycemia, due to insulin excess

1- Diabetic ketoacidosis (DKA)

Diabetic ketoacidosis may be the presenting feature in a patient not previously recognized as having diabetes. In a patient with known diabetes, it may be precipitated by omitting insulin doses, or by the insulin dose becoming inadequate because of an increase in hormones with opposing action. This complication occurs in patients with type I DM.

The major metabolic abnormalities result from hyperglycemia, ketoacidosis, or both which will result in various clinical and laboratory signs. Ketoacidosis results from the accumulation of ketone bodies that are produced from beta-oxidation of fatty acids due to insulin deficiency.

Clinical features

Symptoms

- Polyuria, thirst
- Weight loss
- Nausea, vomiting
- Blurred vision
- Abdominal pain

Signs

- Dehydration
- Hypotension (postural or supine)
- Cold extremities
- Tachycardia
- Air hunger (Kussmaul breathing)
- Smell of acetone
- Confusion, coma (10%)

Laboratory findings usually present in DKA include:

1- high blood glucose, around 250-600 mg/dl.
2- ketones in both plasma and urine.
3- serum Na: normal or low.
4- serum K: high at presentation then severely decreased with treatment.
5- urea: high because of dehydration.
6- acid-base status: metabolic acidosis (pH low, HCO3 low, pCO2 low)

Treatment

When treating patients with DKA, the following points must be considered and closely monitored:

- Correction of fluid loss with intravenous fluids:

Correction of fluid loss makes the clinical picture clearer and may be sufficient to correct acidosis. The presence of even mild signs of dehydration indicates that at least 3 L of fluid has already been lost.

- Correction of hyperglycemia with insulin infusion, followed by subcutaneous insulin.
- Correction of electrolyte disturbances, particularly potassium loss.
- Correction of acid-base balance
- Treatment of concurrent infection, if present:

It is essential to maintain extreme concern for any concomitant process, such as infection, cerebrovascular accident, myocardial infarction, sepsis, or deep venous thrombosis.

The rate of production of H ions that accompanies ketone bodies formation exceeds the ability of all buffer systems leading to accumulation of H ions and development of metabolic acidosis.

Hyperkalemia occurs secondary to acidosis and lack of insulin, although there is a total body deficit due to increased urinary potassium loss in the presence of an osmotic diuresis.

With the correction of the initial causes of hyperkalemia, a profound life-threatening hypokalemia might ensue.

If the potassium level is greater than 6 mEq/L, avoid potassium infusion. If the potassium level is 4.5-6 mEq/L, 10 mEq/h of potassium chloride is administered. If the potassium level is 3-4.5 mEq/L, 20 mEq/h of potassium chloride is administered.

In severe hypokalemia, insulin should be delayed till correction serum potassium to avoid serious cardiac dysrhythmia.

The rate of glucose lowering should be around 100mg/hour.

Only short acting insulin is allowed to be used in treatment of DKA.

10% dextrose should be used when glucose level reaches 250 mg/dl to permit further insulin infusion.

The initial dose of insulin is 0.1 U/KG/h. This dose to be continued till glucose level reaches 180 mg/dl, then half the dose is used till ketoacidosis fully treated.

Cerebral edema may develop with rapid correction of hyperglycemia.

2- Hyperosmolar Hyperglycemic State

Hyperosmolar hyperglycemic state (HHS) is one of two serious metabolic derangements that occur in patients with diabetes mellitus DM and can be a life-threatening emergency.

It is less common than the other acute complication of diabetes, diabetic ketoacidosis (DKA). HHS was previously termed hyperosmolar hyperglycemic nonketotic coma (HHNC); however, the terminology was changed because coma is found in fewer than 20% of patients with HHS.

HHS usually presents in older patients with type 2 DM and carries higher mortality than DKA, estimated at approximately 10-20%. Infection is the most common precipitating factor of HHS.

Laboratory findings usually present in HHS include:
1- high blood glucose, usually > 600 mg/dl.
2- ketones are absent or very minimal in both plasma and urine.
3- serum Na: usually high due to hemoconcentration secondary to severe water loss.
4- serum K: usually normal.
5- urea: higher than that of DKA.
6- acid-base status: pH is normal or slightly decreased (> 7.30)
7- serum osmolality of 320 mOsm/kg or greater.
8- HCO3 > 18 mmol/l

Cerebral cellular dehydration, which contributes to the coma, may also cause hyperventilation, and a respiratory alkalosis, although sometimes plasma lactic acid may rise, evoking a metabolic acidosis and thus a mixed acid–base disturbance may occur.
insulin activity is sufficient to suppress lipolysis but insufficient to suppress hepatic gluconeogenesis or to facilitate glucose transport into cells.
There may also be an increased risk of thrombosis.

Treatment
The main goals in the treatment of hyperosmolar hyperglycemic state (HHS) are as follows:

- To vigorously rehydrate the patient while maintaining electrolyte homeostasis
- To correct hyperglycemia
- To treat underlying diseases
- To monitor and assist the cardiovascular, pulmonary, renal, and central nervous system (CNS) function.

Rapid and aggressive intravascular volume replacement is always indicated as the first line of therapy for patients with HHS. Isotonic sodium chloride solution is the fluid of choice for initial treatment because sodium and water must be replaced in these severely dehydrated patients.

Although many patients with HHS respond to fluids alone, IV insulin in dosages similar to those used in diabetic ketoacidosis (DKA) can facilitate the correction of hyperglycemia. Insulin used without concomitant vigorous fluid replacement increases the risk of shock. Adjust insulin or oral hypoglycemic therapy based on the patient's insulin requirement once the serum glucose level has been relatively stabilized.

3- Hypoglycemia

Hypoglycemia is defined as plasma glucose of less than 45 mg/dl. Symptoms are often related more to the rate of fall of blood glucose than to the absolute value observed.
Hypoglycemia may be caused by many conditions other than excess insulin, but these conditions are unrelated to DM.
Treatment with either insulin or insulin secretion stimulant drugs may lead to hypoglycemia.
Urgent treatment is mandatory as prolonged hypoglycemia can lead to brain damage.

4- Lactic acidosis

In basic terms, lactic acid is the normal endpoint of the anaerobic breakdown of glucose in the tissues. The lactate exits the cells and is transported to the liver, where it is oxidized back to glucose. In the setting of decreased tissue oxygenation, lactic acid is produced as the anaerobic cycle is utilized for energy production. With a persistent oxygen deficiency and overwhelming of the body's buffering abilities, lactic acidosis ensues.

Lactic acidosis is a life-threatening condition characterized by the accumulation of lactic acid in the body with low pH, it is a known cause of metabolic acidosis caused by many medical disorders.

Diabetes mellitus itself and many drugs used for its treatment (metformin) are the causes of lactic acidosis that require urgent intervention because lactic acidosis could be fatal.

To make a diagnosis, the plasma level of lactic acid with blood gas analysis are essential in addition to the clinical features of the patient.

Inborn Errors of Carbohydrates Metabolism

1- Galactosemia

Hereditary galactosemia is among the most common carbohydrate metabolism disorders and can be a life-threatening illness during the newborn period. The incidence varies widely and reaches 1:16,000 in certain countries compared to 1:7,000 in others. It is usually diagnosed during routine neonatal screening. Galactose-1-phosphate uridyltransferase (GALT) deficiency is the most common enzyme deficiency that causes hypergalactosemia as it is responsible for converting ingested galactose to glucose. Galactose will be converted into galactitol which is a toxic substance leading to:

- Hepatomegaly
- Cirrhosis
- renal failure
- feeding intolerance
- vomiting
- hypoglycemia
- seizure
- lethargy
- intellectual impairment
- cataracts.

If untreated, the mortality rate is extremely high while removing lactose largely eliminates the toxicity associated with the newborn disease, but long-term complications routinely occur. Treatment includes cessation of breastfeeding or the standard formula and using a specialized formula.

2- Glycogen storage diseases

Glycogen storage disease is a generic name encompassing at least 10 rare inherited disorders of glycogen storage in tissue. Because the liver and skeletal muscle have the highest rates of glycogen metabolism, these are the structures most affected.
The liver forms are marked by hepatomegaly (caused by increased liver glycogen stores) and hypoglycemia (caused by an inability to convert glycogen to glucose).
The muscle forms, in contrast, have mild symptoms that usually appear in young adulthood during strenuous exercise owing to the inability to provide energy for muscle contraction.

3- Fructose-1,6-bisphophatase deficiency

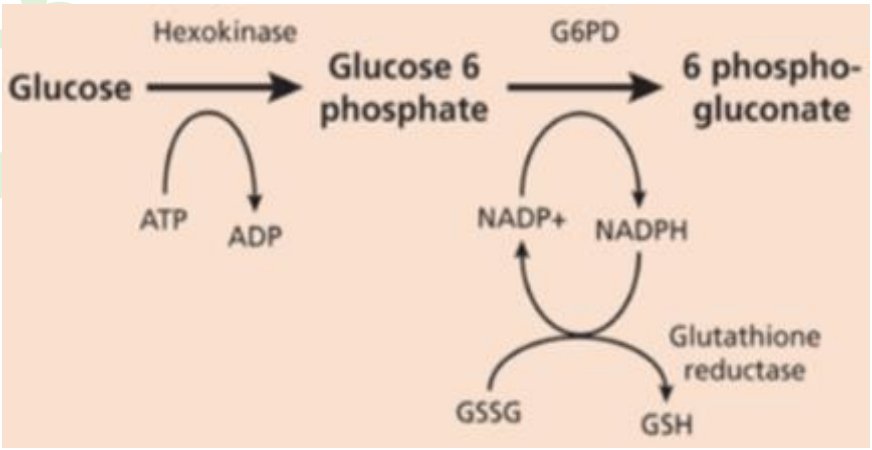
Patients with this deficiency have episodes of apnea, hyperventilation and hypoglycemia, ketosis, and lactic acidosis caused by severe impairment of gluconeogenesis.
The condition is diagnosed by demonstrating the enzyme defect in liver biopsy specimens.

4- Hereditary fructose intolerance

A deficiency of fructose-1-phosphate aldolase produces this rare disorder with hypoglycemia and liver failure. Fructose ingestion inhibits glycogenolysis and gluconeogenesis, producing hypoglycemia. Early detection is important because this condition responds to a diet devoid of sucrose and fructose.

5- Glucose-6-phosphate dehydrogenase deficiency

This is an X-linked defect in the first, irreversible step of the pentose phosphate pathway. It represents the most common enzyme defect encountered in clinical practice affecting around 400 million people worldwide.



A decrease in NADPH production makes red blood cell membranes vulnerable to oxidative stress, leading to hemolysis. NADPH serves as a substrate for glutathione reductase. The reduced glutathione has the ability to convert hydrogen peroxide into water and prevent damage to cellular structures, particularly the cell wall of red blood cells (RBCs) since they have limited capacity for repair once they mature. The most common manifestations are early neonatal unconjugated jaundice and acute hemolytic anemia. The patient presents with anemia, jaundice, dark colour urine and splenomegaly.

However, most individuals with the deficiency are clinically asymptomatic. The hemolytic crises are usually in response to an exogenous trigger such as certain drugs (e.g., antimalarials), food (broad beans) or an infection.

Female heterozygotes may have symptoms but the severity varies. The highest frequency is in those of Mediterranean, Asian or African origin. The diagnosis is by measurement of the enzyme activity in erythrocytes.

Hypoglycemia

Hypoglycemia is defined as a blood glucose level below the target value although there is no consensus about this value. Different values have been applied to define hypoglycemia such as: < 50 mg/dl, < 60 mg/dl and even < 70 mg/dl.
Generally speaking, symptoms do not develop till the level of blood glucose is less than 55 mg/dl although many factors influence the appearance of symptoms and their severity. Hypoglycemia reflects an abnormality in glucose homeostasis which might be caused by various conditions but the most common cause of hypoglycemia is an iatrogenic cause, specifically in patients with diabetes mellitus, commonly in those on insulin therapy as patients with type 1 diabetes are 3 times more likely to develop hypoglycemia compared to patients with type 2.

The classical diagnosis of hypoglycemia depends on confirming the presence of Whipple's triad:

- the presence of the typical symptoms of hypoglycemia
- confirming low blood glucose level
- immediate recovery after administration of glucose

Causes of hypoglycemia in non-diabetic individuals include critical illness, alcohol, cortisol deficiency, malnourishment and insulinoma.

Clinical features

Neuroglycopenic (Result from direct CNS deprivation of glucose)

- Confusion
- Fatigue
- Seizure
- Coma
- Death

Neurogenic (Result from sympathetic stimulation in response to hypoglycemia)

- Adrenergic
  - ✓ tremor, palpitations, anxiety
- Cholinergic
  - ✓ (hunger, diaphoresis, paresthesias)

Surprisingly, patients with diabetes mellitus might develop signs and symptoms of hypoglycemia at higher levels of blood glucose. This phenomenon is called pseudohypoglycemia and is thought to be due to an altered set point at which neuroglycopenic and neurogenic used to occur as a result of chronic hyperglycemia.

At the same time, diabetic patients with a history of recurrent hypoglycemia might experience asymptomatic hypoglycemia despite the significant low blood glucose level. This condition is called hypoglycemia-associated autonomic failure (HAAF) and its pathophysiology is not well understood although defective adrenomedullary adrenaline response to hypoglycemia is a characteristic finding.

Significant impairment of cardiovascular function is expected in patients with recurrent hypoglycemia due to the associated increase in blood pressure, stroke volume and myocardial contractility.

Approach consideration

The direct cause of hypoglycemia needs to be diagnosed after resuscitating the patient as a serious underlying condition might be overlooked. In most cases, the history is highly suggestive of the underlying cause, especially in diabetic patients. When the cause is not clear, a systematic workup is essential which includes:

1- blood glucose
2- serum insulin
3- serum C-peptide
4- serum cortisol
5- serum ACTH
6- renal function
7- liver function

Many factors have been suggested to be associated with severe hypoglycemia such as:

- Age (both extremes of age)
- Strict glycemic control
- Increasing the duration of diabetes
- Sleep
- History of previous severe hypoglycemia
- Associated renal impairment

Hypoglycemia-induced cardiac arrhythmia has been implicated to be the direct cause of sudden death during sleep that might be encountered in young patients with type 1 diabetes "dead in bed syndrome". Furthermore, within 5 years of the onset of type 1 diabetes mellitus, the secretion of the counter-regulatory hormones will be impaired significantly making the recovery of hypoglycemia more difficult.

Management

The severity of hypoglycemia and the patient's level of consciousness are the main factors that determine the mode of treatment.

For mild cases where the level of consciousness is intact:

- Oral fast-acting carbohydrate (10–15 g) is taken as a glucose drink or tablet.
- This should be followed by a snack containing complex carbohydrates.

For severe cases:

- Intravenous 75‑100 mL 20% dextrose over 15 mins (= 15 g; give 0.2 g/kg in children)
  Or
- Intravenous 150‑200 mL 10% dextrose over 15 mins
  Or
- Intramuscular glucagon (1 mg; 0.5 mg in children) ‑ may be less effective in patients on sulphonylurea or under the influence of alcohol.

Full recovery may not occur immediately and reversal of cognitive impairment may not be complete until 60 minutes after normoglycemia is restored. When hypoglycemia has occurred in a patient treated with long- or intermediate-acting insulin or a long-acting sulphonylurea, such as glibenclamide, the possibility of recurrence should be anticipated; to prevent this, infusion of 10% dextrose, titrated to the patient’s blood glucose, or provision of additional carbohydrate may be necessary.

Failure to regain consciousness after the restoration of blood glucose level is a clue to the development of cerebral oedema which is associated with a high mortality rate.

For those on insulin therapy, the next dose should not be omitted but reduced by 10-20% and medical advice should be sought.

23-24

جامعة الفراهيدي – كلية الصيدلة

Disorders of Lipid Metabolism

Clinical Chemistry for 5th-year Pharmacy Students

2

ا.م.د. رائد ضياء هاشم

Disorders of Lipid Metabolism

Introduction

'Lipid' is the term used to describe a number of substances of diverse chemical structures that bear little functional relationship to each other but which have in common the property of being soluble in organic solvents and virtually insoluble in water.

Lipoproteins are macromolecular protein complexes that allow hydrophobic lipids to be transported within the hydrophilic environment of the circulation.

Lipids are essential for health, but excessive concentrations of cholesterol and triglycerides in the circulation, whether due to lifestyle factors or inherited disorders of lipoprotein metabolism, are major factors in the development of atherosclerosis and cardiovascular disease.

Lipids are ubiquitous in the body tissue and play a vital role in virtually all aspects of life:

(1) as hormone precursors

(2) aiding in digestion

(3) providing a source of metabolic fuel and energy storage

(4) acting as functional and structural components in cell membranes

(5) forming insulation to prevent heat loss.

Major groups of lipids and their functions are shown in the following table:

Lipid	Functions
Cholesterol	1- Structural component of the cell membrane. 2- A precursor of bile acid and steroid hormones
Fatty acids	Energy source
Triglycerides	Energy source
Eicosanoids	Blood coagulation, bronchial and vascular contractility, reproduction
Sphingolipids	Central nervous system, blood group substances
Fat-soluble vitamins A D K E	 Vision Calcium homeostasis Activation of clotting factors Antioxidant

Examples of major lipids

Cholesterol

Cholesterol is the major sterol in humans, being present in all body cells and most body fluids. It is found almost exclusively in animals.

Cholesterol is the starting point in many different metabolic pathways including vitamin D synthesis, steroid hormone synthesis, and bile acid metabolism.

Cholesterol enters the intestinal lumen from three sources:

1- the diet (300-450 mg/day)
2- bile
3- the intestine (from slaughtered mucosal cells)

Animal products especially meat, egg yolk, seafood, and whole-fat dairy products provide the bulk of dietary cholesterol, while bile secretion and intestinal cells are responsible for a similar amount of cholesterol to that of diet. At the same time, endogenous cholesterol represents another 400 mg daily.

To be absorbed, unesterified cholesterol must be first solubilized. This occurs through the formation of mixed micelles that contain unesterified cholesterol, fatty acids, monoglycerides (derived from triglycerides), phospholipids, and conjugated bile acids.

The formation of mixed micelles promotes cholesterol absorption by both solubilizing the cholesterol and facilitating its transport to the surface of the luminal cell, where it is absorbed by an active process involving the enterocyte protein NPC1L1, which is the drug target for the cholesterol absorption inhibitor ezetimibe.

There are 3 main factors responsible for the proper digestion and hence proper absorption of cholesterol (and other forms of lipids as well):

1- bile secretion (the most important factor)
2- pancreatic secretion
3- the peristaltic movement of the intestine

Cholesterol is present in the diet but most cholesterol in the body is made by de novo synthesis from acetate. The rate-limiting step in the synthetic pathway is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, catalyzed by the enzyme **HMG-CoA reductase**. In 1960, the use of triparanol for the treatment of hypercholesterolemia resulted in disastrous sequelae. This drug inhibits the final step in the synthesis of cholesterol, not the rate-limiting step, which has led to the accumulation of the

cholesterol precursor substance (desmosterol) in many tissues causing cataract, alopecia, and accelerated atherosclerosis.

The liver is responsible for most cholesterol synthesis. Although only a small amount of the body's cholesterol pool comes from dietary cholesterol, this has an important role in regulating the rate of cholesterol synthesis. The liver is the key organ in maintaining cholesterol balance; any excess cholesterol is excreted by the liver into the bile, either directly, or after conversion into bile acids.

Triglycerides

Triglycerides comprise three fatty acids esterified with a glycerol backbone. Triglycerides are the major dietary fat and constitute 95% of tissue storage fat. They are hydrolyzed in the gut by lipases to fatty acids and monoglycerides.
Triglycerides are digested in the duodenum and the proximal ileum. Through the action of pancreatic and intestinal lipases and in the presence of bile acids, which activate lipases, they are hydrolyzed to glycerol, monoglycerides, and fatty acids. The monoglycerides undergo re-esterification in enterocytes and subsequent incorporation into chylomicrons.

The major sites of endogenous triglyceride synthesis are the liver and adipose tissue. In normal circumstances, hepatic triglycerides are secreted in very low-density lipoproteins (VLDL). In certain pathological states, triglyceride accumulates in hepatocytes, leading to hepatic steatosis.

Adipose tissue triglycerides represent the major energy store of the body. Fatty acids are mobilized from adipose tissue triglycerides by the action of hormone-sensitive lipase (HSL), which is activated by glucagon and adrenaline (epinephrine) and inhibited by insulin.

Eicosanoids

This group includes prostaglandins, thromboxanes, and leukotrienes. These were originally named because they were found in the prostate, platelets (thrombocytes), and white cells (leukocytes), respectively.

They have major effects on the immune response, reproductive function (including the induction of labour), cholesterol metabolism, smooth muscle function (causing vasoconstriction or dilatation), platelet aggregation, and thrombosis.

LIPOPROTEINS

Lipids synthesized in the liver and the intestine must be transported to various tissues to accomplish their metabolic functions. Because of their relative insolubility in aqueous solutions, they are transported in the plasma in macromolecular complexes called *lipoproteins.*

The lipoproteins are macromolecular complexes of lipids (cholesterol, triglycerides, phospholipids) and proteins (apolipoproteins, enzymes), held by non-covalent forces.

The basic structure of lipoproteins is a hydrophobic core of triglycerides and/or cholesteryl esters surrounded by a layer of amphipathic phospholipids, unesterified cholesterol, and proteins. The hydrophilic surface protects the hydrophobic core from the aqueous environment.

Lipoproteins differ in their relative concentrations of protein to lipids and their constituent lipids and proteins. The densities of lipoproteins are inversely related to their size.

The nomenclature of the lipoproteins is based on their density:

Chylomicrons ⟶ (<0.95 g/mL);

VLDL ⟶ (0.95–1.006 g/mL);

Intermediate density lipoproteins (IDL) ⟶ (1.006–1.019 g/mL);

Low-density lipoproteins (LDL) ⟶ (1.019–1.063 g/mL)

High-density lipoproteins (HDL) ⟶ (1.063–1.210 g/mL).

Variable	Chylomicron	VLDL	IDL	LDL	HDL
Lipid-lipoprotein ratio	99: 1	90: 10	85: 15	80: 20	50: 50
Major lipids	Exogenous triglycerides	Endogenous triglycerides	Endogenous triglycerides, cholesteryl esters	Cholesteryl esters	Phospholipids
Major proteins	A-I … B-48.. C	B-100 .. C	B-100 .. E	B-100	A-I … A-II

1- Chylomicrons

Chylomicrons are the largest class of lipoprotein. The major protein component is apo B-48 but they also contain apo A-I, apo A-II, and apo A-IV. After secretion, they acquire apo E and apo C from HDL. Chylomicrons are formed in the intestine and are the transport vehicle of dietary fat.

2- Very low-density lipoproteins

These are the largest of the lipoproteins containing endogenously produced lipids. The major protein component of VLDL is apo B-100 but they also contain apo C-I, apo C-II, apo C-III, apo E, and small amounts of apo A. Like chylomicrons, VLDLs acquire the majority of their component apo E and apo C from HDL in the circulation; the core of VLDLs is composed predominantly of triglycerides.

In contrast to chylomicrons, the triglycerides in VLDL are endogenous in origin.

3- Intermediate density lipoproteins

These particles are produced during the conversion of VLDL to LDL after the removal of most of TG; their densities lie between those of these lipoproteins. The core of IDLs contains cholesteryl esters and triglycerides.

4- Low-density lipoproteins

These are the major cholesterol-containing lipoproteins and represent the end-product of VLDL catabolism. The core of LDL comprises mainly cholesteryl esters; the protein component is apo B-100.

5- High-density lipoproteins

These are the smallest and densest of the lipoproteins. They may be sub-classified based on size, density, shape, surface charge, and electrophoretic mobility, as well as apolipoprotein composition.

High-density lipoprotein is usually divided into three major subclasses:

(1) Nascent, discoidal, or pre-β1HDL comprises predominantly apo A-I and phospholipid. It actively exports free cholesterol from peripheral cells and macrophages.

(**2**) HDL3 is formed from pre-β1HDL by the acquisition of free cholesterol. It is the preferred substrate for lecithin cholesterol acyltransferase (LCAT), which esterifies free cholesterol,

increasing the size of the particle and following the uptake of more free cholesterol, producing.

(3) the larger and more cholesterol-rich HDL2.

6- Lipoprotein(a)

Lipoprotein an (Lp a) consists of LDL with its apo B-100 bound by a disulfide bond to apolipoprotein(a) (apo a). The plasma concentration of Lp(a) is genetically determined and is inversely related to the length of the apo(a), so that the greater the chain length, the lower the concentration.

Epidemiological studies suggest that a high Lp(a) concentration is an independent risk factor for cardiovascular disease (CVD), particularly in subjects with familial hypercholesterolemia.

7- Lipoprotein X

Lipoprotein X is a lipoprotein that is found only in the plasma of subjects with cholestasis or who have familial lecithin cholesterol acyltransferase deficiency.

It is composed of phospholipids, free cholesterol, and proteins; the major protein is albumin but small amounts of apo C and apo D are also present. It contains no apo B.

Unlike all other lipoproteins, it migrates towards the cathode on agarose gel electrophoresis.

In the fasting state, most plasma triglycerides are present in VLDL. In the postprandial state, chylomicrons appear transiently and contribute significantly to the total plasma triglyceride concentration. LDL normally carries about 70% of total plasma cholesterol but very little triglyceride. HDL contains about 20 to 30% of plasma cholesterol.

APOLIPOPROTEINS

These are proteins that bind lipids to form lipoproteins. They transport the lipids through the lymphatic and circulatory systems.

They have three functions:

(1) they provide the structural element to the lipoprotein particles.

(2) they act as ligands for specific receptors.

(3) they also act as activators or inhibitors of specific enzymes involved in lipoprotein metabolism.

Apolipoprotein A

Apolipoprotein A-I (apo A-I) is the major protein of HDL, constituting 70–80% of HDL protein. It is synthesized primarily in the liver and small intestine. It is essential in the reverse cholesterol transport pathway.

Epidemiological studies have shown that plasma apo A-I concentrations, like those of HDL-cholesterol (HDL-C), are inversely related to cardiovascular risk.

Apolipoprotein B

This lipoprotein exists in two forms; apolipoprotein B-100 (apo B-100), which is made in the liver and is the structural protein of VLDL, IDL, and LDL, and apo B-48, which is synthesized in the intestine and is incorporated into chylomicrons.

Increased plasma concentrations of apo B-containing lipoproteins confer an increased risk for the development of atheroma.

Apolipoprotein C

There are three apolipoprotein Cs, all of which are synthesized in the liver. In plasma, they are transferred between the triglyceride-rich lipoproteins (chylomicrons, VLDL, and their remnants) and HDL.

Many studies have confirmed the stronger association of high apo B and low apo A with the development of coronary atherosclerosis compared to high LDL and low HDL respectively.

It has been shown that only 14.5% of patients with myocardial infarction younger than the age of 60 years have LDL-C above the 95th percentile.

In contrast, 35% of these patients have apo B-100 above the 95th percentile.

Unfortunately, these markers have not been yet been including in the current guidelines as screening tests.

Lipoprotein Metabolism

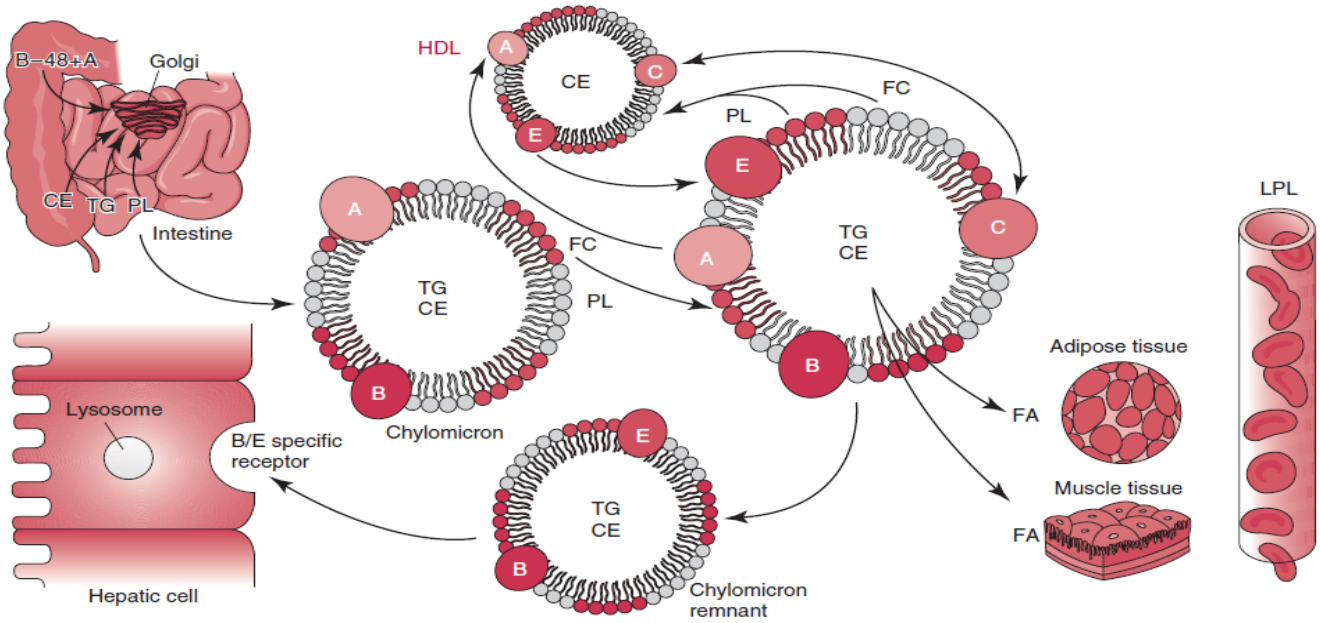
The pathways of lipoprotein metabolism are complex and intersect at several points. They include exogenous and endogenous pathways based on whether they carry lipids from the dietary or hepatic origin.

A- Exogenous Pathway

The primary function of the exogenous pathway is the absorption of dietary lipid and its delivery, particularly triglycerides, to peripheral tissues and the liver. The absorbed lipid is packaged into chylomicron which enters the circulation via the thoracic duct. Apo C and Apo E will be transported from HDL to chylomicron as they are essential for further steps of chylomicron metabolism.

Apo C is responsible for the activation of LPL at the adipose or muscle cells, this enzyme will allow these cells to obtain the free fatty acids present in chylomicron.

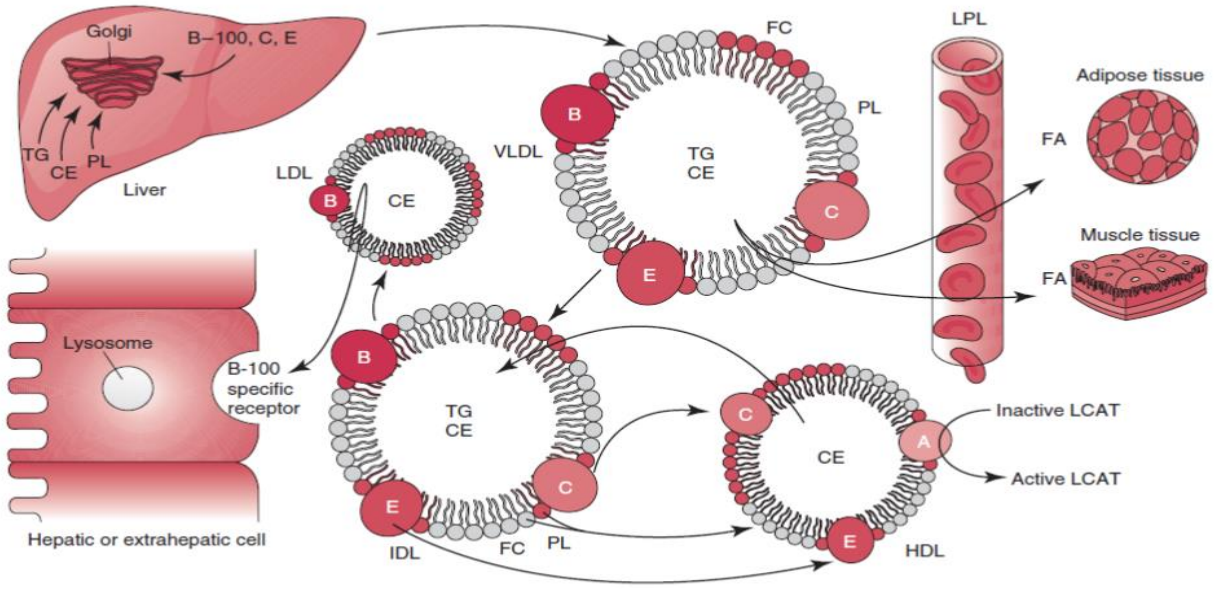
Apo E will be recognized by certain receptors in the liver to remove chylomicron remnants from the circulation.



B- Endogenous Pathway

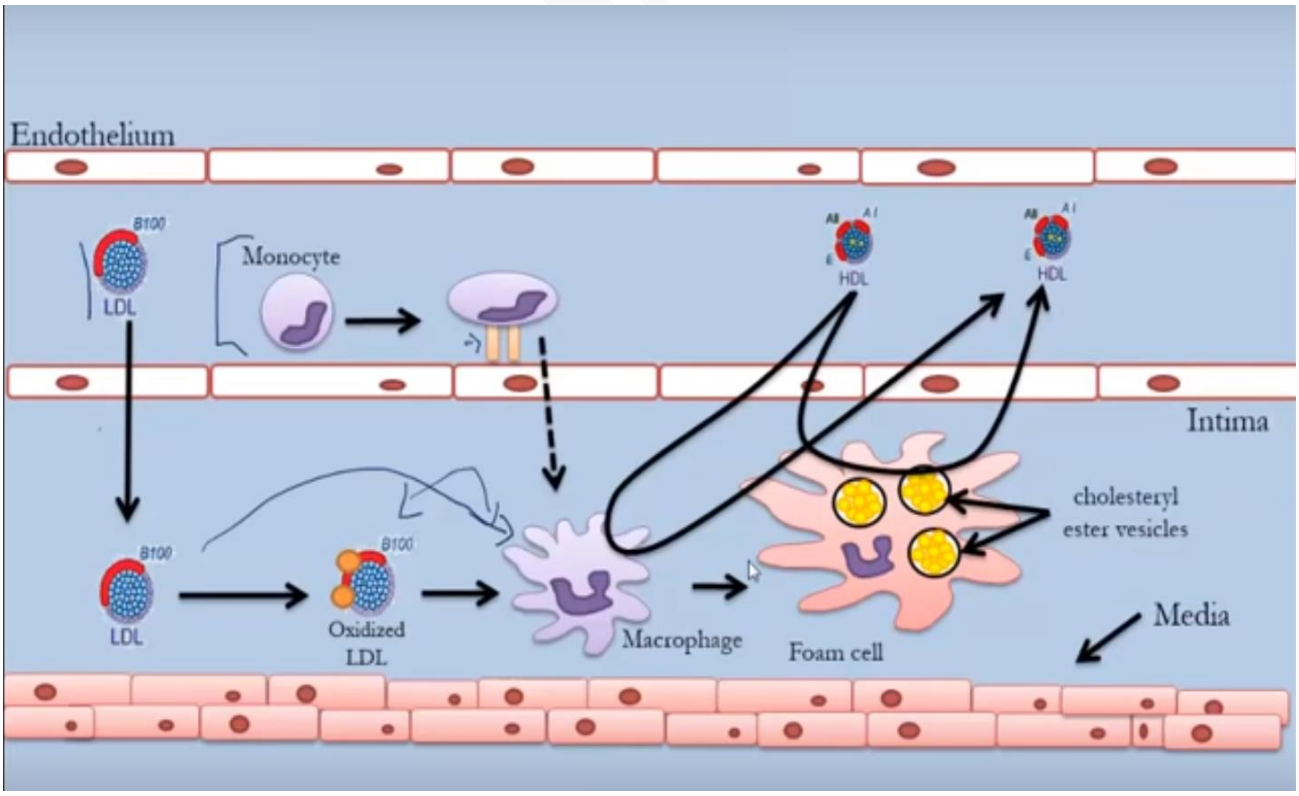
The endogenous pathway involves the delivery of lipids that are packaged in the liver to peripheral cells. Hepatocytes have the ability to synthesize triglycerides from carbohydrates or fatty acids.

In addition, when dietary cholesterol acquired from the receptor-mediated uptake of chylomicron remnants is insufficient, hepatocytes can synthesize their own cholesterol by increasing the activity of HMG-CoA reductase.



C- Reverse Cholesterol Transfer Pathway

The reverse cholesterol transport pathway helps the body maintain cholesterol homeostasis by removing excess cholesterol from peripheral cells and delivering it to the liver for excretion. It is mediated mostly by HDL; this in part accounts for the antiatherogenic property of HDL.



CLINICAL SIGNIFICANCE

The clinical significance of lipids is, primarily, the association with CAD, but it is also associated with other vascular disorders, such as thrombotic stroke and peripheral vascular disease. This association was first described in 1910 and confirmed later with many subsequent studies.

The following notes should be taken into consideration:

1- Increased cholesterol is a critical factor in the pathogenesis of atherosclerotic disease.

2- The relationship between cholesterol and atherosclerotic coronary disease is curvilinear (i.e. if a risk ratio of 1.0 is assigned at a cholesterol value of 200 mg/dL, the risk ratio increases to 2.0 at 250 mg/dL and 4.0 at 300 mg/dL).

3- Cholesterol-lowering is beneficial in people with established disease and even in those with normal or moderately increased cholesterol concentrations (185 to 240 mg/dL).

4- More recent studies have shown that individuals with a preexisting disease may show a reversal of atherosclerosis if they are aggressively treated so that they achieve LDL-C concentrations below 70 mg/dL.

Disorders of Lipoprotein Metabolism

Dyslipoproteinemia is diagnosed in most patients using plasma lipid and lipoprotein cholesterol concentrations. Causes of Dyslipoproteinemia are divided into two major groups: primary and secondary.

When hyperlipidemia is evaluated, it first should be determined whether it is from a primary lipoprotein disorder or is secondary to a wide variety of metabolic diseases. The diagnosis of primary hyperlipidemia is made after secondary causes have been ruled out.

Exogenous factors, such as dietary and alcohol intake, oral contraceptives, diabetes mellitus, and pharmacologic agents [e.g., steroids, isotretinoin, and β-blockers], are the main secondary causes of hyperlipidemia in adults.

Acquired or secondary hypolipidemia is much less common than its hyperlipidemic equivalent. It is of no direct clinical consequence, being only a manifestation of the underlying condition.

Causes of Secondary Hyperlipidemia and Dyslipoproteinemia include:

Disorder	Cause
Exogenous	Drugs: corticosteroids, isotretinoin, thiazides, anticonvulsants, beta-blockers, anabolic steroids, certain oral contraceptives Alcohol Obesity
Endocrine and metabolic	Acute intermittent porphyria, Diabetes mellitus, Hypopituitarism, Hypothyroidism, Pregnancy
Renal	Chronic renal failure, Nephrotic syndrome
Hepatic	Hepatitis
Others	Anorexia nervosa, Starvation, Systemic lupus erythematosus, Burns, Acute trauma (surgery)

Primary Dyslipoproteinemias

Polygenic hypercholesterolemia represents the most common cause of hypercholesterolemia. It is characterized by high serum cholesterol and LDL levels in the setting of normal serum TG. LDL increase ranges between 140-300 mg/dl. It is a genetically determined condition that is thought to be aggravated by numerous factors including:

- *Fatty diet*
- *Obesity*
- *Sedentary lifestyle*

The main examples of primary dyslipoproteinemias are:

1- Familial Combined Hyperlipidemia

About 10 to 15% of patients with premature CAD have familial combined hyperlipidemia (FCHL), thus making it one of the more common forms of dyslipidemia. It is inherited as an autosomal dominant trait but usually does not become symptomatic until adulthood.

Premature coronary artery disease is defined as CAD that occurs in males before the age of 55 years, or in females before the age of 65 years.

The typical findings are:

(1) a raised plasma apo B-100 concentration

(2) an increase in plasma concentrations of LDL (high total cholesterol), VLDL (high triglycerides) or both.

(3) Plasma HDL-cholesterol concentration is usually low

Overproduction of apo B-100 occurs in FCH so affected individuals have raised plasma apo B concentrations even though their lipid concentrations may be normal sometimes.

2- Familial hypertriglyceridemia

The production of large VLDL with abnormally high triglyceride content appears to be responsible for familial hypertriglyceridemia (FHTG). Plasma LDL cholesterol and apo B-100 concentrations are normal. Administration of estrogen and corticosteroids aggravates hypertriglyceridemia in these patients and sometimes can lead to acute pancreatitis.

In patients with a fasting plasma triglyceride concentration >10 mmol/L, there is a risk of acute pancreatitis, and reducing this risk is usually the first priority of treatment, before addressing residual cardiovascular risk.

This disorder appears to be inherited in an autosomal dominant pattern with delayed expression and an estimated frequency in the population of about 1: 500 persons, making this a relatively common disorder.

The newest ACC/AHA Guidelines have minimized the importance of TG for CHD risk prediction or management.

3- Familial hypercholesterolemia

This term encompasses a group of disorders due to mutations causing reduced clearance of LDL by the LDL receptor resulting in marked hypercholesterolemia and premature atherosclerosis.

It manifests in the heterozygous state as marked hypercholesterolemia, owing to high LDL-cholesterol concentration, and premature cardiovascular disease. Heterozygous FH is one of the most common inherited metabolic diseases, with a frequency of about 1 in 500 in most populations. Tendon xanthomata occur in approximately 70% of untreated heterozygotes.

Homozygous familial hypercholesterolemia (HoFH) is a rare and life-threatening disease originally characterized clinically by plasma cholesterol levels >13 mmol/L (>500 mg/dL), extensive xanthomas, and marked premature and progressive atherosclerotic cardiovascular disease (ACVD). Untreated, most patients with markedly elevated LDL-C levels develop overt atherosclerosis before the age of 20 years and generally do not survive past 30 years.

4- Tangier disease

Tangier disease is a rare autosomal recessive condition associated with low total plasma cholesterol concentrations (typically <3 mmol/L) but, unlike the apo B deficiency states, plasma triglycerides concentrations are normal or increased. Virtually no HDL-cholesterol is present (<0.1 mmol/L). Clinically, the patient develops splenomegaly and yellow tonsils with a variable increase in cardiac risk.

The main pathophysiological feature of Tangier disease is increased catabolism of HDL, rather than a defect in biosynthesis.

5- Type V Hyperlipoproteinemia

This disorder is characterized by an increase in both chylomicrons and VLDL and has an incidence of about 1 in 500.

Clinical presentations in adult patients include eruptive xanthomas, lipemia retinalis, pancreatitis, and abnormal glucose tolerance with hyperinsulinism. Premature atherosclerotic complications are not as commonly seen as with FH.

This heterogeneous syndrome appears to be inherited in an autosomal dominant mode.

6- Dysbetalipoproteinemia

This disorder is caused by a primary genetic defect in the removal of remnants of both intestinal chylomicrons and hepatic VLDL. The disease is characterized by increased plasma cholesterol and triglycerides, and the concentrations of the two lipids are about the same. The most distinctive clinical presentation of dyslipoproteinemia is the presence of palmar xanthomas, yellow deposits that occur in the creases of the palms.

INVESTIGATION OF LIPID DISORDERS

1- Total cholesterol

This is the most commonly used single measure of lipid status, it is inadequate on its own for the diagnosis of lipid disorders, or as the only measure prior to starting treatment. A full fasting lipid profile including triglycerides and HDL cholesterol should be measured at least once to avoid missing significant dyslipidemias with normal total cholesterol. LDL-cholesterol, non-HDL-cholesterol, and apo B measurements may have benefits in assessing cardiovascular risk and the adequacy of treatment.

The simple appearance of a serum or plasma sample may indicate a lipid disorder. Chylomicrons and VLDL are large enough to scatter light.

Fasting or non-fasting makes little difference (± 3%) to measurements.

2- Triglycerides

Triglycerides increase up to 2–3-fold after a meal, so samples should be taken after an overnight (>12 h) fasting in order to obviate difficulties of interpretation resulting from the presence of chylomicrons or chylomicron remnants.

Plasma triglyceride concentration has a much greater biological variation than does that of cholesterol, at ~20% even in fasting samples.

3- High-density lipoprotein cholesterol

Increasing concentrations of HDL particles are strongly associated with decreasing accumulation of atherosclerosis within the walls of arteries, this makes the measurement of HDL an essential component of assessing the cardiovascular risk for patients.

4- Low-density lipoprotein cholesterol

LDL-cholesterol (LDL-C) can be derived by substituting the results of the analysis of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) (fasting) into the Friedewald formula: (all measurements being in mmol/L).

LDL- C = T C − H D L-C - TG/2.2

This formula is not applicable to subjects with plasma triglyceride concentrations >4.5 mmol/L since, at these levels, VLDL contains a greater proportion of triglycerides, and so the formula overestimates VLDL-cholesterol and underestimates LDL-cholesterol.

5- Non-HDL-cholesterol

Non-HDL-cholesterol is derived simply using the formula (total cholesterol – HDL-cholesterol).

The major difference from LDL-cholesterol is that it includes cholesterol in VLDL.

In some epidemiological studies, it has been shown to predict vascular risk almost as well as apo B and better than LDL-cholesterol. This may simply be because VLDL is atherogenic in its own right alone. Other advantages of non-HDL-cholesterol are that it can be measured in non-fasting samples and that its measurement has better precision and accuracy than Friedewald-derived LDL-cholesterol.

Clinical tips about the prevention of coronary heart disease (CHD)

1- For the primary prevention of CAD, adults 20 years of age or older should have their fasting lipoprotein profile (total cholesterol, triglycerides, HDL-C, and LDL-C) measured once every 5 years. If a fasting sample is not available, then only total cholesterol and HDL-C should be considered.

2- The risk factors for the development of CAD are:

- Cigarette smoking
- Hypertension (blood pressure ≥140/90 mm Hg or on antihypertensive medication)
- Low HDL cholesterol (<40 mg/dL)
- Family history of premature CAD (CAD in male first-degree relative <55 years; CAD in female first-degree relative <65 years)
- Age (men ≥45 years; women ≥55 years)

3- The goal LDL according to risk factors:

Risk Category	LDL Goal, mg/dL
CAD and CAD risk equivalents (high risk) *	<70
Multiple (2+) risk factors (moderate risk)	<130
0-1 risk factor (low risk)	<160

*

Other forms of atherosclerotic disease: peripheral arterial disease, abdominal aortic aneurysm, and symptomatic carotid artery disease or diabetes.

These goals of LDL were updated when further studies have shown that around 40% of patients who developed CAD had LDL < 130 mg/dl. So, the moderate risk group was subcategorized into two groups:

Moderately high risk: LDL goal <130 mg/dl **(<100 mg/dl optional)**

Moderate risk: LDL goal <130 mg/dl

The factors that determine the moderately high-risk group are:

a- advanced age
b- severe risk factors (continued smoking, strong family history of premature CAD)
c- high TG level (>200 mg/dl) with elevated non-HDL cholesterol (>160 mg/dl)
d- low HDL (<40 mg/dl)
e- metabolic syndrome

4- Children over the age of 2 should be screened for hypercholesterolemia when they have a parent with hypercholesterolemia (>240 mg/dL) or positive family history (mother, father, uncle, aunt, or grandparent) with early documented CAD (at 55 years or less) such as myocardial infarction, angina pectoris, peripheral vascular disease, cerebrovascular disease, or sudden cardiac death.

5- Once patients begin a treatment plan, providers should reassess at 4 to 12 weeks with a fasting or non-fasting lipid test and check for statin intolerance, with retesting every 3 to 12 months if needed.

CAD risk equivalent is the risk of developing a major coronary event (MI + coronary death) over 10 years >20%.

According to 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease:
-The main objective of primary prevention is to reduce the risk of atherosclerotic cardiovascular disease (ASCVD).
- The initial step should be estimating the 10-year ASCVD risk of.
- many equations are available to calculate the risk such as Framingham risk score calculation.
-Individuals will be categorized into low (<5%), borderline (5 to <7.5%), intermediate (≥7.5 to <20%), or high (≥20%) 10-year risk.
- For those with borderline risk, other risk factors will determine the indication of statin use.

Management of Lipoprotein Disorders

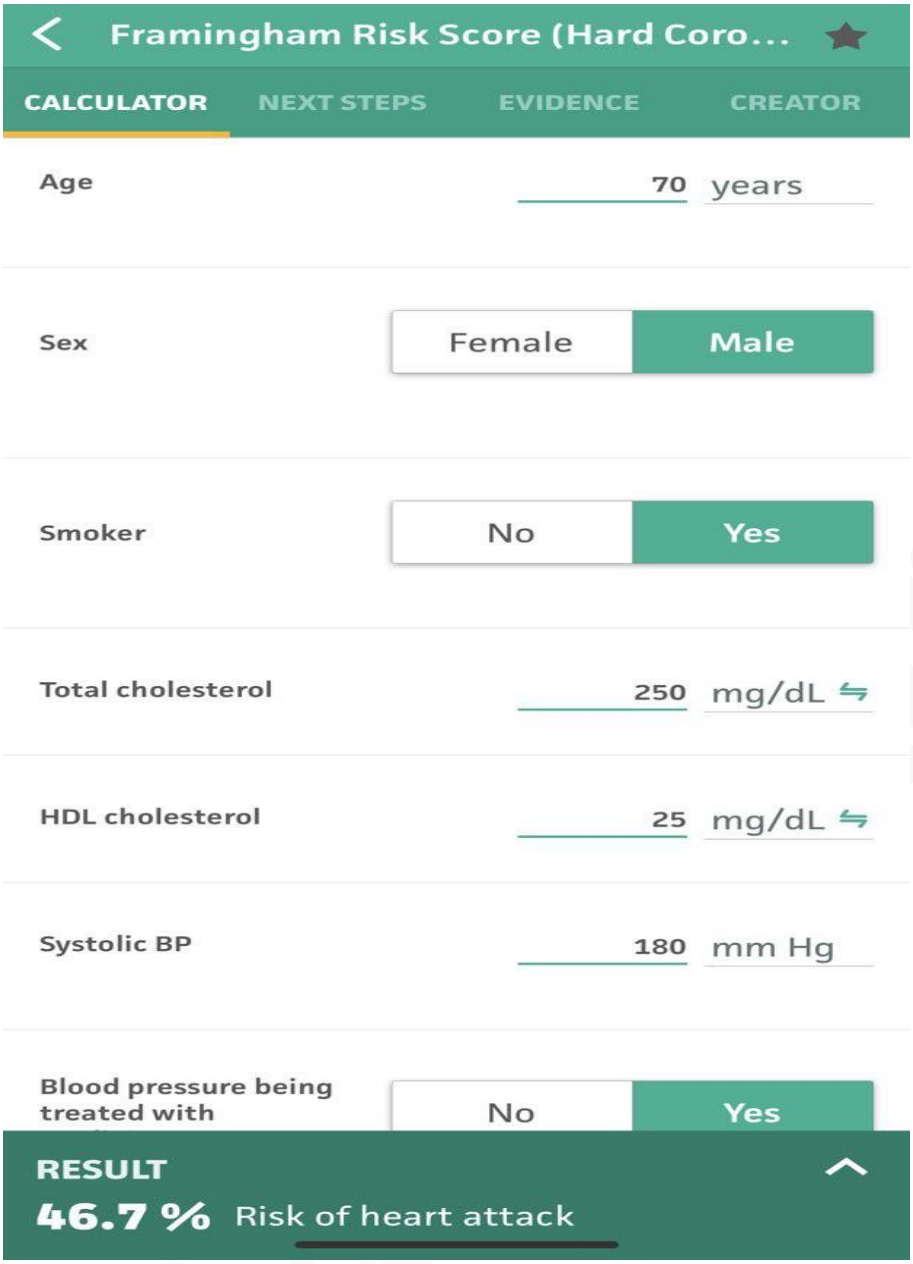
1- reduction in saturated fat and cholesterol intake

2- increased physical activity

3- weight control.

4- Drugs:

A wide variety of pharmacologic agents are available for cholesterol lowering in adults, including:
(1) bile acid– binding resins (cholestyramine and colestipol),
(2) niacin,
(3) gemfibrozil,
(4) ezetimibe,
(5) HMG-CoA reductase inhibitors (e.g., atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin); the latter group has been found to reduce LDL cholesterol by as much as 40%.
Some of these drugs are better tolerated by individual patients than others, and all have demonstrated long-term safety.
Niacin is the only FDA approved drug that can significantly raise HDL cholesterol. Its use has been limited by side effects from flushing, although newer formulations have reduced this problem.
These drugs can be used individually or in combination.



Example of calculating cardiovascular risk score using Framingham equation.

Used for:

Patients aged 30-79 years with no prior history of coronary heart disease.

Do not use in patients with intermittent claudication or diabetes.

23-24

جامعة الفراهيدي – كلية الصيدلة

Liver Function Tests

Clinical Chemistry for 5th-year Pharmacy Students

Objectives:

1. **Reviewing anatomy, biochemistry and physiology of the liver.**
2. **Learning the clinical application of the main components of liver function test.**
3. **Learning the diagnostic laboratory alterations of liver function tests in the most common liver diseases.**

ا.م.د. رائد ضياء هاشم

Liver function tests

The liver is the second largest (after the skin) organ in the human body and the largest gland (weighing an average of 1500 g). It lies under the diaphragm in the right upper abdomen and mid-abdomen.

The liver has a central and critical biochemical role in the:

(1) metabolism

(2) digestion

(3) detoxification

(4) elimination of substances from the body.

All blood from the intestinal tract initially passes through the liver, where products derived from the digestion of food are processed, transformed, and stored. These include amino acids, carbohydrates, fatty acids, cholesterol, lipids, vitamins, and minerals.

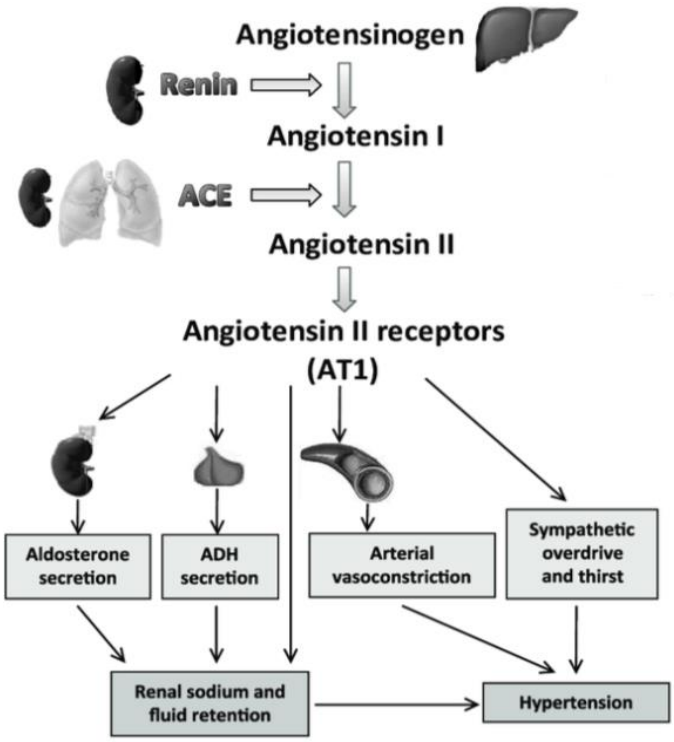
The liver responds to multiple hormonal and neural stimuli to regulate blood glucose concentration. Not only does it extract glucose from the blood for use in generating energy, but it also stores dietary glucose as glycogen for later use. The liver is also the major site for gluconeogenesis, which is critical for maintaining blood glucose in the fasting state.

The liver is central in lipid metabolism; it extracts and processes dietary lipids, and it is the principal site of cholesterol, triglycerides, and lipoprotein synthesis.

Another major liver function is the synthesis of bile acids from cholesterol with the secretion of these compounds into the bile, facilitating the absorption of dietary fat and fat-soluble vitamins.

The liver is also the primary site of metabolism of both endogenous substances and exogenous compounds, such as drugs and toxins. This process, known as biotransformation, converts lipophilic substances to hydrophilic ones for subsequent elimination.

The liver is a major site of the catabolism of hormones and thus participates in the regulation of plasma hormone concentrations. The liver is also involved in hormone synthesis, producing such hormones as insulin-like growth factor 1, angiotensinogen, hepcidin, thrombopoietin, erythropoietin, and prohormone 25-OH vitamin D.



- Hepcidin is the principal regulator of iron absorption and its distribution to tissues.
- Its concentration is inversely related to iron stores.
- Its concentration is increased during inflammation leading rapid decrease in circulating iron as it impairs iron absorption in GIT and iron release

In many cases, individuals with liver disease maintain normal function despite extensive liver damage. In such cases, liver disease may be recognized only by using tests that detect injury. Most commonly, this is accomplished by measuring plasma activities of enzymes found within liver cells, released in somewhat specific patterns with different forms of injury.

Liver disease is relatively common, and the measurements of serum levels of bilirubin, hepatic enzymes and albumin, as well as the prothrombin time (PT), provide simple tests to determine whether a disease is present and give some guidance as to its nature.

Structure of the liver

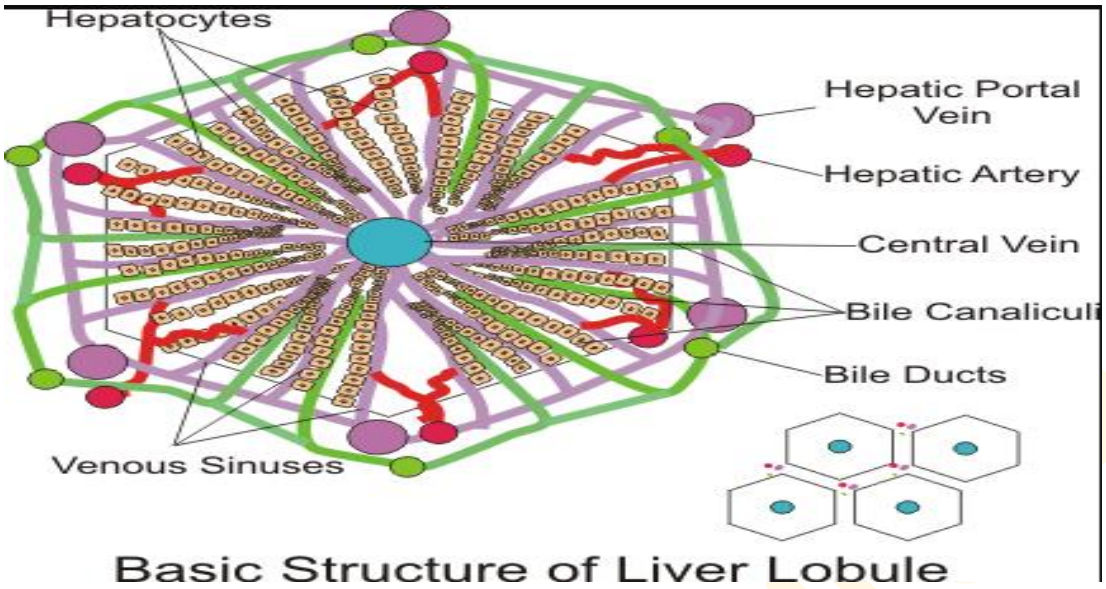
The adult, liver weighs approximately 1.2 to 1.5 kg, and it has a dual blood supply:

(a) the portal vein, which carries blood from the spleen and nutrient-enriched blood from the gastrointestinal (GI) tract, supplies approximately 70% of the blood supply;

(b) the hepatic artery.

Only about 80% of the cells in the liver are hepatocytes; the remainder consists of endothelial (Kupffer) cells lining the hepatic sinusoids and vascular and supporting tissue cells.

The functional unit of each liver acinus consists of the portal tract, surrounded by radiating cords of hepatocytes. Blood enters the acinus via the portal tract and passes along the sinusoids towards the central vein.



Basic Structure of Liver Lobule

Biochemical functions of the liver

The liver is involved in various excretory, synthetic, and metabolic functions. Clinical laboratories perform numerous tests that are useful in the biochemical assessment of these functions.

I- Hepatic Excretory Function

Organic compounds of both endogenous and exogenous origin are extracted from the sinusoidal blood, biotransformed, and excreted into the bile or urine. Assessment of this excretory function provides valuable clinical information.

The most frequently used tests involve the measurement of plasma concentrations of endogenously produced compounds, such as bilirubin and bile acids, and the determination of the rate of clearance of exogenous compounds, such as aminopyrine, lidocaine, and caffeine.

1- Bilirubin

Bilirubin is the orange-yellow pigment derived from heme, mainly as a product of red blood cell turnover. It is extracted and biotransformed in the liver and excreted in bile and urine.

Bilirubin is a product of heme catabolism. Red cell hemoglobin accounts for approximately 85% of all bilirubin. Heme is catabolized to unconjugated bilirubin in the reticuloendothelial system. Unconjugated bilirubin is bound to albumin in the plasma and transported bound to

albumin to the liver and is conjugated with glucuronic acid in the hepatocytes; the conjugation is catalyzed by glucuronyl transferase.

Conjugated bilirubin is secreted into the bile and enters the duodenum. In the intestinal tract, bilirubin glucuronides are hydrolyzed and reduced by bacteria to form colourless urobilinogen, which undergoes enterohepatic circulation.

A small fraction (2 to 5%) of urobilinogen escapes the liver and is excreted in the urine. In the colon, urobilinogen spontaneously oxidized to stool pigments stercobilin, mesobilin, and urobilin.

Clinical application

Increased plasma bilirubin (hyperbilirubinemia) typically is classified as:

1- indirect (unconjugated bilirubin)

2- direct (conjugated bilirubin).

Causes of hyperbilirubinemia are usually classified into:

1- prehepatic

Due to overproduction of bilirubin, usually caused by hemolysis (Thalassemia), or decreased metabolism by the liver. This condition is associated with increased indirect bilirubin and urobilinogen in urine.

2- hepatic

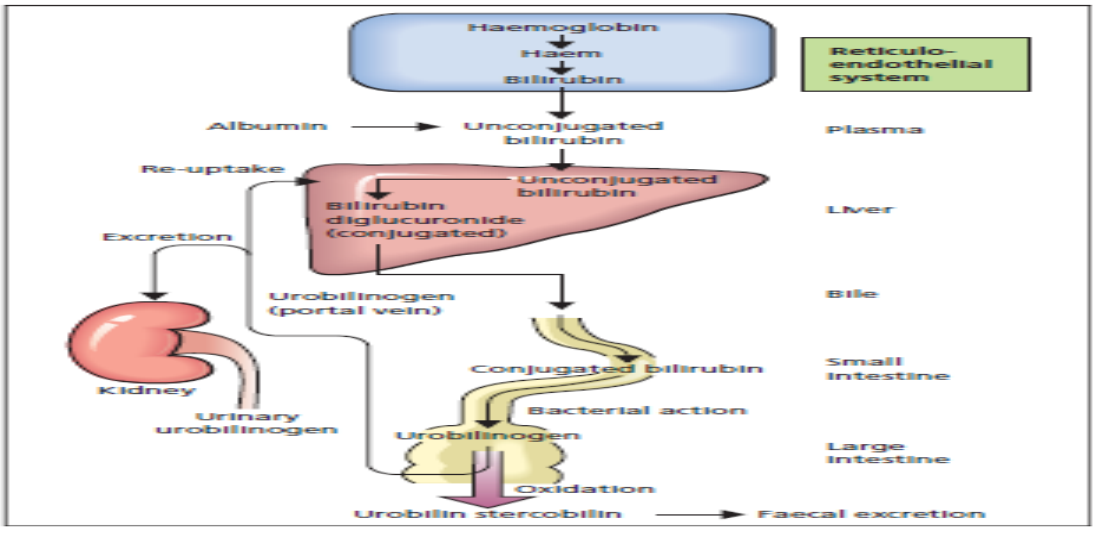
Due to an inflammatory process in the liver tissue itself as in hepatitis and liver cirrhosis.

In this case, both direct and indirect bilirubin are elevated.

3- posthepatic

Due to functional or mechanical impairment in bilirubin excretion from the hepatocyte.

This condition is associated with a marked increase in direct bilirubin and the presence of bilirubin in urine.



2-Bile Acids

Regulation of bile acid metabolism is a major function of the liver. Alterations in bile acid metabolism are usually a reflection of liver dysfunction.

Cholesterol homeostasis is in large part maintained by the conversion of cholesterol to bile acids and subsequent regulation of bile acid metabolism. Bile acids themselves provide surface-active detergent molecules that facilitate both hepatic excretion of cholesterol and solubilization of lipids for intestinal absorption.

Bile acid homeostasis requires normal terminal ileum function to absorb bile acids for recirculation (enterohepatic circulation). Alterations in hepatic bile acid synthesis, intracellular metabolism, excretion, intestinal absorption, or plasma extraction are reflected in derangements of bile acid metabolism.

II- Hepatic Synthetic Function

The liver has an extensive synthetic capacity and plays a major role in the regulation of protein, carbohydrate, and lipid metabolism. The liver is also involved in hormone synthesis, producing such hormones as insulin-like growth factor 1, angiotensinogen, hepcidin, thrombopoietin, erythropoietin, and the prohormone 25-OH vitamin D. Protein, triglycerides, fatty acid, cholesterol, and bile acid synthesis occurs within the liver.

1- Protein Synthesis

The liver is the primary site of the synthesis of plasma protein. Although disturbances of protein synthesis occur as a consequence of impaired hepatic function, a variety of other factors may affect plasma protein concentrations. These include:

(1) decreased availability of amino acids (malnutrition, maldigestion, and malabsorption)

(2) catabolic states (hyperthyroidism, Cushing's syndrome, burns)

(3) protein-losing states (nephrotic syndrome and protein-losing enteropathy)

(4) the action of hormones (such as growth hormone, cortisol, estrogen, androgens, and thyroid hormones).

As previously mentioned, most plasma proteins are synthesized in the liver, examples include:

a- Albumin

b- Immunoglobulins

c- Ceruloplasmin

d- Coagulation Proteins

2- Lipid and Lipoprotein Synthesis

The liver plays a key role in the metabolism of lipids and lipoproteins. On a daily basis, approximately 33% of the fatty acids originating from adipose tissue enter the liver, where they undergo esterification into triglycerides or are oxidized.

Oxidation is favoured in the fasting state and esterification is favoured in the nonfasting state. Excessive esterification results in "fatty liver" a disorder in which excess triglycerides are deposited in large vacuoles that displace other cellular components.

Most cholesterol is endogenously synthesized in the liver. Endogenous cholesterol and cholesterol of dietary origin enter the hepatic pool, where they are:

1- converted to bile acids.

2- incorporated into lipoproteins, or

3- used in the synthesis of liver cell membranes.

Fatty liver is a condition associated with accumulation of fat in hepatocytes.
Two main types of fatty liver are present:
Alcohol related.
Non-alcohol related fatty liver disease (NAFLD).
- Risk factors of NAFLD are: obesity, DM, age, smoking, hypercholesterolemia and metabolic syndrome.
- Early stages are not harmful but in few numbers of cases it can eventually progress to serious liver disease, stages of NAFLD are:
1- steatosis
2- nonalcoholic steatohepatitis (NASH)
3- fibrosis
4- cirrhosis
- Early diagnosis can be done by ultrasonic examination.
- Elevated liver enzymes is an alarming sign of progression.
- No specific treatment is available.

3- Urea Synthesis

The urea cycle mediates the removal of ammonia as urea in the amount of 10 to 20 g per day in a healthy adult. The absence of a fully functional urea cycle may result in hyperammonemic encephalopathy and irreversible brain injury in severe cases.

Patients with end-stage liver disease may have low concentrations of urea in plasma, and the rate of urea excretion in urine becomes lower than in healthy individuals with increased plasma concentrations of urea precursors (ammonia and amino acids).

These findings suggest that patients with liver disease have an impaired ability to metabolize protein nitrogen and synthesize urea. The rate of hepatic urea synthesis also depends on the exogenous intake of nitrogen and endogenous protein catabolism.

III- Hepatic Metabolic Function

It represents the mainstay of the whole function of the liver. The metabolism of most drugs (activation and detoxification) and the disposal of exogenous and endogenous substances, such as galactose and ammonia occur in the liver.

1-Ammonia Metabolism

The major source of circulating ammonia is the GI tract. Plasma ammonia concentration in the hepatic portal vein is typically fivefold to tenfold higher than that in the systemic circulation. It is derived from the action of bacterial enzymatic action on the contents of the colon and the hydrolysis of glutamine in both the small and large intestines.

Elevated concentration of ammonia (hyperammonemia) exerts toxic effects on the central nervous system. Severe or chronic liver failure (as occurs in fulminant hepatitis or cirrhosis, respectively) leads to significant impairment of normal ammonia metabolism, leading to hepatic encephalopathy which is a serious and probably fatal complication of liver disease. This explains the use of wide-spectrum oral antibacterial drugs in addition to laxatives in patients with hepatic encephalopathy.

2- Carbohydrate Metabolism

Because the liver is a major processor of dietary and endogenous carbohydrates, liver disease affects carbohydrate metabolism in a variety of ways.

However, none of the conventional modes of evaluating carbohydrate metabolism has value in the diagnosis of liver disease.

Because the liver is the major site of both glycogen storage and gluconeogenesis, hypoglycemia is a common complication in certain liver diseases, particularly fulminant hepatic failure, advanced cirrhosis, and hepatocellular carcinoma.

IV- Hepatic Storage Function

Because individual cells are unable to store a sufficient supply of energy-rich carbohydrate substrates, the liver serves as the major site for their storage. For example, hepatic storage of glycogen allows the release of glucose to other tissues when the need exists (e.g., when plasma concentrations of glucose decrease). Many other substances are usually stored in the liver such as triglycerides, and vitamin A.

Clinical manifestations of liver disease

Various characteristics indicate the presence of liver disease, including:

(1) jaundice: due to both impaired conjugation and excretion of bilirubin.

(2) itching: mainly in cholestatic liver disease, possibly due to deposition of bile salts in the skin (although the exact cause is not yet understood).

(3) bleeding tendency: due to 1- impaired synthesis of clotting factors, 2- esophageal varices.

(4) edema (leg edema, ascites): due to hypoalbuminemia and portal hypertension.

(5) weight loss

Liver function tests

Several biochemical tests can be used to:

1- Detect the presence of liver disease

2- Distinguish among different types of liver disorders

3- Assess the extent of known liver disease

4- Follow the response to treatment

Liver function tests are classified according to the various functions of the liver:

A- Tests based on excretory function:

1- Serum bilirubin

2- Urine bilirubin

3- Urine urobilinogen

B- Tests based on detoxification function

1- Hippuric acid test

2- Determination of blood ammonia

C- Tests based on synthetic function

1- Serum albumin

2- Prothrombin time

3- Transthyretin (prealbumin)

D- Tests based on metabolic function

1- Galactose tolerance test

2- aminoaciduria

- Albumin synthesis is inhibited in case of acute inflammation by the action of IL-6.
- Although albumin synthesis is impaired in case of liver cirrhosis, a significant number of patients with cirrhosis might have normal or even increased albumin synthesis.
- Loss of albumin into ascitic fluid seems responsible for the decrease in albumin in many cases.
- Transthyretin has a short half-life of 24 to 48 hours, making it a sensitive indicator of current synthetic ability compared to albumin which has a half-life of 21 days.

Measurement of certain enzymes is considered the cornerstone in the evaluation of liver function but, actually, they are indicators for the presence of hepatocyte destruction or cholestasis.

Enzymes that are indicators of the presence of hepatocyte destruction are:

AST & ALT (Aspartate Aminotransferase, Alanine Aminotransferase)

Enzymes that are indicators of the presence of cholestasis or biliary obstruction are:

1- ALP (alkaline phosphatase)

2- GGT (Gamma-Glutamyl Transferase)

3- 5'NT (5'-nucleotidase)

The liver has a limited number of ways of responding to injury. Acute injury to the liver may be asymptomatic but often presents as jaundice.

The major acute liver diseases are:

(1) acute hepatitis and (2) cholestasis.

Chronic liver injury generally takes the clinical form of chronic hepatitis; its long-term complications include cirrhosis and hepatocellular carcinoma. The discussion of liver disease will focus mainly on these patterns.

The natural history of liver disease

With an acute injury to the liver, several outcomes are possible. In many individuals, the damage is clinically unapparent and recovery occurs with the clearance of the causative agent.

In some, clinical acute hepatitis occurs. In the overwhelming majority of these, clearance of the causative agent results in complete recovery; in a very small minority, the damage is so severe that acute liver failure (fulminant hepatitis) develops, which is usually fatal without liver transplantation.

A variable percentage of persons with acute liver injury (dependent on the cause) progress to chronic hepatitis. In some, recovery eventually occurs naturally or following the treatment of the underlying cause.

Among those in whom chronic hepatitis persists, many will never progress to cirrhosis.

A majority of those who do will remain well for many years, but about 3% per year develop decompensated cirrhosis (bleeding varices, ascites, hepatic encephalopathy) or hepatocellular carcinoma. These are the most common causes of death from liver disease.

In summary:

An acute hepatic injury might be asymptomatic, but when leads to acute hepatitis, the outcomes might be either:

1- Full recovery

2- Fulminant hepatitis & death (require urgent liver transplantation)

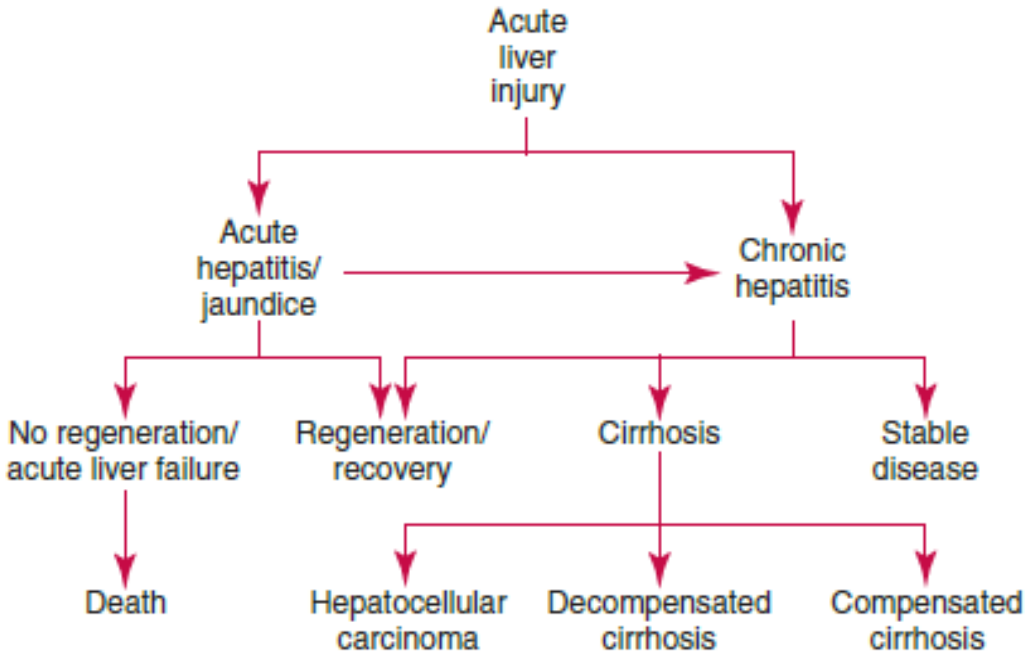
3- Chronic hepatitis

In turn, the outcomes of chronic hepatitis might be either:

1- Full recovery

2- Liver cirrhosis, which in turn can lead to hepatocellular carcinoma

3- Stable chronic hepatitis



Jaundice

Jaundice is defined as yellowish discoloration of skin and sclera due to hyperbilirubinemia.

It is the commonest sign of presentation of various types of liver disease, although it might be a sign of certain disorders other than liver disease such as hemolytic disorders (known as prehepatic jaundice).

Hyperbilirubinemia cannot be presented as jaundice unless the total serum bilirubin exceeds 2.5-3 mg/dl. When hyperbilirubinemia is discovered in a patient, the next step is to determine the cause of hyperbilirubinemia by performing the following laboratory tests:

1- direct bilirubin

2- urinary bilirubin and urobilinogen

3- ALP

4- ALT & AST

Jaundice is usually classified according to the different anatomical sites of pathology:

1- Prehepatic jaundice (increased bilirubin production)

In this type of hyperbilirubinemia, the liver has a normal function, but the rate of bilirubin production is very high which exceeds the capacity of the liver to uptake the whole amount of bilirubin.

- Never treat a patient with jaundice blindly with increasing CHO intake.
- Avoid considering any case of jaundice as a case of hepatitis A, you might miss a more serious cause.

Common causes:

A- malaria,

B- hemolytic anemia:

- neonatal jaundice
- sickle cell crisis
- spherocytosis
- thalassemia
- autoimmune disorders
- glucose-6-phosphate dehydrogenase deficiency (G6PD)

The expected changes in liver function tests:

1- TSB >>> high but composed mainly of indirect bilirubin ***(direct bilirubin will be normal)***

2- ALP >>> normal ***(beware that ALP is physiologically high in children)***

3- Bilirubin in urine >>> absent

4- Urobilinogen in urine >>> increased

5- ALT >>> normal

6- AST >>> normal or slightly increased

2- Hepatic jaundice (liver dysfunction)

In this type of hyperbilirubinemia, the liver function is impaired mostly by a direct inflammatory process (other causes are present) that leads to impairment of conjugation of bilirubin. In most cases, this is associated with cholestasis.

Common causes:

- hepatitis (commonly viral or alcohol related),
- cirrhosis,
- drugs
- Crigler-Najjar syndrome
- Gilbert's syndrome
- cancer.
- metabolic (Wilson's disease, hemochromatosis)

The expected changes in liver function tests:

1- TSB >>> high ***(both direct and indirect bilirubin will***

2- ALP >>> slightly high ***(beware that ALP is physiologi***

3- Bilirubin in urine >>> present

4- Urobilinogen in urine >>> normal or absent

5- ALT >>> extremely high

6- AST >>> extremely high

Gilbert's syndrome is a common autosomal recessive disorder associated with impaired conjugation of bilirubin due to decreased activity of UDG transferase.
- unconjugated hyperbilirubinemia occurs during fasting, menstruation or illness.
- usually TSB doesn't exceed 3.5 mg/dl although it rarely reaches 6 mg/dl.
- it is either asymptomatic or associated with non-specific symptoms.
- no treatment is required but phenobarbital can be used in severe cases.
- males are more affected.
- diagnosis is made by exclusion.
- liver enzymes are within normal.

Wilson's disease is an autosomal recessive disorder associated with ceruloplasmin deficiency that leads to increased levels of free copper that will precipitate in various tissues including liver and nervous system and causing serious damage.
- it is treated with chelating agents to increase urinary excretion of copper.

3- Posthepatic jaundice (obstructive)

In this type of hyperbilirubinemia, initially, the liver has a normal function, but the capacity to excrete conjugated bilirubin is greatly impaired due to obstruction of the biliary tract (totally or partially).

Common causes :

- gallstones in the bile duct
- cancer
- strictures of the bile duct
- cholangitis,
- congenital malformations

The expected changes in liver function tests:

1- TSB >>> high (***direct bilirubin composes most of the total bilirubin)***

2- ALP >>> extremely high ***(beware that ALP is physiologically high in children)***

3- Bilirubin in urine >>> present

4- Urobilinogen in urine >>> absent

5- ALT >>> normal or slightly high

6- AST >>> normal or slightly high

	S. Bilirubin		U. Urobilinogen	U. Bilirubin	F. Urobilinogen
	Conjugated	Unconjug.			
Normal	**0.1-0.4 mg/dl**	**0.2-0.7 mg/dl**	Present	Absent	Present
Prehepatic	Normal	Increased	Increased	Absent	Increased
Hepatic	Increased	Increased	N/Decreased	Present	Decreased
posthepatic	Increased	Normal	Absent	Present	Absent

Examples of common liver disease will be discussed here:

I- Acute Hepatitis

Hepatitis, a general term referring to inflammation of the liver, may result from various causes, these include:

A- Infectious causes:

1- viral (hepatitis A,B,C,D)

2- bacterial, fungal, and parasitic organisms

B- noninfectious

1- alcohol

2-drugs

3- autoimmune diseases

4-metabolic diseases.

Viral hepatitis encounters more than 50% of all cases of acute hepatitis all over the world. The clinical and laboratory presentation of various causes of acute hepatitis is nearly identical with minimal difference in the severity of symptoms but the outcomes are greatly variable, these outcomes are:

1- full recovery

2- chronic hepatitis

3- fulminant hepatitis and death

The most important indicator of prognosis in acute viral hepatitis is impairment in synthetic function, with prothrombin time (PT) a widely accepted indicator.

In acute viral or alcoholic hepatitis, PT more than 3 seconds above normal is associated with a poor prognosis.

Types of Viral Hepatitis

	A	B	C	D	E	G
Type	RNA	DNA	RNA	Partial	RNA	RNA
Incubation period, d	45-50	30-150	15-160	30-150	20-40	Unknown
Transmission						
Fecal-oral	Yes	No	Minimal	No	Yes	No
Household	Yes	Min	Min	Yes	Yes	No
Vertical	No	Yes	Min	Yes	No	Yes
Blood	Rare	Yes	Yes	Yes	Unknown	Yes
Sexual	No	Yes	Min	Yes	Unknown	Yes
Diagnosis	Anti-HAV IgM	HBsAg, PCR, anti-HBc IgM	Anti-HCV, PCR	Anti-HDV	Anti-HEV	Anti-HGV
Carrier state	No	Yes	Yes	Yes	Yes	Yes
Chronic hepatitis	No	Depends on age, immune status	50-70%	Yes	Rare†	No
Liver cancer	No	Yes	Yes	No	No	No
Prevention						
Vaccine	Yes	Yes	No	Yes*	No	No
Immunoglobulin	Yes	Yes	No	Yes*	No	No
Response to interferon	Not used	30%	40-80%	Yes	Not used	Yes

Although jaundice is a key clinical finding in acute hepatitis, it is often absent. Characteristic laboratory findings include:

1- Elevated AST and ALT (*An increase in AST activity to greater than 200 U/L, or in ALT activity to greater than 300 U/L, has sensitivity and specificity greater than 90% for acute hepatitis).*

2- ALP is elevated up to 3 times the upper reference range in 90% of cases.

3- Total serum bilirubin is elevated, the conjugated bilirubin represents the predominant form although the unconjugated form is found to be higher in 15% of cases of acute hepatitis. It is worth mentioning that many patients with acute hepatitis have normal TSB (anicteric hepatitis).

II- Chronic Hepatitis

Chronic hepatitis is defined as chronic inflammation of the liver that persists for at least 6 months, or signs and symptoms of chronic liver disease in the presence of elevated aminotransferases.

It is characterized by ongoing inflammatory damage to hepatocytes, often accompanied by hepatocyte regeneration and scarring.

Clinical features and laboratory changes

The clinical features of chronic hepatitis are highly variable. Most patients are asymptomatic, but nonspecific features such as fatigue, lack of concentration, and weakness may be present.

Most patients are diagnosed because of an unexplained abnormality in aminotransferase activities. Moderate elevations in aminotransferase activities (an average of about twofold, and in most cases less than fivefold) are characteristic, whereas the results of most other tests are normal.

Normal aminotransferase activities do not rule out histologic evidence of chronic hepatitis, especially in the presence of chronic viral hepatitis.

Characteristically, ALT is elevated to a greater degree than AST, although elevations in both are common; reversal of the AST/ALT ratio to greater than 1 suggests coexisting alcohol abuse or development of cirrhosis.

The causes of chronic hepatitis and required investigation are shown in the following table:

Causes of Chronic Hepatitis

Cause	Diagnosis
Hepatitis B	History, HBsAg, anti-HBs, anti-HBc, HBV DNA
Hepatitis C	Anti-HCV, HCV RNA by PCR
Autoimmune type 1	ANA, anti–smooth muscle antibody
Autoimmune type 2	SLA, anti-LKM_1
Wilson's disease	Ceruloplasmin
Drugs	History
α_1-Antitrypsin deficiency	α_1-AT phenotype
Nonalcoholic fatty liver disease (NAFLD)	Metabolic syndrome, liver ultrasound, liver biopsy
Idiopathic	Liver biopsy, absence of markers

III- Liver Cirrhosis

Cirrhosis, defined anatomically as diffused fibrosis with nodular regeneration, represents the end stage of scar formation and regeneration in chronic liver injury.

This response to injury occurs independently of the etiology and thus it is not possible, in most circumstances, to determine the cause of cirrhosis based on histology.

In clinical practice, all chronic liver diseases are known to lead to cirrhosis, but this conversion is gradual and may take years. Nevertheless, in "cryptogenic cirrhosis" the cause remains unknown.

The presence of the fibrous scar will eventually increase the pressure in the portal vein leading to portal hypertension which will be followed by shunting of blood from the portal vein into the hepatic vein bypassing the liver and reaching directly to the circulation.

Portal hypertension and impaired lymphatic drainage in addition to hypoalbuminemia are factors related to the development of ascites.

Clinical features include:

- Hepatomegaly
- Abdominal pain
- Bleeding
- Weight loss, anorexia and easy fatigability
- Ascites and bilateral pitting leg edema
- Jaundice
- Neurological features of hepatic encephalopathy
- Hepatorenal syndrome with decreased urine output and increased serum creatinine.

The earliest laboratory abnormalities to develop in cirrhosis are:

(1) fall in platelet count

(2) increase in PT

(3) a decrease in the albumin-to-globulin ratio to less than 1

(4) increase in the AST/ALT activity ratio to greater than 1

Surprisingly, jaundice is a late finding in decompensated cirrhosis. Cirrhosis, regardless of its cause, is a major risk factor for the development of hepatocellular carcinoma.

The severity of cirrhosis depends on certain clinical and laboratory findings where the severity is classified into 3 groups that predict the risk of mortality over the next 1-2 years. The grading system that is used to determine the severity of cirrhosis is called the Child–Pugh classification".

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/L)	2 to 3 mg/dL (34.2 to 51.3 micromol/L)	>3 mg/dL (>51.3 micromol/L)
Albumin	>3.5 g/dL (35 g/L)	2.8 to 3.5 g/dL (28 to 35 g/L)	<2.8 g/dL (<28 g/L)
Prothrombin time (seconds over control) or	<4	4 to 6	>6
INR	<1.7	1.7 to 2.3	>2.3
Encephalopathy	None	Grades 1 to 2	Grades 3 to 4
5 to 6: A (well-compensated disease), 7 to 9 is class B (significant functional compromise) 10 to 15 is class C (decompensated disease).			
one- and two-year patient survival: class A: 100 and 85%; class B: 80 and 60%; and class C: 45 and 35%.			

Management

- Ascites: sodium and water restriction, diuretics (spironolactone) and paracentesis.
- Hepatic encephalopathy: lactulose and wide spectrum oral antibacterial drug.
- Portal hypertension: non-selective beta-blockers (propranolol)
- Esophageal varices: Endoscopic sclerotherapy
- Consider liver transplantation.

IV- Hepatic failure and hepatic encephalopathy

Both severe acute hepatitis and advanced liver cirrhosis might be associated with various physiological derangements designated as hepatic failure. The condition could be precipitated in acute, acute on chronic or chronic patterns. With this severe impairment of liver functions, all organs and systems will be affected specifically the nervous system. Surprisingly, AST and ALT activity will be greatly reduced as the total number of hepatocytes is diminished. The characteristic findings include:

- Hypoglycemia: due to impaired hepatic gluconeogenesis.
- Hyperammonaemia: due to impaired conversion of ammonia into urea.
- Hypovolaemia and hypotension: due to loss of the intravascular fluid in the peritoneal cavity and interstitial compartment.

V- Reye's syndrome

This condition is characterized by:

- It is a rare disorder that presents as acute hepatitis.
- Occurs in children and is associated with: marked encephalopathy, severe metabolic acidosis and hypoglycaemia.
- Acute fatty infiltration of the hepatocytes with a marked increase in aminotransferases while slight increases in bilirubin.
- maybe precipitated by viral infections, such as influenza A or B, or drugs such as salicylates.

Anicteric hepatitis

In certain circumstances, acute hepatitis might not be associated with jaundice or even asymptomatic. This condition is more commonly seen in acute infection with HCV and in children infected with HAV. The patient presents with a flu-like illness and may remain without diagnosis. Typically, the patient has an increased activity of liver enzymes typical to that of any acute hepatitis, but serum bilirubin is usually normal or minimally increased.

23-24

جامعة الفراهيدي – كلية الصيدلة

RENAL FUNCTION TESTS

Clinical Chemistry for 5th-year Pharmacy Students

Objectives:

1- **Reviewing the biochemical aspects of the renal system.**

2- **Learning how to interpret the results of renal function tests.**

3- **Discussing the main examples of renal diseases.**

4

ا.م.د. رائد ضياء هاشم

RENAL FUNCTION TESTS

The kidneys play a central role in the homeostatic mechanisms of the human body, and reduced renal function strongly correlates with increased morbidity and mortality.

The functional unit of the kidney is the nephron, with each kidney containing between 0.6 and 1.2 million nephrons. In nearly all types of renal disease, impaired function of the kidneys is attributed to a diminished number of functioning nephrons rather than to the compromised function of individual nephrons.

Biochemical investigations, both routine and specialized, are an important part of the clinician's diagnostic plan, and investigations of kidney function constitute a significant element of the workload of most laboratories.

The basic anatomy and physiology of the kidneys are important for understanding the pathophysiology of disease and the rationale for diagnostic and management strategies in kidney disease.

Anatomy and physiology of nephron

The nephron is the functional unit of the kidney, the number of nephrons that an individual is born with (the nephron dose) may determine the individual's susceptibility to renal injury. The nephron consists of a glomerulus, proximal tubule, loop of Henle, distal tubule and collecting duct. The collecting ducts ultimately combine to develop into the renal calyces, where the urine collects before passing along the ureter and into the bladder.

The kidney is divided into several lobes. The outer, darker region of each lobe, the cortex, consists of most of the glomeruli and the proximal and distal tubules. The cortex surrounds a paler inner region, the medulla, which is further divided into a number of conical areas known as the renal pyramids, the apex of which extends toward the renal pelvis, forming papillae.

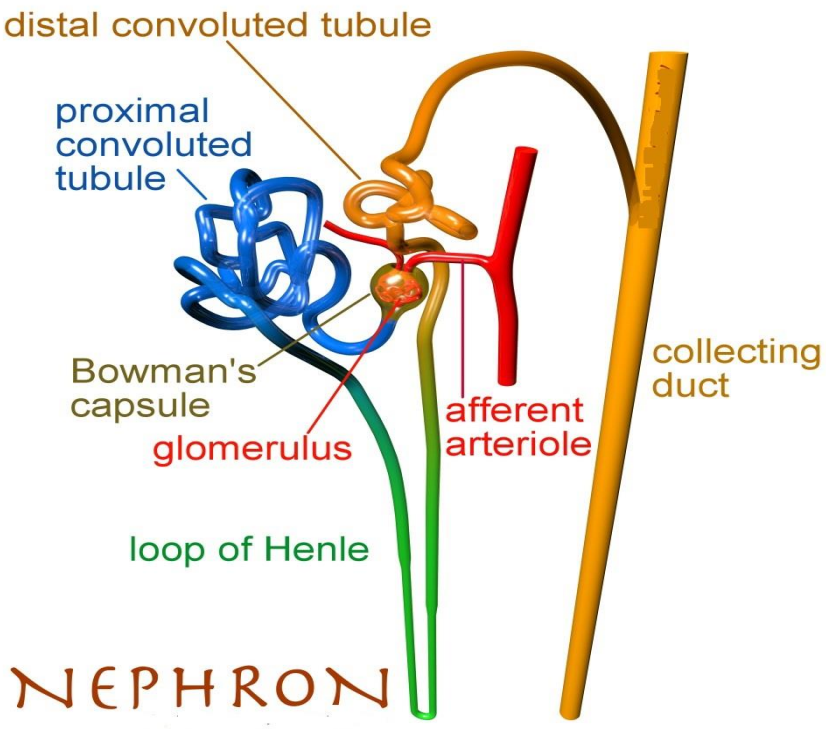
The glomerulus is formed by the invagination of a tuft of capillaries into the dilated, blind end of the nephron (Bowman's capsule).

The capillaries are supplied by an afferent arteriole and drained by the efferent arteriole and it is from the glomerulus that the filtrate is formed. The diameter of the afferent arteriole is larger than the efferent arteriole.

Two cellular layers separate the blood from the glomerular filtrate in Bowman's capsule: the capillary endothelium and the specialized epithelium of the capsule.

Functionally, the glomerular membrane permits the free passage of neutral substances up to 4 nm in diameter and almost totally excludes those with diameters greater than 8 nm. However, the charge on molecules, as well as their diameters, affects their passage into Bowman's capsule.

The proximal convoluted tubule is about 15 mm long and 55 µm in diameter. Its wall is made up of a single layer of cells that interdigitate with one another and are united by apical tight junctions.



Structure of nephron

Functions of PCT

1- PCT reabsorbs 2/3 of the filtered Na or (65-80% of Na) and H2O.

2- It reabsorbs all of the glucose and amino acids.

The proximal convoluted tubule straightens and the next portion of each nephron is the **loop of Henle**. The descending portion of the loop and the proximal portion of the ascending limb are made up of thin, permeable cells. On the other hand, the thick portion of the ascending limb is made up of thick cells containing many mitochondria.

The Loop of Henle reabsorbs 10-20% sodium and chloride and 10% of the filtered water.

The specialized cells at the end of the ascending loop of Henle form the macula densa, which lie in between the efferent and the afferent arteriole.

The macula, the neighboring lacis cells, and the renin-secreting granular cells in the afferent arteriole form the juxtaglomerular apparatus.

The distal convoluted tubule, which starts at the macula densa, is about 5 mm long.

The distal tubules coalesce to form collecting ducts that are about 20 mm long and pass through the renal cortex and medulla to empty into the pelvis of the kidney.

Functions

1- it actively reabsorbs sodium and chloride

2- it is relatively impermeable to water, but in the presence of the antidiuretic hormone (ADH), its permeability to water increases making urine concentrated.

3- it secretes ammonium ions and hydrogen ions.

Collecting Duct

The collecting ducts are formed from approximately six distal tubules. These are successively joined by other tubules to form ducts of Bellini, which ultimately drain into a renal calyx.

Functions:

1- Reabsorbs sodium and water

2- Secretes potassium

Kidney function

The kidneys regulate and maintain the constant optimal chemical composition of the blood and interstitial and intracellular fluids throughout the body through the integration of the major renal functions. The glomeruli have a distinct function that differs from the tubular function, collectively known as renal function.

The main renal functions are classified into:

1- Excretory Functions

The *excretory function* of the kidneys serves to rid the body of many end products of metabolism and of excessive inorganic substances ingested in the diet. Waste products include the nonprotein nitrogenous compounds urea, creatinine, and uric acid; a number of other organic acids, including amino acids, are excreted in small quantities.

2- Reabsorptive Functions

Reabsorption at the renal tubules is mandatory to preserve essential molecules. Both excretory and absorptive functions work together for the homeostasis of most plasma compounds.

The end result of the excretory and the reabsorptive functions of the kidneys is the formation of urine which has a highly variable concentration of its contents.

So, urine formation depends on three renal processes:

1- glomerular filtration.

2- tubular excretion

3- tubular reabsorption

According to the previous facts, it becomes logical to say that the formation of urine depends on the following renal functions:

1- filtration

2- secretion

3- reabsorption

So: **volume of urine = filtrate + secretion – reabsorption**

3- Regulatory Functions

The main regulatory functions of kidneys include:

A- electrolyte homeostasis:

Electrolytes are Na, K, Cl, Ca, HCO3, PO4, Mg.

Kidneys play a crucial role in the homeostasis of these electrolytes via various mechanisms in accordance with hormonal regulation or other mechanisms.

For example, calcium homeostasis depends on renal function as well as the action of vitamin D and parathyroid hormone.

B- water homeostasis:

Approximately 180 L of glomerular filtrate is formed each day, and approximately 99% of this is reabsorbed in the production of urine.

In the kidney, different segments of the nephron show differing permeability to water, enabling the body to both retain water and produce urine of variable concentrations.

4- Endocrine Function

The *endocrine functions* of the kidneys may be regarded as primary because the kidneys are endocrine organs producing hormones, or as secondary because the kidneys are a site of action for hormones produced or activated elsewhere. In addition, the kidneys are a site of degradation for hormones such as insulin and aldosterone.

In their primary endocrine function, the kidneys produce erythropoietin (EPO), prostaglandins and thromboxanes, 1,25(OH2)D3, and renin.

A - The importance of *renin* in the maintenance of systemic blood pressure.

B - *Erythropoietin*: is responsible for stimulating erythroid progenitor cells within the bone marrow to produce red blood cells, this is why a patient with chronic renal failure usually develops anemia.

C - *Prostaglandins and Thromboxanes***:** have an important role in regulating the physiologic action of other hormones on renal vascular tone, mesangial contractility, and tubular processing of salt and water.

*D- 1,25(OH2)D3***:** The kidneys are primarily responsible for producing 1,25(OH2)D3 from 25-hydroxycholecalciferol as a result of the action of the enzyme 25-hydroxycholecalciferol 1α-hydroxylase found in proximal tubular epithelial cells. Vitamin D is responsible for calcium homeostasis.

Glomerular Filtration Rate (GFR)

The GFR is considered to be the most reliable measure of the functional capacity of the kidneys and is often thought of as indicative of the number of functioning nephrons.

As a physiological measurement, it has proved to be the most sensitive and specific marker of changes in overall renal function.

GFR describes the flow rate of filtered fluid through the kidney. Its reference range is: 90-125 ml/min, and the severity of kidney disease is usually related to the degree of the decrease in GFR.

Chronic kidney disease is classified in accordance with GFR into:

Stage	GFR	Description
1	90+	Normal kidney function but urine findings or structural abnormalities or genetic traits point to kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3A 3B	45-59 30-44	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15 or on dialysis	Very severe, or end-stage kidney failure (sometimes called established renal failure)

There are many physiological causes that lead to increased GFR such as:

1- pregnancy

2- fever

3- exercise

4- increased intravascular volume

Kidney diseases can be classified into:

A- acute and chronic

B- glomerular and tubular

Components of renal function tests

I- Urine analysis

Examination of the urine is often the first step in the assessment of a patient suspected of having a deterioration in kidney function. Both macroscopic and microscopic examinations could be helpful.

Examples of macroscopic changes that might indicate significant pathology include:

Macroscopic changes	Clinical significance
pink-red-brown colour	Hematuria, Myoglobinuria
Turbidity	Infection, fat particles in a patient with nephrotic syndrome
Excessive foaming when shaken	proteinuria.

Reagent Strip (Dipstick) Testing

With this type of test, different methods detect substances that overflow into the urine, such as glucose, ketones, bilirubin, nitrite, pH, and urobilinogen, changes in the concentration of which reflect a change in another organ system in the body.

Microscopic Examination of Urine

Microscopic examination of the sediment obtained from centrifugation of a fresh urine sample shows the presence of (1) a few cells (erythrocytes, leukocytes, and cells derived from the kidney and urinary tract), (2) casts.

An increase in red cells or casts implies hematuria possibly caused by glomerular disease; white cells or casts imply the presence of white cells in the tubules.

Quantitative Assessment of Proteinuria: Total Protein and Albumin

Higher molecular weight proteins are retained within the circulation by the glomerular filter, and lower molecular weight proteins are freely filtered, reabsorbed and catabolized within the tubular cells.

Consequently, the appearance of notable amounts of protein in the urine suggests renal disease.

Commonly, proteinuria is classified as:

1- Tubular proteinuria

Tubular proteinuria occurs most commonly in disease processes affecting the tubulointerstitial component of the kidney. It comprises low molecular proteins such as beta-2 microglobulin, which in normal conditions are completely reabsorbed by proximal tubules. The amount of proteinuria is less than 2 g and the dipstick may be negative.

2- Glomerular proteinuria

Glomerular proteinuria can be categorized according to whether pathological damage of the glomerulus is present. Types in which the patient has no pathological damage to the glomerulus include transient and orthostatic proteinuria.

3- Overflow proteinuria

Overflow proteinuria is most commonly associated with increased production of abnormal low molecular weight proteins (e.g., light chains in multiple myeloma, myoglobin in rhabdomyolysis) that exceeds the reabsorption capacity of the tubules, leading to spilling of the protein into the urine.

Transient proteinuria occurs in patients with normal renal function, the quantitative protein excretion is less than 1 g/day. Causes are usually physiological and include:

1- fever

2- exercise

3- orthostatic proteinuria

4- pregnancy

The typical urinary total protein loss is less than 150 mg/d. The proteins lost are made up of albumin (typically <30 mg/d) and some smaller proteins, together with proteins secreted by the tubules.

Glomerular proteinuria associated with pathological damage to the glomerulus is categorized by protein quantity. In non-nephrotic proteinuria, the amount of proteinuria is < 3.5 g/24 h and is persistent as in glomerulonephritis.

Nephrotic-range proteinuria is defined as >3.5 g/24 h of proteinuria.

Microalbuminuria

In health, relatively small amounts of albumin (less than 30 mg/d) are lost in the urine. Because of this, and because total protein assays are imprecise at low concentrations, relatively large increases in urine albumin loss can occur without a significant measurable increase in urinary total protein.

Microalbuminuria is a term used to describe an increase in urinary excretion of albumin above the reference interval for healthy nondiabetic subjects, but at an excretion that is not generally detectable by less sensitive clinical tests such as reagent strips designed to measure total protein. Approximately, this equates to urinary albumin concentrations between 30 and 300 mg/L.

Microalbuminuria is considered a clinically important indicator of deteriorating renal function in diabetic subjects. The albumin/creatinine ratio in a random urine sample can replace the use of microalbuminuria as an indicator of impairment of renal function.

II- Creatinine

Creatinine is a metabolite of creatine, which is synthesized in the kidneys, liver, and pancreas, then transported in the blood to other organs such as muscle and brain, where it is phosphorylated to phosphocreatine, a high-energy compound.

Most commonly, GFR is assessed by measuring serum creatinine. Creatinine is freely filtered at the glomerulus, and its concentration in serum is inversely related to GFR while its concentration in urine is directly related to GF.

As a marker of GFR, it is convenient and inexpensive to measure but is affected by:

(1) age,
(2) gender,
(3) exercise,
(4) certain drugs (e.g., cimetidine, trimethoprim),
(5) muscle mass,
(6) nutritional status.

Because of all these limitations, it is recommended that serum creatinine measurement alone is not used to assess kidney function.

GFR and Creatinine Clearance

In the past, a 24-hour urine creatinine clearance has been regarded as a more sensitive tool for the detection of kidney failure than a single plasma creatinine measurement. However, the inconvenience of a timed urine collection, failure to collect the entire specimen, and the wide (11%) within-subject variability, restrict the usefulness of this procedure. Furthermore, there is some tubular secretion of creatinine, hence, overestimating GFR and masking any future renal impairment.

In general, 4 equations are commonly used to assess GFR, these are:

1- Creatinine Clearance:

$$C_{cr} = \frac{Ucr\ X\ V}{Pcr\ X\ 1440} \quad \text{ml/minute}$$

2- Cockcroft- Gault equation

$$eGFR=\{((140–age) \times weight) / (72 \times S_{Cr})\} \times 0.85 \text{ (if female)}$$

3- MDRD study equation

$$eGFR\ (ml/min) = 175 \times (\text{serum creatinine})^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if the patient is female})$$

4- CKD-EPI creatinine (the most accurate)

eGFR = 141 x min (SCr/κ, 1) α x max (SCr /κ, 1)-1.209 x 0.993Age x 1.018 [if female] x 1.159 [if Black]

III- Urea

Catabolism of proteins and nucleic acids results in the formation of urea and ammonia; more than 90% of urea is excreted through the kidneys, with losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction. Consequently, kidney disease is associated with the accumulation of urea in the blood.

An increase in serum urea concentration characterizes the uremic (azotemic) state.

In a normal kidney, 40 to 70% of the highly diffusible urea moves passively out of the renal tubule and into the interstitium, ultimately reentering plasma.

Clinical Significance

Numerous extrarenal factors influence the circulating urea concentration, limiting its value as a test of kidney function.

For example, plasma urea concentration is increased by:

1- a high-protein diet,

2- increased protein catabolism,

3- reabsorption of blood proteins after gastrointestinal hemorrhage,

4- treatment with cortisol

5- dehydration

6- decreased perfusion of the kidneys (e.g., heart failure).

The measurement of both urea and creatinine in the blood can differentiate the type of renal impairment whether prerenal, renal, or postrenal.

IV- Uric acid

In humans, uric acid is the major product of catabolism of the purine nucleosides, adenosine and guanosine.

Hyperuricemia could be the result or the cause of renal disease. Asymptomatic hyperuricemic patients are at risk for kidney disease; few of these patients ever developed the clinical syndrome of gout.

Kidney disease associated with hyperuricemia may take one or more of several forms:

(1) gouty nephropathy with urate deposition in the renal parenchyma,

(2) acute intratubular deposition of urate crystals

(3) urate nephrolithiasis.

EXAMPLE OF RENAL DISORDERS

Chronic renal disease

The guidelines define CKD as either kidney damage or a decreased glomerular filtration rate (GFR) for at least 3 months. Whatever the underlying etiology, once the loss of nephrons and reduction of functional renal mass reaches a certain point, the remaining nephrons begin a process of irreversible sclerosis that leads to a progressive decline in the GFR.

It is an increasing medical problem all over the world and has specific clinical features which include:

1- hypertension
2- leg edema
3- earthy colour
4- loss of appetite
5- vomiting
6- oliguria or polyuria

Serum potassium and phosphorus, urea, and creatinine are significantly high in a patient with chronic renal disease while hypocalcemia is a characteristic feature. Anemia is a cardinal feature due to erythropoietin deficiency. Serum sodium is usually low.

Causes include DM, hypertension, and autoimmune diseases.

The classical biochemical findings in patients with CKD:

Laboratory test	Finding
eGFR	low
Creatinine	High
Urea	High
Calcium	Low
PTH	High
Na	Low
PO4	High
K	High
Hb	Low
ALP	High
Albuminuria	Positive
HCO3	Low

Outlines of treatment

1- Correct the underlying cause if possible
2- Control blood pressure and blood glucose.
3- Avoid nephrotoxic drugs.
4- Treat associated metabolic disorders:
5- Diet: restriction of salt, water, potassium and phosphorus is obligatory. Protein restriction is controversial and aggressive restriction of protein in diet should avoided as it seems to increase morbidity and mortality.
6- Renal replacement therapy when indicated (clinically or biochemically).

Treatment of CKD associated metabolic disorders:

Disorder	Suggested treatment
Anemia	EPO
Hypocalcemia	Activated vit. D
Hyperphosphatemia	Ferrous citrate
Dyslipidemia	Statin

Common causes of end-stage renal failure

Disease	Proportion	Comments
Congenital and inherited	5%	Polycystic kidney disease, Alport's syndrome
Renovascular disease	5%	Mostly atheromatous, may be more common
Hypertension	5–20%	Causality controversial, much may be renal disease
Glomerular diseases	10–20%	IgA nephropathy is most common
Interstitial diseases	20–30%	Often drug-induced
Systemic inflammatory diseases	5–10%	Systemic lupus erythematosus, vasculitis
Diabetes mellitus	20–40%	Large racial and geographical differences
Unknown	5–20%	

Prognosis of CKD by GFR and albuminuria categories: KDIGO 2012

GFR categories (ml/min per 1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	⩾90			
	G2	Mildly decreased	60–89			
	G3a	Mildly to moderately decreased	45–59			
	G3b	Moderately to severely decreased	30–44			
	G4	Severely decreased	15–29			
	G5	Kidney failure	<15			

Green: low risk (if no other markers of kidney disease, no CKD); yellow: moderately increased risk; orange: high risk; red, very high risk.

Acute kidney injury

Acute kidney injury is associated with a high rate of adverse outcomes; mortality rates range between 25 and 80 per cent, depending on the cause and the clinical status of the patient.
Acute kidney injury is defined as an abrupt or rapid decline in renal filtration function (within 48 hours) that leads to any of the following conditions:

1- Increase in serum creatinine level 3 doubles its previous level.
2- Decrease in GFR by 75% of its previous level.
3- Serum creatinine level ≥ 4 mg/dL with an acute increase of >0.5 mg/dL.
4- Urine output < 0.3 mL/kg/h for 24 hours.
5- Anuria for 12 hours.

Any of the previous 5 features is enough to define the patient as having AKI.
AKI may be reversible and renal function may be restored completely or partially, but if:
- the patients have a complete loss of kidney function for more than 4 weeks then the patient is considered to have persistent AKI;
- while if the patients have a complete loss of kidney function for more than 3 months then the patient is considered to have end-stage kidney disease ESKD.

Normal urine output
0.5 to 1.5 cc/kg/hour
A patient should be urinating at least every 6 hours.
Oliguria
Decreased urine output < 300cc/m^2/24 hours
< 0.5 cc/kg/hour in children
< 1.0 cc/kg/hour in infants
Usually < 500 cc/day in adults
Anuria
No or minimal urine output
Usually < 100 mL/day in adults

The term acute kidney injury should replace terms such as acute renal failure and acute renal insufficiency, which previously has been used to describe the same clinical condition.

Causes

The causes of acute kidney injury can be divided into three categories:

1- Prerenal (caused by decreased renal perfusion, often because of volume depletion).
In these cases, underlying kidney function may be normal, but decreased renal perfusion associated with intravascular volume depletion (e.g., from vomiting or diarrhea) or decreased arterial pressure (e.g., from heart failure or sepsis) results in a reduced glomerular filtration rate.

2- Intrinsic renal (caused by a process within the kidneys).

Intrinsic renal causes are also important sources of acute kidney injury and can be categorized by the component of the kidney that is primarily affected (i.e., tubular, glomerular, interstitial, or vascular).

Causes include glomerulonephritis, drug toxicity, and viral or bacterial infection.

3- Postrenal (caused by inadequate drainage of urine distal to the kidneys).

Postrenal causes typically result from obstruction of urinary flow, and prostatic hypertrophy is the most common cause of obstruction in older men. The prompt diagnosis followed by early relief of obstruction is associated with improvement in renal function in most patients.

In patients who already have underlying chronic kidney disease, any of these factors, but especially volume depletion, may cause acute kidney injury in addition to the chronic impairment of renal function.

Laboratory findings:

1- urine examination:

Looking for the presence of hematuria, RBC cast, or proteinuria.

2- blood urea & serum creatinine:

Both are usually elevated and the ratio of elevated blood urea to elevated serum creatinine differs according to the cause of AKI.

In prerenal and postrenal types: the level of elevation of blood urea is much higher than the level of elevation of serum creatinine.

In the intrinsic renal type: elevation of both urea and creatinine is comparable.

3- electrolytes:

-Serum potassium is high,

-Serum calcium is normal initially

-Serum sodium is reduced (dilutional hyponatremia)

- metabolic acidosis

-hyperphosphatemia & hypermagnesemia

Nephrotic Syndrome

Nephrotic syndrome is a disorder characterized by urinary excretion of > 3.5 g of protein/day due to a glomerular disorder plus edema and hypoalbuminemia. It is more common among children and has both primary and secondary causes.

In nephrotic syndrome, the glomeruli are affected by an inflammation or a hyalinization (the formation of a homogenous crystalline material within cells) that allows proteins such as albumin, antithrombin or immunoglobulins to pass through the cell membrane and appear in the urine.

Diagnosis is by determination of urine protein/creatinine ratio in a random urine sample or measurement of urinary protein in a 24-hour urine collection.

Causes

A- Primary glomerulonephrosis

Primary causes of nephrotic syndrome are usually described by their histology:

- Minimal change disease
- Focal segmental glomerulosclerosis
- Membranous glomerulonephritis
- Membranoproliferative glomerulonephritis

B- Secondary glomerulonephrosis

Secondary causes of nephrotic syndrome have the same histologic patterns as the primary causes. Secondary causes include:

- Diabetic nephropathy
- Hepatitis B
- Genetic disorders
- Multiple myeloma

Clinical presentation

1- The first sign of nephrotic syndrome in children is usually swelling of the face; this is followed by swelling of the entire body.
2- Foamy urine may be a presenting feature.
3- A thrombotic complication, such as deep venous thrombosis of the calf veins or even a pulmonary embolus, maybe the first clue to nephrotic syndrome.

Hypercoagulopathy is related to increased plasma levels of factors V and VIII, and of fibrinogen with blood hyperviscosity; decreased plasma levels of natural anticoagulants: free protein S, and antithrombin III.

Additional historical features can be related to the cause of nephrotic syndrome. Thus, the recent start of a nonsteroidal anti-inflammatory drug (NSAID) suggests such drugs as the cause and a more than 10-year history of diabetes with symptomatic neuropathy indicates diabetic nephropathy.

Lab. findings

1. proteinuria: > 3.5 g/24 hour
2. protein/ creatinine > 3.5
3. hypoalbuminemia
4. hypercholesterolemia
5. hypocalcemia

Treatment of nephrotic syndrome:	
Corticosteroids	**Immune suppressive drugs**
Statin	Diuretics
ACE inhibitors	Calcium and vit. D

****

****

23-24

جامعة الفراهيدي – كلية الصيدلة

Endocrine Disorders -I-_

Clinical Chemistry for 5th-year Pharmacy Students

5

ا.م.د. رائد ضياء هاشم

Endocrine Disorders

Introduction

Four systems in the human body are responsible for the integration and coordination of various body functions; these systems are:

1- Nervous system

2- Circulatory system

3- Immune system

4- Endocrine system

It is impossible for the endocrine system to properly function in the homeostatic maintenance of the body without its functional integration with the central nervous and circulatory systems. The definition of a hormone as a signalling substance that uses blood circulation to reach the target tissues clarifies the role of the circulatory system in endocrine function.

The hypothalamus represents the main mediator between the CNS and the endocrine system, which is simultaneously an integral part of the central nervous system and an endocrine gland.

The hypothalamus controls the function of the “peripheral” endocrine glands such as adrenal and thyroid glands via the pituitary gland.

The hypothalamic-pituitary axis

A. Hypothalamus

The hypothalamus contains neurosecretory neurons which synthesize peptides and catecholamines; these are released into the circulatory system and act as hormones. Some hypothalamic hormones are released into the systemic circulation to target distant tissues. Other hypothalamic hormones are released in the portal circulation for delivery to the anterior pituitary where they stimulate or inhibit the release of the anterior pituitary hormones.

All the hypothalamic hormones are peptides except dopamine, which is a catecholamine.

The hypothalamic hormones are shown in the following table:

Hormones that act on the anterior pituitary	Action
GnRH (gonadotropin-releasing hormone)	stimulates the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH)
GHRH (growth hormone-releasing hormone)	stimulates the release of growth hormone (GH)
Somatostatin	inhibits GH release
TRH	stimulates the release of thyroid-stimulating hormone (TSH) and prolactin
Dopamine	inhibits prolactin release
CRH (corticotropin-releasing hormone)	stimulates ACTH (adrenocorticotropin) release
Hormones acting on distant tissues	Action
ADH (antidiuretic hormone)	decreases the free water clearance in kidneys and stimulates thirst
Oxytocin	uterine smooth muscle contraction

Pituitary Gland

The pituitary gland is a complex gland consisting of hormone-producing adenoid (glandular) cells (anterior pituitary) and the axon terminals of neurosecretory cells originating in the hypothalamus (posterior pituitary). It is a pea-sized endocrine gland located at the base of the brain, often referred to as the “master gland”.

Posterior pituitary hormones

The posterior pituitary contains mostly axon terminals of the hypothalamic neurosecretory cells. These axon terminals are surrounded by the inferior hypophyseal artery capillary bed. ADH and oxytocin are the two hormones released by the posterior pituitary.

ADH is responsible for osmotic homeostasis (it decreases the free water clearance in kidneys and stimulates thirst) and, it is also a very potent vasoconstrictor when present at higher plasma concentrations.

Oxytocin mostly acts on the uterine smooth muscle and the smooth muscle in the mammary glands.

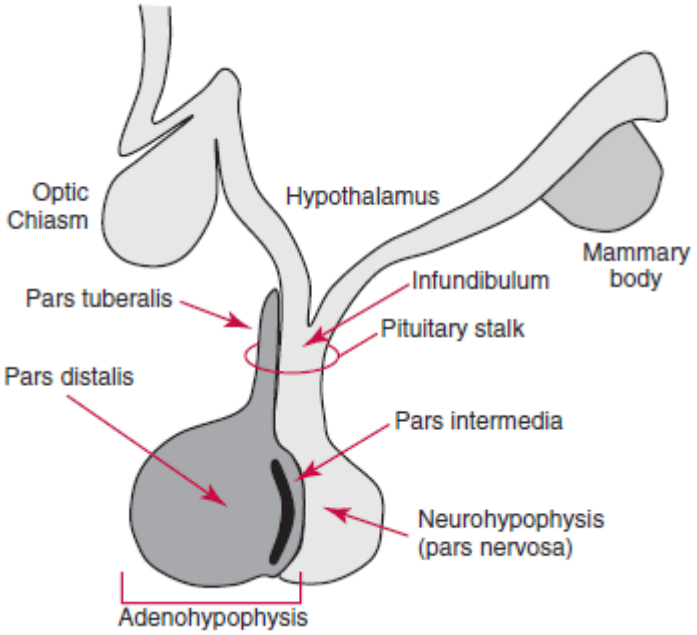
Anterior pituitary hormones

There are six types of cells in the anterior pituitary, named after the primary peptide/protein hormones they produce:

1- **ACTH** (adrenocorticotropin)
2- **TSH** (thyroid-stimulating hormone; thyrotropin)
3- **FSH** and **LH** (follicle-stimulating and luteinizing hormone or gonadotropins)
5- **GH** (growth hormone or somatotropin)
6- **prolactin** (lactotropin)

Upon stimulation of the anterior pituitary by hypothalamic hormones, these hormones are released and diffuse into the second portal capillary bed.

A newly recognized product of the pituitary gland detected in some perimenopausal and postmenopausal women is human chorionic gonadotropin (hCG). Usually, hCG is associated with pregnancy or gestational trophoblastic disease. In early pregnancy, hCG doubles approximately every 48 hours, whereas the concentration of hCG from pituitary or gestational trophoblastic disease origin is relatively stable and does not increase in the pattern seen in pregnancy.



Regulation of pituitary hormone secretion

a. Neural control

Secretion of pituitary hormones can be controlled by neural stimuli originating at the peripheral or in the central nervous system. An excellent example of the peripheral control of pituitary hormones is the release of oxytocin and prolactin during nursing.

The suckling of a baby stimulates sensory nerves and activates the afferent pathway to the hypothalamus. The hypothalamus responds with the release of oxytocin in the posterior pituitary and the release of TRH (along with the decrease of dopamine release) to stimulate the release of prolactin in the anterior pituitary. Oxytocin acts on the smooth muscle cells in the ducts of the mammary gland to expel the milk and prolactin acts on the glandular tissue in the breast to stimulate milk production.

b. Negative feedback

In many cases, the target tissues for the anterior pituitary hormones are the glands themselves.

The hormones secreted by these glands can inhibit the release of their tropic hormone or its tropic hormone-releasing hormone. For example, FSH and LH stimulate the production of sex hormones. Estradiol, progesterone and testosterone can inhibit both the release of gonadotropins as well as the release of GnRH.

Presenting problems in endocrine disease

Endocrine diseases present in many different ways and to clinicians in many different disciplines.

Although endocrinal disorders can present as a classical syndrome, the presentation is sometimes with non-specific symptoms or with asymptomatic biochemical abnormalities.

Examples of non-specific symptoms that might indicate an endocrinal disorder include:

Symptom	Most likely endocrine disorder(s)
Lethargy and depression	Hypothyroidism, diabetes mellitus, hyperparathyroidism, hypogonadism, adrenal insufficiency, Cushing's syndrome
Weight gain	Hypothyroidism, Cushing's syndrome
Weight loss	Thyrotoxicosis, adrenal insufficiency, diabetes mellitus
Polyuria and polydipsia	Diabetes mellitus, diabetes insipidus, hyperparathyroidism, Conn's syndrome
Heat intolerance	Thyrotoxicosis, menopause
Headache	Acromegaly, pituitary tumour, phaeochromocytoma
Muscle weakness (usually proximal)	Thyrotoxicosis, Cushing's syndrome, hypokalaemia (e.g. Conn's syndrome), hyperparathyroidism, hypogonadism
Coarsening of features	Acromegaly, hypothyroidism

Presenting problems in hypothalamic and pituitary disease

There is a wide range of clinical features that might be associated with hypothalamic and pituitary disease. These clinical features are usually divided into three main categories:

A- Clinical features related to local complications

- Headache
- Visual field defect
- Diplopia

B- Clinical features related to hormone excess

1- Hyperprolactinaemia

- Galactorrhoea
- Amenorrhoea
- Hypogonadism

2- Acromegaly

- Headache
- Sweating
- Change in shoe and ring size

3- Cushing's disease

- Weight gain
- Bruising
- Myopathy
- Hypertension
- Striae
- Depression

C- Clinical features related to hypopituitarism

1- Growth hormone

- Lethargy

2- Gonadotrophins

- Lethargy
- Loss of libido
- Hair loss
- Amenorrhoea

3- ACTH

- Lethargy
- Postural hypotension
- Pallor
- Hair loss

4- TSH

- Lethargy

5- Vasopressin (ADH)

- Thirst and polyuria

Clinical tips

1- In most cases, a patient with an endocrinal disorder presents with multiple complaints rather than a single one.

2- The duration of symptoms is very essential factor for development of symptoms and directly correlated with the severity.

3- Many conditions including cancers might be associated with endocrinal abnormalities.

4- Certain drugs might mask the symptoms of the endocrinal diseases such as B- blockers in hyperthyroidism, while others might exacerbate the symptoms such thiazides in hyperparathyroidism.

5- Medications may also directly alter a laboratory test, such as use of oral contraceptives in attempting to diagnose hyperthyroidism or hypothyroidism.

6- The current laboratory techniques used to measure hormones in body fluid are quite sensitive and specific that many endocrinal disorders are diagnosed even when the patient is asymptomatic.

7- Although most endocrinal disorders are related to an increase or decrease in a certain hormone, many other endocrinal disorders are due to receptors dysfunction (nephrogenic DI).

Examples of common disorders of the anterior pituitary include

Hyperprolactinemia

Hyperprolactinemia is a common abnormality that usually presents with hypogonadism and/or galactorrhoea (lactation in the absence of breastfeeding). Since prolactin stimulates milk secretion but not breast development, galactorrhoea rarely occurs in men and only does so if gynecomastia has been induced by hypogonadism.

Many non-pathological causes for hyperprolactinemia are present and should be always kept in mind during the management of hyperprolactinemia. Pituitary tumors can cause hyperprolactinemia by directly secreting prolactin (prolactinomas).

Common causes of hyperprolactinemia include:

-The half-life of prolactin is 25-50 minutes.
-Serum prolactin level is higher in females than in males.

A- Physiological

• Stress (e.g., post-seizure)
• Pregnancy
• Lactation
• Exercise

B- Drug-induced

1- Dopamine antagonists

• Antipsychotics: (phenothiazines)
• Antidepressants
• Antiemetics (e.g., metoclopramide, domperidone)

2- Dopamine-depleting drugs

• Methyldopa

3- Oestrogens

• Oral contraceptive pill

C- Pathological

- Prolactinoma (usually microadenoma)
- Primary hypothyroidism
- Polycystic ovarian syndrome
- Chronic kidney disease
- Liver cirrhosis

-Prolactinoma is a benign tumor representing 40% of all pituitary tumors.
- The level of serum prolactin can be extremely high in prolactinoma (50,000 ng/ml).
-Other causes of hyperprolactinemia are rarely associated prolactin of more than 200 ng/ml.
-Idiopathic hyperprolactinemia is diagnosed when no apparent cause is found.
-Lactation results in amenorrhea and secondary infertility because of prolactin-mediated suppression of gonadotropins.
-The presentation of the patient is highly related to the level of serum prolactin.

Clinical assessment

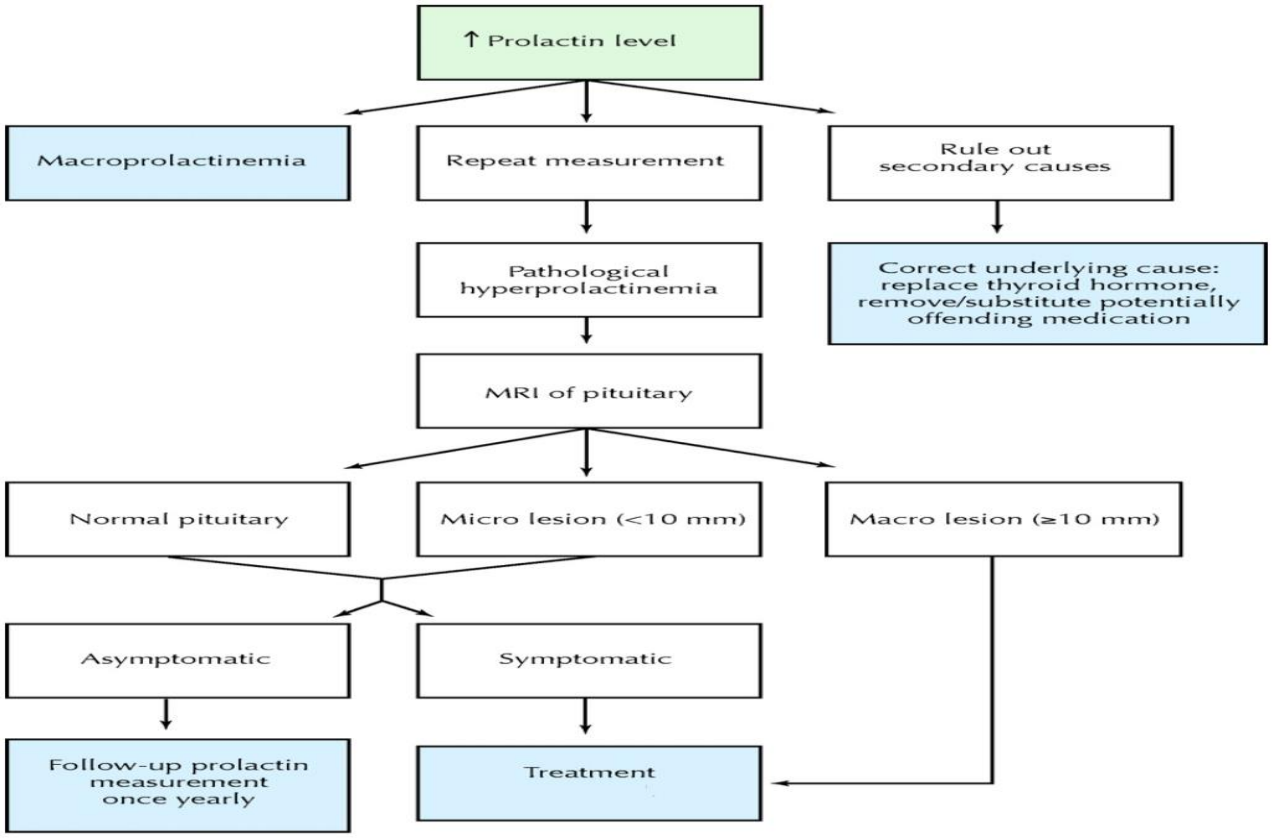
In women, in addition to galactorrhea, hypogonadism associated with hyperprolactinemia causes secondary amenorrhea and anovulation with infertility. Important points in history include drug use, recent pregnancy and menstrual history.

In men, there is decreased libido, reduced shaving frequency and lethargy.

Investigations

- You should always keep in mind that a slight elevation of serum prolactin may be related to non-pathological causes.
- Patients with prolactin excess should have tests of gonadal function, and T4 and TSH measured to exclude primary hypothyroidism.
- Serum prolactin of > 1000 mU/L is an indication for an MRI or CT scan of the hypothalamus and pituitary.

Whereas serum prolactin levels between 20 and 200 µg/L can be found in patients with hyperprolactinemia due to any cause, prolactin levels above 200 µg/L usually indicate the presence of a lactotroph adenoma.



(Approach to patients with hyperprolactinemia)

In the absence of an apparent cause of hyperprolactinemia, imaging (preferably MRI) of the pituitary fossa is recommended to establish whether a prolactin-secreting pituitary tumour or another lesion is present.

Management

1- Drug therapy with Dopamine agonist:

Bromocriptine, Cabergoline, Quinagolide

2- Surgery and radiotherapy

Biochemical and clinical improvements in response to dopamine agonist therapy are readily apparent in most patients. In addition, tumor shrinkage can be expected in about 80% of macroadenomas. However, a major drawback of medical therapy is the potential need for lifelong treatment. Discontinuation of bromocriptine therapy has been shown to lead to the recurrence of hyperprolactinemia in most patients and tumor regrowth if the treatment duration has been less than 2 years.

Cabergoline can be used in this schedule:

Initially: 0.25 mg PO 2x/week

May increase by 0.25 mg q4Weeks (or longer) up to 1 mg 2x/week.

Once the diagnosis has been established and therapy initiated, fasting prolactin levels should be monitored monthly. Later, prolactin levels can be monitored every 3-6 months. Shrinkage of the tumor should be followed by formal visual-field testing and MRI.

Acromegaly

Acromegaly is caused by excess growth hormone (GH) secretion from a pituitary tumor, usually a macroadenoma. In fact, the whole clinical features occur due to excess IGF-1 which is synthesized in the liver and its synthesis is stimulated by GH.

Clinical features

If GH hypersecretion occurs before puberty and the growth plate has not been fused yet, then the presentation is with gigantism with a longitudinal acceleration of linear growth. More commonly, GH excess occurs in adult life and presents with acromegaly.

If hypersecretion starts in adolescence and persists into adult life, then the two conditions may be combined.

- Headache - Hypertension - Increased sweating - Cardiomyopathy
- Hepatomegaly - Diabetes (10%) - Colonic cancer - Skull growth
- Enlargement of hands
- prominent supraorbital ridges with large frontal sinus.
- Enlargement of lips, nose and tongue

Investigations

The clinical diagnosis must be confirmed by measuring GH levels during an oral glucose tolerance test. In normal subjects, plasma GH suppresses to below 0.5 µg/L (approximately 2 mU/L).

In acromegaly, GH is not suppressed and in about 50% of patients, there is a paradoxical rise.

Measurement of insulin-like growth factor (IGF-1) can be used instead as it is more convenient and reliable than GH, elevated IGF-1 levels are consistent with acromegaly. Laboratory confirmation of acromegaly is followed by radiological imaging to localize the tumour.

Management

- Surgery or radiotherapy as a primary treatment.
- Somatostatin analogues (octreotide), as surgical treatment, can cure 60% of patients only.

THE ADRENAL GLANDS

The adrenals comprise several separate endocrine glands within a single anatomical structure. The adrenal medulla is an extension of the sympathetic nervous system which secretes catecholamines into capillaries rather than synapses.

Most of the adrenal cortex is made up of cells that secrete cortisol and adrenal androgens, and form part of the hypothalamic–pituitary–adrenal (HPA) axis. The small outer glomerulosa of the cortex secretes aldosterone under the control of the renin-angiotensin system. These functions are important in the integrated control of cardiovascular, metabolic and immune responses to stress.

Layers of the adrenal cortex and their synthetic function

Zona glomerulosa	(outer layer)	Synthesis of aldosterone
Zona fasciculata	(middle layer)	Synthesis of glucocorticoids
Zona reticularis	(innermost layer)	Synthesis of androgens

Presenting problems in adrenal disease

Cushing's syndrome

Cushing's syndrome is caused by excessive activation of glucocorticoid receptors. By far the most common cause is iatrogenic, due to prolonged administration of synthetic glucocorticoids such as prednisolone.

Etiology

Amongst endogenous causes, pituitary-dependent cortisol excess (by convention, called Cushing's disease) accounts for approximately 80% of cases. Both Cushing's disease and cortisol-secreting adrenal tumors are four times more common in women than men.

Causes of Cushing's syndrome include:

A- ACTH-dependent

• Pituitary adenoma secreting ACTH (Cushing's disease)

• Ectopic ACTH syndrome (small-cell lung carcinoma)

B- Non-ACTH-dependent

• Iatrogenic (chronic glucocorticoid therapy)

• Adrenal adenoma or carcinoma

Pseudo-Cushing's syndrome

• Alcohol excess (biochemical and clinical features)

• Major depressive illness (biochemical features only)

• Primary obesity (mild biochemical features, some clinical overlap)

Clinical Features

Hair thinning	Acne	Moon face
Peptic ulcer	Hyperglycemia	Menstrual disturbance
Osteoporosis	Tendency to infections with poor wound healing	
Hypertension	Striae	Cataracts & Mild exophthalmos
Bruising	Plethora	Hirsutism

Investigations

A- Establishing the presence of Cushing's syndrome:

1- Overnight-low dose dexamethasone suppression >>>>> failure of suppression of serum cortisol level indicates the presence of Cushing's syndrome. 1 mg dexamethasone to be given orally at 11 pm and serum cortisol level to be measured at 8-9 am the next morning.

or

2- 24-hr urinary free cortisol (high level of urinary cortisol)

B- Determining the underlying cause

Plasma ACTH:

In the presence of excess cortisol secretion, an undetectable ACTH indicates an adrenal tumor, while any detectable ACTH suggests a pituitary cause or ectopic ACTH.

4- *Localizing the tumor by radiological imaging (MRI or CT scan).*

Management

Untreated Cushing's syndrome has a 50% 5-year mortality. Most patients are treated surgically with medical therapy given for a few weeks prior to the operation. Several drugs are used to inhibit corticosteroid biosynthesis, including metyrapone and ketoconazole. The dose of these agents is best titrated against 24-hour urine-free cortisol.

Adrenal insufficiency

Adrenal insufficiency results from inadequate secretion of cortisol and/or aldosterone. It is potentially fatal with variable presenting features. A high index of suspicion is therefore required in patients with:

unexplained fatigue, hyponatremia or hypotension.

The most common cause is ACTH deficiency (secondary adrenocortical failure), usually because of inappropriate withdrawal of chronic glucocorticoid therapy or a pituitary tumour. Congenital adrenal hyperplasias and Addison's disease (primary adrenocortical failure) are rare.

Causes

Secondary (↓ACTH)

- Withdrawal of suppressive glucocorticoid therapy
- Hypothalamic or pituitary disease

Primary (↑ACTH)

Addison's disease

Common causes
- Autoimmune
  - Sporadic
  - Polyglandular syndromes
- Tuberculosis
- HIV/AIDS
- Metastatic carcinoma
- Bilateral adrenalectomy

Rare causes
- Lymphoma
- Intra-adrenal haemorrhage (Waterhouse–Friedrichsen syndrome following meningococcal septicaemia)
- Amyloidosis
- Haemochromatosis

Corticosteroid biosynthetic enzyme defects
- Congenital adrenal hyperplasias
- Drugs
  Metyrapone, ketoconazole, etomidate

Clinical Features

Weight loss Anorexia Nausea &Vomiting

Hyperpigmentation (in primary type only)

Diarrhoea or constipation Postural hypotension

Shock Hypoglycaemia

Hyponatraemia Hypercalcaemia

Investigation

- Random serum cortisol is misleading.

- ACTH stimulation test (synacthen stimulation test: failure of cortisol level to increase after administration ACTH is indicative of adrenocortical insufficiency.

- ACTH assay: high ACTH indicates Addison's disease, while normal or low ACTH is indicative of secondary adrenocortical insufficiency.

Management

Patients with adrenocortical insufficiency always need glucocorticoid replacement therapy and usually, but not always, mineralocorticoid (in primary type only).

1- Glucocorticoid replacement

Hydrocortisone (cortisol) is the drug of choice. In someone who is not critically ill, hydrocortisone should be given by mouth, 15 mg on waking and 5 mg at around 1800 hrs. The dose may need to be adjusted for the individual patient but this is subjective. Excess weight gain usually indicates over-replacement, whilst persistent lethargy or hyperpigmentation may be due to an inadequate dose.

Equivalent doses of glucocorticoids
Hydrocortisone: 20 mg
- **Cortisone acetate: 25 mg**
- **Prednisolone: 5 mg**
- **Dexamethasone: 0.5 mg**

2- Mineralocorticoid replacement

Fludrocortisone is administered in a dose of 0.05–0.1 mg daily and adequacy of replacement may be assessed objectively by measurement of blood pressure, plasma electrolytes and plasma renin activity.

3- Management of adrenal crisis

1- Correct volume depletion

• Intravenous saline as required to normalize blood pressure and pulse.

2- Replace glucocorticoids

• Intravenous hydrocortisone succinate 100 mg.

• Continue parenteral hydrocortisone (50–100 mg i.m. 6-hourly) until the patient is well enough for reliable oral therapy

3- Correct other metabolic abnormalities

• Acute hypoglycaemia: i.v. 10% glucose

• Hyperkalaemia: should respond to volume replacement, but occasionally requires specific therapy.

4- Identify and treat the underlying cause

• Consider acute precipitant, e.g., infection

• Consider adrenal or pituitary pathology.

Primary hyperaldosteronism

Most individuals with primary hyperaldosteronism have bilateral adrenal hyperplasia (idiopathic hyperaldosteronism), while only a minority have aldosterone-producing adenoma (APA; Conn's syndrome).

Clinical features

Individuals with primary hyperaldosteronism are usually asymptomatic, but they may have features of sodium retention or potassium loss. Sodium retention may cause edema, while hypokalemia may cause muscle weakness and polyuria secondary to renal tubular damage which produces nephrogenic diabetes insipidus) and occasionally tetany.

Blood pressure is elevated but accelerated phase hypertension is rare.

Investigations

Routine blood tests may show hypokalemic alkalosis. Sodium is usually at the upper end of the normal range in primary hyperaldosteronism but is characteristically low in secondary hyperaldosteronism.

The key measurements are plasma renin and aldosterone, where the plasma renin-aldosterone ratio is low.

Management

spironolactone or amiloride to control both hypertension and electrolyte disturbance.

Phaeochromocytoma

A rare neuroendocrine tumor that may secrete catecholamines (adrenaline, noradrenaline) and is responsible for less than 0.1% of cases of hypertension. It is benign in most cases but approximately 15% show malignant features. Around 25% of phaeochromocytomas are associated with inherited disorders, including neurofibromatosis.

Clinical features

These depend on the pattern of catecholamine secretion. Some patients present with a complication of hypertension, such as stroke, myocardial infarction, left ventricular failure, hypertensive retinopathy or accelerated phase hypertension. The apparent paradox of postural hypotension between episodes is explained by 'pressure natriuresis' during hypertensive episodes so that intravascular volume is reduced.

1- Hypertension (usually paroxysmal; often a postural drop of blood pressure)

2- Paroxysms of Pallor (occasionally flushing), Palpitations, Sweating, Headache, and anxiety (fear of death)

3- Abdominal pain, vomiting

4- Constipation

4- Weight loss

5- Glucose intolerance

Investigations

1- Excessive secretion of catecholamines can be confirmed by measuring metabolites in plasma and/or urine (metanephrine and normetanephrine).

2- Serum chromogranin A is often elevated.

Management

Medical therapy is required to prepare the patient for surgery, preferably for a minimum of 6 weeks to allow restoration of normal plasma volume. The most useful drug in the face of very high circulating catecholamines is the α-blocker phenoxybenzamine (10–20 mg orally 6–8 hours) because it is a non-competitive antagonist, unlike prazosin or doxazosin. If α-blockade produces a marked tachycardia, then a β-blocker (e.g. propranolol) or combined α- and β-antagonist (e.g. labetalol) can be added. On no account should the β-antagonist be given before the α-antagonist, as it may cause a paradoxical rise in blood pressure due to unopposed α-mediated vasoconstriction.

Congenital Adrenal Hyperplasia CAH

CAH represents a group of disorders of steroid synthesis that is initiated in intrauterine life. It is transmitted in an autosomal recessive pattern affecting the production of sex hormones, glucocorticoids and mineralocorticoids from cholesterol in the adrenal glands.

The excessive production of one or more of these hormones or the deficiency of another is associated with various patterns of presentation including altered sexual development and/or severe loss of salt and water.

CAH has an incidence of 1:15000 and both sexes are equally affected although the presentation is usually different between male and female neonates. The diagnosis is usually made at birth although atypical cases might be diagnosed at puberty.

Pathophysiology

Glucocorticoids, mineralocorticoids and sex hormones are synthesized from cholesterol in the adrenal cortex. This process involves the presence of many enzymes where a deficiency of any of these enzymes will lead to the altered synthesis of one or more of these hormones.

The most common enzyme deficiency is 21-hydroxylase which is essential for the production of all steroids because it is required for the conversion of 17 hydroxyprogesterone (17OHP) to 11-deoxycortisol (the precursor for cortisol) and also the conversion of progesterone to 11 deoxycorticosterone (the precursor of aldosterone).

The deficiency of 21-hydroxylase will lead to cortisol deficiency with a subsequent increase in ACTH. This increase will be associated with a shift in the pathway of steroid synthesis towards androgen leading to a significant increase in its concentration.

This process is initiated during fetal life which explains the presence of virilization at birth. In certain cases, enzyme deficiency is mild leading to a delayed presentation as excessive sex hormone in children or adolescents or even infertility in adults.

Clinical features

The clinical presentation is directly related to the type of hormonal alteration:

1- Signs of mineralocorticoid deficiency

Repeated vomiting with severe dehydration, hypovolemia and shock.

2- Signs of excess androgens

- Precocious puberty
- Ambiguous genitalia, menstrual irregularity and infertility in females
- Excessive facial hair and extreme virilization in males

In general, the diagnosis of CAH is made much earlier in females than in males because of the prominent ambiguous genitalia present in females at birth while most male neonates have no signs of CAH at birth.

Diagnosis

The diagnosis of CAH relies on confirming the presence of elevated serum 17-alpha hydroxyprogesterone on two occasions

Other associated findings include:

- Hypoglycemia
- Hyponatremia
- hypokalemia

Management

Oral glucocorticoids will be sufficient to control the overproduction of androgens.

Mineralocorticoids are to be given if already deficient.

CAH has a good prognosis if diagnosed early with proper treatment and close follow-up.

23-24

جامعة الفراهيدي – كلية الصيدلة

The Reproductive System

Clinical Chemistry for 5th-year Pharmacy Students

Objectives:

1- **Review of the reproductive system of both male and female.**
2- **Discussing the major examples of reproductive system disorders.**
3- **Learning the common pitfalls in the diagnosis of reproductive disorders.**

6

ا.م.د. رائد ضياء هاشم

The Reproductive System (Endocrine II)

Introduction

In clinical practice, disorders of the reproductive system in both males and females are shared among several specialties, including gynecology, urology, pediatrics, psychiatry and endocrinology. In many situations, a multidisciplinary team is required for proper management.

The physiology of the reproductive system should be discussed before discussing the common disorders of the male and female reproductive systems.

The reproductive system of male

In the male, the testis is responsible for two principal functions:

1- synthesis of testosterone by the interstitial Leydig cells under the control of luteinizing hormone (LH).

2- spermatogenesis by Sertoli cells under the control of follicular stimulating hormone (FSH) (but also requiring adequate testosterone).

Negative feedback suppression of LH is mediated principally by testosterone, while secretion of another hormone by the testis, inhibin, suppresses FSH.

It is obvious that if we want to assess this axis, testosterone, FSH, and LH should be investigated.

Testosterone is better to be measured in the morning when its values are somewhat higher.

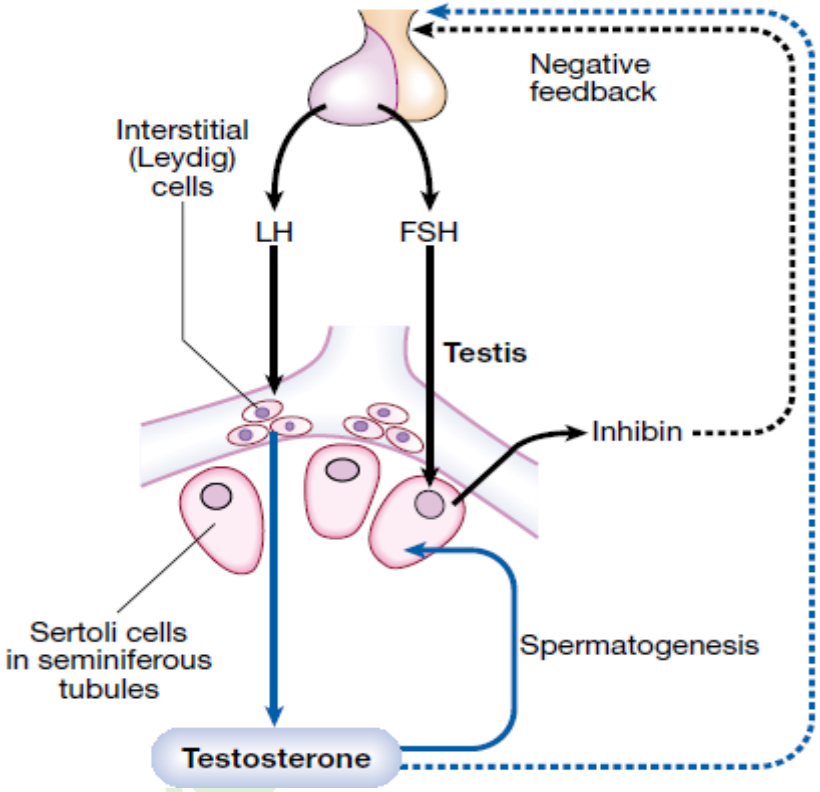
The testicular function can also be tested by a seminal fluid analysis which can be very informative.

There is no equivalent of menopause in men, although testosterone concentrations decline slowly from the fourth decade onwards.

The functions (effects) of testosterone include:

- Facial, axillary and body hair growth
- Scalp balding
- Skin sebum production
- Prostate development and function
- Laryngeal enlargement

• Muscle power
• Bone metabolism/epiphyseal closure
• Libido



Male reproductive physiology

The reproductive system of female

In the female, physiology is complicated by variations in function during the normal menstrual cycle.

The menstrual cycle can be divided into two phases:

(1) follicular or proliferative phase

(2) the luteal or secretory phase.

The median duration of a menstrual cycle is 28 days, with most cycle lengths ranging from 25 to 30 days. Patients with menstrual cycles occurring at intervals of less than 21 days are called polymenorrheic, while patients with prolonged menstrual cycles of more than 35 days

are called oligomenorrheic. Due to anovulation and insufficient follicular growth, the menstrual cycle is usually most irregular around the extremes of reproductive life (menarche and menopause).

All women have a relatively constant luteal phase of the cycle with a period of 14 days. The variation in the duration of the cycle is generally related to the varying lengths of the follicular phase which can range from 10 to 16 days.

The menstrual cycle can be summarized as follows:

1- During the first 14 days after the previous menses FSH stimulates the growth and development of ovarian follicles. This leads to a gradual increase in estradiol production from granulosa cells.

2- The high level of estradiol initially suppresses FSH secretion (negative feedback) but then, above a certain level, stimulates an increase in both the frequency and amplitude of gonadotropin-releasing hormone (GnRH) pulses.

3- The high level of GnRH will result in a marked increase in LH secretion (positive feedback of estradiol) which occurs in the middle of the cycle.

4- The midcycle 'surge' of LH induces ovulation.

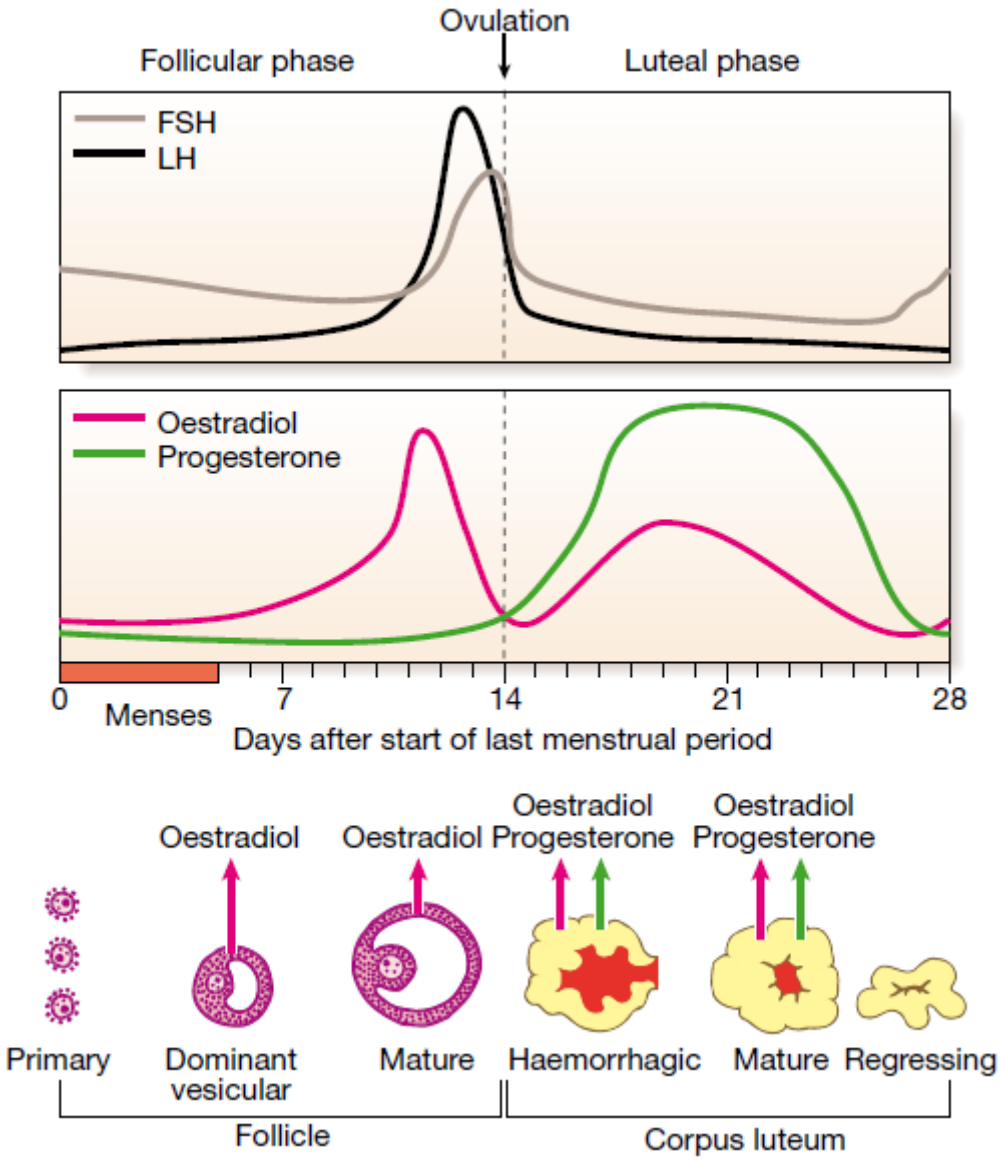
5- After the release of the ovum, the follicle differentiates into a corpus luteum which is a transient endocrine organ that secretes progesterone. The latter is responsible for preparing the endometrium of the uterus for implantation.

6- Corpus luteum function decreases by the end of the luteal phase unless a pregnancy produces human chorionic gonadotropin. If pregnancy does not occur and under the influence of estradiol and prostaglandins, the corpus luteum undergoes luteolysis and forms a scar tissue called the corpus albicans.

7- Withdrawal of progesterone results in menstrual bleeding.

Circulating levels of estrogen and progesterone in pre-menopausal women are, therefore, critically dependent on the time of the cycle.

The most useful 'test' of ovarian function is a careful menstrual history. In addition, ovulation can be confirmed by measuring plasma progesterone levels during the luteal phase ('day 21 progesterone') or by tracking changes in oestrogen and progesterone metabolites in urine specimens collected at weekly intervals.



The normal menstrual cycle

Cessation of menstruation (menopause) occurs at an average age of approximately 50 years in developed countries. In the 5 years before, there is a gradual increase in the number of anovulatory cycles and this is referred to as the climacteric. Estrogen and inhibin secretion falls and negative feedback results in the increased pituitary secretion of LH and FSH.

Effects of estradiol include:

- Endometrial proliferation
- Genital development and lubrication
- Breast proliferation
- Bone epiphyseal closure and mineral content
- Body fat distribution
- Skin sebum

Effects of progesterone include:

- Endometrial secretory change
- Decreased myometrial contractility
- Thermogenesis
- Breast swelling

Presenting problems in reproductive disease

I- Delayed puberty

Puberty is defined as a stage of physical maturation in which an individual becomes physiologically capable of sexual reproduction. The main factor responsible for the initiation of puberty is the activation of the hypothalamic-pituitary-gonadal axis that induces and enhances the progressive ovarian and testicular sex hormone secretion that is responsible for the significant biological, morphological, and psychological changes to which the adolescent is subjected.

Puberty is considered to be delayed if the onset of the physical features of sexual maturation has not occurred by a chronological age that is 2.5 standard deviations above the national average.

In the UK this is by the age of 14 in boys and 13 in girls.

The most influencing factor that determines the timing and onset of puberty is the genetic factors, although other factors including nutritional status and chronic illness should be considered.

Clinical assessment

During the assessment of an individual with delayed puberty, the key issue is to determine whether the delay in puberty is simply because:

1- the 'clock is running slow' (constitutional delay of puberty), or

2- there is pathology in the hypothalamus/pituitary (hypogonadotropic hypogonadism) or

3- there is pathology in the gonads (hypogonadotropic hypogonadism).

A careful history and physical examination are essential and should be performed with particular reference to previous or current medical disorders, social circumstances and family history.

Body proportions, sense of smell and pubertal stage should be carefully documented and, in boys, the presence or absence of testes in the scrotum noted. Current weight and height may be plotted on centile charts along with parental heights. Previous growth measurements in childhood, which can usually be obtained from health records, are extremely useful.

A- Constitutional delay of puberty

This is the most common cause of delayed puberty. Affected children are healthy and have usually been more than 2 standard deviations below the mean height for their age throughout childhood. There is often a history of delayed puberty in siblings or parents.

Bone age is lower than chronological age. Constitutional delay of puberty should be considered a normal variant as puberty will commence spontaneously. However, affected children can experience significant psychological distress.

B- Hypogonadotropic hypogonadism

This may be due to structural, inflammatory, or infiltrative disorders of the pituitary and/or hypothalamus. In such circumstances, other pituitary hormones, such as growth hormone, are also likely to be deficient.

'Functional' gonadotropin deficiency is caused by a variety of factors including:

1- Chronic systemic illness (e.g. asthma, malabsorption, coeliac disease, renal failure)

2- Psychological stress

3- Anorexia nervosa

4- Hyperprolactinaemia

5- Other endocrine diseases (e.g. Cushing's syndrome, primary hypothyroidism).

Isolated gonadotropin deficiency is usually due to a genetic abnormality that affects the synthesis of either GnRH or gonadotropins.

The most common form is:

Kallmann's syndrome, in which there is primary GnRH deficiency with anosmia or hyposmia. If isolated gonadotropin deficiency is left untreated, the epiphyses fail to fuse, resulting in tall stature with disproportionately long arms and legs relative to trunk height. Hormonal evaluation reveals low levels of testosterone, FSH, and LH.

Cryptorchidism (undescended testes) and gynecomastia are commonly observed in all forms of hypogonadotropic hypogonadism.

C- Hypergonadotropic hypogonadism

Hypogonadotropic hypogonadism associated with delayed puberty is usually due to Klinefelter's syndrome in boys and Turner's syndrome in girls.

Causes of acquired Hypergonadotropic hypogonadism:

1- Chemotherapy/radiotherapy to gonads
2- Trauma/surgery to gonads
3- Autoimmune gonadal failure
4- Mumps orchitis
5- Tuberculosis
6- Haemochromatosis

Investigation

1- LH and FSH, testosterone (in boys) and estradiol (in girls).

2- Chromosome analysis is performed if gonadotropin concentrations are elevated.

3- If gonadotropin concentrations are low, then the differential diagnosis lies between constitutional delay and hypogonadotropic hypogonadism.

4- General tests (complete blood count, renal function, liver function)

Management

Low doses of oral estrogen in girls (e.g. ethinylestradiol 2 μg daily) or testosterone in boys (e.g. depot testosterone ester injections 50 mg i.m. titrated according to serum testosterone level).

In children with constitutional delay, this 'priming' therapy can be discontinued when endogenous puberty is established, usually in less than a year.

In children with hypogonadism, the underlying cause should be treated and reversed if possible. If hypogonadism is permanent, then sex hormone doses are gradually increased during puberty and full adult replacement doses are given when development is complete.

Amenorrhea

Primary amenorrhea describes the condition of a female patient who has never menstruated; this usually occurs as a manifestation of delayed puberty, but may also be a consequence of anatomical defects of the female reproductive system. Secondary amenorrhea describes the cessation of menstruation.

The causes of this common condition:

1- Physiological

• Pregnancy

• Menopause

• lactation

2- Hypogonadotropic hypogonadism (mentioned above)

3- Ovarian dysfunction

• Hypergonadotropic hypogonadism

• Polycystic ovarian syndrome

• Androgen-secreting tumors

3- Uterine dysfunction

• Asherman's syndrome

In non-pregnant women, secondary amenorrhea is almost invariably a consequence of either ovarian or hypothalamic/pituitary dysfunction. Premature ovarian failure is considered when amenorrhea occurs before 40 years of age.

Investigations

1- Pregnancy should be excluded in women of reproductive age by measuring human chorionic gonadotropin (hCG) in a sample of urine.

2- Serum LH, FSH, estradiol, prolactin, testosterone.

3- T4 and TSH

-High concentrations of LH and FSH with low or low normal estradiol suggest primary ovarian failure.

-Elevated LH, prolactin and testosterone levels with normal estradiol are common in PCOS.

-Low levels of LH, FSH and estradiol suggest hypothalamic or pituitary disease.

-Treatment depends on the cause.

Male hypogonadism

The clinical features of both hypo- and hypergonadotropic hypogonadism include:

1- loss of libido

2- lethargy

3- muscle weakness

4- decreased frequency of shaving.

5- gynecomastia

6- infertility

7- delayed puberty

8- anemia of chronic disease.

Investigations

Male hypogonadism is confirmed by demonstrating a low serum testosterone level. The distinction between hypo- and hypogonadotropic hypogonadism is by measurement of random LH and FSH.

High levels of LH and FSH indicate hypogonadotropic hypogonadism.

Treatment

Testosterone replacement

Infertility

Infertility affects around 1 in 7 couples of reproductive age, often causing substantial psychological distress.

In women, infertility may result from anovulation or from abnormalities of the reproductive tract that prevent fertilization or embryonic implantation, most commonly damaged fallopian tubes from a previous infection.

In men, infertility may result from impaired sperm quality (e.g. reduced motility) or number. Azoospermia or oligospermia is usually idiopathic but may be a consequence of hypogonadism.

In many couples, more than one factor causing subfertility is present, and in a substantial proportion, no cause can be identified.

Causes of infertility

A- Female factor (35–40%)

1- Ovulatory dysfunction

- Polycystic ovarian syndrome
- Hypogonadotropic hypogonadism
- Hypergonadotropic hypogonadism

2- Tubular dysfunction

- Pelvic inflammatory disease
- Endometriosis

3- Cervical and/or uterine dysfunction

- Congenital abnormalities
- Fibroids
- Asherman's syndrome

B- Male factor (35–40%)

1- Reduced sperm quality or production

- Y chromosome microdeletions
- Varicocoele
- Hypogonadotropic hypogonadism
- Hypergonadotropic hypogonadism

2- Tubular dysfunction

- Varicocoele
- Congenital abnormality of vas deferens/epididymis
- Previous sexually transmitted infection (chlamydia, gonorrhoea)

C- Unexplained or mixed factor (20–35%)

Investigations

Investigations are generally performed after a couple has failed to conceive after 12 months of marriage unless there is an obvious abnormality (e.g. amenorrhea).

Investigations are guided by the history and examination of both partners.

The male partner should provide at least two fresh semen samples, over an interval of several weeks, for analysis of sperm count and quality.

In women with regular periods, ovulation can be confirmed by an elevated serum progesterone concentration on day 21 of the menstrual cycle.

In women with irregular periods, such as in PCOS, weekly urine samples can be collected over several months to detect any peaks in progesterone metabolites that indicate ovulation.

Ultrasound can be used to assess uterine and ovarian anatomy.

Management

In women with anovulatory cycles secondary to PCOS, anti-estrogen therapy with clomifene or tamoxifen blocks negative feedback of oestrogen on the hypothalamus/ pituitary and encourages gonadotropin secretion and thus ovulation.

In women with gonadotropin deficiency or in whom anti-estrogen therapy is unsuccessful, ovulation may be induced by direct stimulation of the ovary by daily injection of FSH and an injection of hCG to induce follicular rupture at the appropriate time. In hypothalamic disease, pulsatile GnRH therapy is used.

Men with hypogonadotropic hypogonadism who wish fertility are usually given injections of hCG several times a week (recombinant FSH may also be required in men with hypogonadism of pre-pubertal origin); it may take up to 2 years to achieve satisfactory sperm counts.

Extraction of sperm from the epididymis for IVF, and intracytoplasmic sperm injection (ICSI, when single spermatozoa are injected into each oöcyte), are being used increasingly in men with oligospermia or poor sperm quality who have a primary testicular disease.

Gynecomastia

Gynecomastia is the presence of glandular breast tissue in males. Normal breast development in women is estrogen-dependent, while androgens oppose this effect. Gynecomastia results from an imbalance between androgen and estrogen activity, which may reflect androgen deficiency or estrogen excess. It should be always differentiated from pseudogynecomastia which is caused by the deposition of fat under and around the nipple.
The most common causes are physiological: for example, in the newborn baby (due to maternal and placental estrogens), in pubertal boys (in whom estradiol concentrations reach adult levels before testosterone) and in elderly men (due to decreasing testosterone concentrations).

Causes of gynecomastia

Idiopathic

Physiological

Drug-induced

- Cimetidine
- Digoxin
- Anti-androgens (cyproterone acetate, spironolactone)
- Some exogenous anabolic steroids (diethylstilbestrol)

Hypogonadism

Androgen resistance syndrome

Oestrogen excess

• Liver failure (impaired steroid metabolism)

• An estrogen-secreting tumor (for example, of testis)

• an hCG-secreting tumor (for example, of testis or lung)

Investigations

If a clinical distinction between gynecomastia and adipose tissue cannot be made, then ultrasonography or mammography is required.

A random blood sample should be taken for testosterone, LH, FSH, estradiol, prolactin and hCG. Elevated estrogen concentrations are found in testicular tumors and hCG-producing neoplasms.

Hirsutism

Hirsutism refers to the excessive growth of thick terminal hair in an androgen-dependent distribution in women (upper lip, chin, chest, back, lower abdomen, thigh, forearm) and is one of the most common presentations of endocrine disease.

The terminal hairs are thick, pigmented and fully matured that are distributed mainly on the scalp, pubic area, axilla and face (in males only) which differ completely from the villous hairs which are thin, short, and contain nearly no pigmentation.

Even a mild form of hirsutism might be noticed by the patient and might lead to serious mental stress. The main goals of hirsutism management are to exclude any serious underlying medical condition and to establish a treatment plan.

Causes

1- Idiopathic

2- Polycystic ovarian syndrome

3- Exogenous androgen administration

4- Androgen-Secreting Adrenal Tumors

5- Androgen-secreting Ovarian Tumors

6- Congenital Adrenal Hyperplasia

7- Iatrogenic Cushing Syndrome

Most cases are idiopathic but investigations are required to exclude other causes.

Investigations

Up to 50% of patients including those with mild hirsutism have excess androgen. Laboratory studies are directed to confirm the clinical impression of hyperandrogenism and to identify the source of excess androgens, either adrenal or ovarian.

A random blood sample should be taken for testosterone, prolactin, LH and FSH. If there are clinical features of Cushing's syndrome, an overnight 1 mg dexamethasone suppression test should be performed.

If the total testosterone level is normal, serum-free testosterone is recommended. An extremely high serum testosterone level might be caused by ovarian or adrenal tumors. In idiopathic hirsutism, testosterone level is within the normal range and the condition is thought to be due to increased sensitivity of the skin to testosterone.

If testosterone levels are elevated above twice the upper limit of the normal female range, especially if this is associated with low LH and FSH, then causes other than idiopathic hirsutism and PCOS are more likely, and the source of the androgen excess should be established.

Dehydroepiandrosterone sulfate (DHEAS): This type of androgen is synthesized exclusively in the adrenal cortex in contrast to testosterone which originates from the ovaries and the adrenal cortex. Accordingly, when both DHEAS and testosterone are increased, this indicates an adrenal pathology, while when testosterone is increased in the presence of normal DHEAS, this indicates an ovarian pathology.

Treatment recommendations (for premenopausal)

1- both pharmacological therapy and direct hair removal can be used.

2- even in the absence of a hormonal disturbance, pharmacological therapy is still an option.

3- oral combined contraceptives are suggested for most patients, with the addition of an antiandrogen agent after 6 months if the response is unsatisfactory.

4- monotherapy with an anti-androgen is not recommended.

5- monotherapy with insulin-lowering drugs is not recommended.

- Combined contraceptive pills are the first choice of treatment as they decrease androgen level.
- 6 months might be required for an improvement to be noticed.
- Patients should continue their treatment for years.
- Weight loss is of great importance in obese women.

Polycystic ovarian syndrome (PCOS)

PCOS describes a collection of clinical and biochemical features for which the primary cause remains uncertain. PCOS often affects several family members and is aggravated by obesity. Women with PCOS vary in the severity and combination of features that they manifest; the diagnosis is usually made during the investigation of patients presenting with hirsutism or amenorrhea/oligomenorrhea with or without infertility. It is associated with abnormalities in the metabolism of both androgen and estrogen and an abnormal hypothalamic-pituitary-ovarian (HPO) axis. it is an extraordinarily common disorder affecting 5-20% of women of childbearing age.

There is no universally accepted definition, but it has been recommended that PCOS requires the presence of two of the following three features:

1- menstrual irregularity

2- clinical or biochemical androgen excess

3- multiple follicles in the ovaries (most readily detected by ultrasound).

It is of great importance to differentiate between polycystic ovaries and PCOS where polycystic ovaries is a term used to describe an ultrasonic finding where 20 or more follicles in at least 1 ovary are present while the PCOS represents a metabolic disorder with certain clinical, laboratory and radiological features with or without polycystic ovaries.

Women with PCOS are at increased risk of glucose intolerance and some authorities recommend screening for type 2 diabetes and other cardiovascular risk factors associated with the metabolic syndrome.

Clinical and laboratory features

Mechanisms	Manifestations
Pituitary dysfunction	High serum LH High serum prolactin
Anovulatory menstrual cycles	Oligomenorrhoea Secondary amenorrhoea Cystic ovaries Infertility
Androgen excess	Hirsutism Acne
Obesity	Hyperglycaemia Elevated oestrogens
Insulin resistance	Dyslipidaemia Hypertension

Management

This depends on the clinical problem, but all overweight women with PCOS should be encouraged to lose weight as this may improve the clinical manifestations (especially menstrual irregularity) and reduce the risk of type 2 diabetes.

1- Menstrual irregularity and infertility

The menstrual disorder may not require treatment unless fertility is desired. **Metformin**, by reducing insulin resistance, may restore regular ovulatory cycles in overweight women, although it is less effective than **clomifene** at restoring fertility as measured by a successful pregnancy.

2- Hirsutism

A- Hair removal.

B- Anti-androgen therapy:

The life cycle of each hair follicle is at least 3 months, so no improvement is likely before this time when previous follicles have all shed their hair and replacement hair growth has been suppressed. Unless the patient has lost weight, the hirsutism will return if therapy is discontinued.

^^

^^

^^

23-24

جامعة الفراهيدي – كلية الصيدلة

Disorders of Calcium Metabolism

Clinical Chemistry for 5th-year Pharmacy Students

ا.م.د. رائد ضياء هاشم

Disorders of Calcium Metabolism

Introduction

At the beginning, and before starting to discuss the disorders of calcium metabolism, it is important to discuss a few concepts.

Total body water in an adult of 70 kg varies from 60 to 70% (36-49 litres) of total body weight when expressed as a percentage of lean body mass, i.e. the sum of the fat-free tissue. The body water is known to be distributed mainly in two compartments:

1- Intracellular fluid (ICF):

The fluid present within the cells is approximately 2/3 of total body water (28 L in 70 kg subject). Total body water = 0.6 x wt. (kg), so a subject with 70 kg weight has around 42 L of water in his body.

2- Extracellular fluid (ECF):

The fluid present outside the cells constitutes approximately 1/3 of total body water (14 L in a 70 kg subject). The extracellular fluid (ECF) is considered to be present in the two compartments as follows:

A- Plasma (intravascular):

The fluid that contains blood cells within the vascular system, represents approximately 1/4 of the ECF (3L in 70 kg subject).

B- Interstitial tissue fluid:

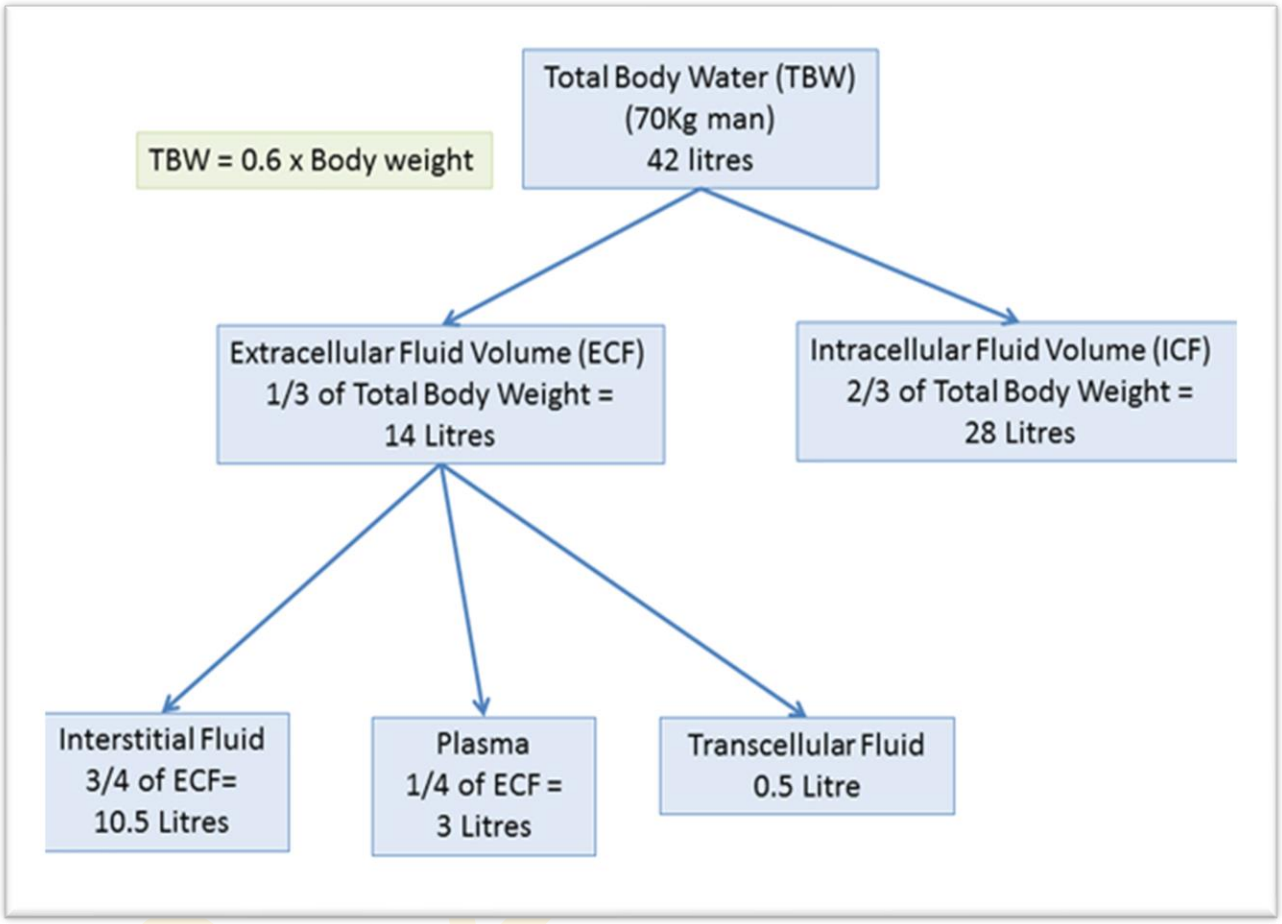
It is the fluid found in the spaces between the blood vessels and surrounding cells and represents approximately 3/4 of the ECF (11L in 70 kg subject).

C- Transcellular fluid:

A variety of extracellular fluid collections are formed by the transport or secretory activity of cells. Examples of transcellular fluids are:

1- Fluids found in salivary glands, pancreas, liver and biliary tract, skin, and the mucous membrane of respiratory and GI tracts.

2- The fluids present in spaces within the eyes (aqueous humour), cerebrospinal fluid (CSF) in the spinal canal and ventricles of the brain, and that within the lumen of the GI tract (mostly reabsorbed and not lost).



Distribution of electrolytes in the body

Non-electrolyte particles such as glucose, urea, etc do not dissociate in solution, while substances like NaCl and KCl in solution dissociate into sodium (Na-), potassium (K-) and chloride (Cl") ions, they are called electrolytes.

Water molecules completely surround these dissociated ions and prevent the union of positively charged particles with negatively charged ones. The positive ions are called cations and the

negatively charged ions are called anions. Fluid in any compartment will contain an equal number of cations and anions.

Electrolytes Composition of ECF:

Both plasma (IVF) and tissue fluid (ISF) may be considered as one single compartment for all practical purposes as both resemble each other and both differ grossly from ICF.

The electrolyte composition of ISF is similar to plasma except that Cl^- largely replaces proteins as the anion.

The predominant cation is Nat, and the predominant anion is Cl"

Electrolytes Composition of ICF:

ICF contains 195 mEq of cations and anions. Values of various electrolytes in ICF differ in different tissues, but the chief cations are K* followed by Mg. These are balanced by the chief anions PO4 and next by proteins.

So, total electrolytes concentration is higher in ICF than ECF (195 vs 155 mEq).

Electrolytes of plasma and tissue fluid

	Cations mEq/L			Anions mEq/L		
(a)	**Plasma**					
	Na^+	=	143	Cl^-	=	103
	K^+	=	5	HCO_3^-	=	27
	Ca^{++}	=	5	$HPO_4^=$	=	2
	Mg^{++}	=	2	$SO_4^=$	=	1
	Total	=	**155**	$Proteins^-$	=	16
				$Organic\ acids^-$	=	6
				Total	=	**155**
(b)	**Tissue fluid**					
	Na^+	=	145	Cl^-	=	116
	K^+	=	5	HCO_3^-	=	27
	Ca^{++}	=	3	$HPO_4^=$	=	3
	Mg^{++}	=	2	$SO_4^=$	=	2
	Total	=	**155**	$Proteins^-$	=	1
				$Organic\ acids^-$	=	6
				Total	=	**155**

Normal water balance

Total Body water is constantly exchanged with the external environment.

The input of Water:

Sources of water include:

- Water intake which is normally absorbed into the body from the bowel.
- Metabolic water: Formed from the oxidation of foodstuffs; each gram of carbohydrates, fats and proteins yields 0.55 gm, 1.06 gm and 0.45 gm of water respectively on complete oxidation.

In ml, on oxidation of 1 gm of carbohydrates, fats and proteins produce 0.56 ml, 1.07 ml, and 0.34 ml water respectively. In general, 10 to 15 ml of water is produced per 100 calories of energy.

The output of Water:

Water is lost from the body constantly from various routes, which are as follows:

1- Via kidney as urine: 1000 to 1500 ml in 24 hours. Scientifically speaking, the normal urine output of an adult is 1ml/kg/h.

2- Via skin as insensible perspiration: 600 to 800 ml of water in 24 hrs.

Frank sweating differs from insensible perspiration. Sweat is a "hypotonic" solution, 30 to 90 mEq/litre of NaCl is lost in sweating. In insensible perspiration there is no loss of salts, it is equivalent to distilled water.

3- Via lungs in the expired air: approximately 400-600 ml of water is lost in 24 hours.

4- Via feces: approximately 100 to 150 ml of water is lost in 24 hours from the large intestine in feces.

Average water intake and output in an adult			
Intake		Output	
Drinking	1000-1500 ml	Urine	1000-1500 ml
Water in food	700 ml	Lung	400 ml
Metabolic water	400 ml	Skin	600 ml
		Feces	100 ml
Total	2100 – 2600 ml	Total	2100 – 2600 ml

Normally in a healthy subject, the intake of water is more than the loss via skin, lungs and feces and the excess water is excreted by the kidneys.

Thus, the urinary volume largely depends on the intake of water.

If the intake of water is low or excessive amounts are lost via extrarenal channels, the excretion of urine is diminished. Urinary volume may be reduced to 500-600 ml in 24 hours and this is called the minimum excretory volume.

The loss through expired air (minimum 400 ml), by insensible perspiration through the skin (minimum 600 ml), loss through feces (minimum 100 ml) and the minimum excretory volume of the kidney to eliminate waste products,(i.e. 500 ml) is called as obligatory losses (approximately 1600 ml).

This loss will continue as long as the individual is surviving.

Calcium homeostasis

A healthy adult body has a total of 1 kg of calcium; about 99% of this is present within the crystal structure of the bone mineral, and less than 1% is in soluble form in the extracellular and intracellular fluid compartments. In the extracellular fluid compartment (ECF), about half of the total calcium is ionized, and the rest is principally bound to albumin or complexed with counter-ions.

Ionized calcium (which is the active form) in the ECF plays an important role in many physiologic pathways, including:

1- muscle contraction

2- secretion of neurotransmitters and hormones

3- coagulation pathways.

Ionized serum calcium concentrations range from 4.65 to 5.25 mg/dl, and the total serum calcium concentration ranges from 8.5 to 10.5 mg/dL. However, the usual 2: 1 ratio of total to ionized calcium may be disturbed by disorders such as:

1- metabolic acidosis, which reduces calcium binding by proteins.

2- changes in protein concentration, caused by cirrhosis, dehydration, venous stasis, or multiple myeloma.

In acidosis, the protonation of albumin reduces its ability to bind calcium, leading to an increase in unbound [Ca2+], and vice versa, without any change in total [calcium]. Thus, hyperventilation with respiratory alkalosis can reduce plasma [Ca2+], with the development of tetany.

Given this fact, total serum calcium concentrations are adjusted, or "corrected" to a reference albumin concentration: the actual total serum calcium value is adjusted according to this equation:

Corrected Calcium mg/dl = Serum Calcium + 0.08(40 - serum albumin g/l)

The control of body calcium involves a balance between:

1- the amounts of calcium that are absorbed from the gut.

2- the amounts of calcium that are deposited into the bone and cells.

3- the amounts of calcium that are excreted from the kidney.

This fine balance, involving three organs, is chiefly under the control of parathyroid hormone (PTH), which is synthesized and secreted by the parathyroid glands. Hypocalcemia leads to increased secretion of PTH, whereas hypercalcemia results in diminished PTH secretion. Regulation of extracellular calcium takes place through complex interactions at the target organs of the major calcium-regulating hormone, PTH, and vitamin D and its active metabolites, 1,25-dihydroxy vitamin D (1,25[OH]2D).

The renal actions of PTH:

(1) stimulate the activity of the one-alpha hydroxylase

(2) increase reabsorption of calcium by the cells of the distal tubule

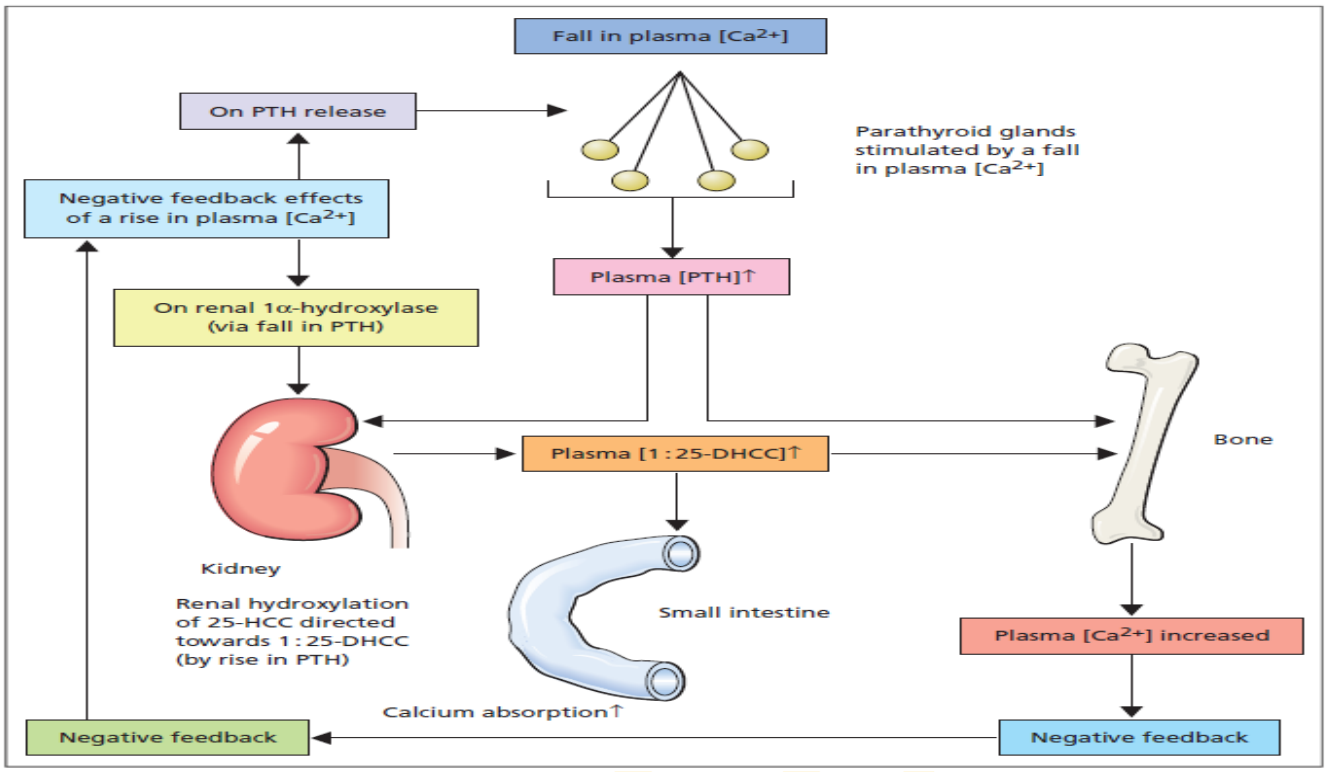
(3) inhibit phosphate reabsorption by proximal tubular cells.

Skeletal Actions of PTH

PTH acts directly on osteoblasts and indirectly on osteoclasts to increase their numbers and activity, thereby enhancing bone turnover and the release of stored calcium.

Intestinal actions of PTH

PTH exerts indirect actions on intestinal calcium absorption by increasing the circulating 1,25(OH)2D concentrations. The increased 1,25(OH)2D concentrations facilitate calcium absorption.



Hypercalcemia

Hypercalcemia is defined as total serum calcium above 10.5 mg/dL and ionized serum calcium above 5.25 mg/dL. There is no formal grading system for defining the severity of hypercalcemia, but mild, moderate, and severe hypercalcemia is generally considered for total serum calcium concentrations less than 12 mg/dL, between 12 and 14 mg/dL, and greater than 14 mg/dL, respectively.

Three mechanisms that may lead to hypercalcemia are:

1- increased bone resorption.

2- increased gastrointestinal absorption of calcium.

3- decreased renal calcium excretion.

Causes of hypercalcemia

I- High parathyroid hormone levels

1- Primary hyperparathyroidism (adenoma, hyperplasia, or carcinoma).

2- Tertiary hyperparathyroidism (hyperplasia or adenoma in chronic renal failure).

II- Low parathyroid hormone levels

1- Malignancy

A- Primary

- Parathyroid hormone-related peptide (PTHrP): bronchogenic carcinoma, renal cell carcinoma
- Excess production of $1,25(OH)_2D$ (lymphoma)

B- Secondary

- Lytic bone metastases (multiple myeloma and breast carcinoma)

2- Excess vitamin D

A commonly encountered cause these days because of the obsession with vitamin D deficiency

3- Drugs

- Thiazide diuretics - Lithium -Total parenteral nutrition
- Estrogens/antiestrogens, testosterone - Milk-alkali syndrome- Vitamin A toxicity

4- Non-parathyroid endocrine disorders

- Thyrotoxicosis - Pheochromocytoma - Acute adrenal insufficiency

5- Immobilization

Clinical features of hypercalcemia

The clinical features depend on the severity of hypercalcemia and vary from a mild, asymptomatic, biochemical abnormality detected during routine screening to a life-threatening medical emergency. Symptoms do not usually develop when serum calcium is below 12 mg/dL and are invariably present when the hypercalcemia exceeds 14 mg/dL.

The classical manifestations of hypercalcemia include:

1- Renal

Stones (nephrolithiasis) and nephrocalcinosis, polyuria, polydipsia. Polyuria is thought to be due to two main mechanisms;

1- calcium deposition in the medulla leads to secondary tubulointerstitial injury.
2- activation of the calcium-sensing receptor by hypercalcemia can directly impair the concentrating ability by affecting both the loop of Henle and collecting tubules.

2- Musculoskeletal

Bone pain, osteopenia, fractures, muscular weakness, especially proximal myopathy.

3- Gastrointestinal

Nausea, vomiting, lack of appetite, constipation, peptic ulcers, and pancreatitis. Peptic ulcer might occur due to increased gastrin secretion in response to hypercalcemia. Surprisingly, oral administration of calcium can increase gastrin secretion even in the absence of hypercalcemia. Hypercalcemia (of any cause) is a rare cause of a severe form of acute pancreatitis due to deposition of calcium in the pancreatic ducts.

4- Neurological

Tiredness, lethargy, inability to concentrate, increased sleepiness, depression, confusion, coma. It is thought that the changes in serum calcium level slow down nerve function and neurotransmission rate, inducing psychosis.

5- Cardiac

Bradycardia, first-degree atrioventricular block, arrhythmias, shortened QT interval

Investigations for hypercalcemia

A- Blood

1- Estimations of serum calcium, phosphate, albumin, urea and electrolytes, creatinine, alkaline phosphatase
2- Parathyroid hormone
3- Complete blood count
4- Serum protein electrophoresis
5- 25-OH-D3 (and if indicated, 1,25[OH]2D3)
6- Thyroid function tests
7- Parathyroid hormone-related peptide (if malignancy suspected)

B- Urine

Estimations of 24-hr urinary calcium and creatinine clearance.

C- Imaging

Chest radiograph

Radiograph of hands (subperiosteal bone resorption in hyperparathyroidism)

Ultrasound of kidneys

Treatment

1- Asymptomatic patients with mild hypercalcemia do not usually need urgent treatment.

2- Patients with severe hypercalcemia would require treatment regardless of symptoms.

3- patients with moderate hypercalcemia would require urgent treatment if symptomatic.

4- The first step in treatment is the correction of the cause if possible.

5- The acute management of hypercalcemia involves:

A- general measures to enhance hydration (with normal saline)

B- diuresis (with furosemide)

C- specific measures using drugs to lower serum calcium according to the cause.

D- potent bisphosphonates (parenteral pamidronate)

E- intravenous hydrocortisone (120 mg/d)

F- dialysis

Familial benign hypocalciuric hypercalcaemia (FBHH)

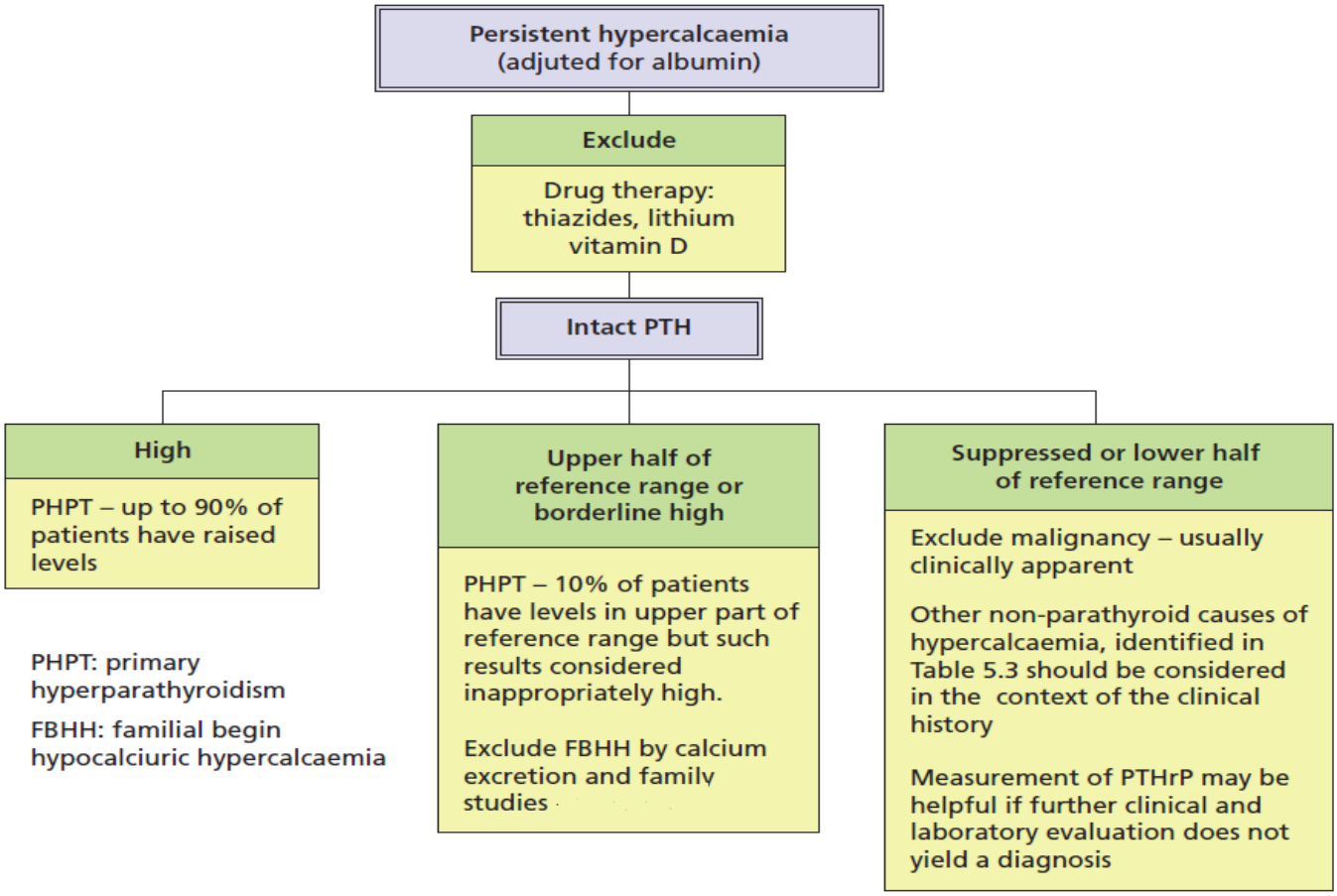
This is an autosomal dominant disorder that is usually asymptomatic and may have a population prevalence of up to 1:16000. It arises from an inactivating mutation in the calcium-sensing receptor gene in the parathyroid gland, kidney and other organs, and results in a high plasma [Ca2+] that is sensed as ‘normal’, with normal or marginally elevated plasma [PTH].

FBHH must be distinguished from primary hyperparathyroidism since parathyroidectomy does not reduce plasma [Ca2+], and no active treatment is indicated. While there is no single biochemical test that can always distinguish, unequivocally, FBHH from primary hyperparathyroidism, in patients with FBHH, urinary calcium is usually low and serum [magnesium] tends to be high normal. Therefore, a combination of family studies and the measurement of calcium excretion together with serum [magnesium] is helpful in identifying the condition. This condition is benign and requires no treatment as it is associated with mild hypercalcemia.

Milk–alkali syndrome

Milk consumption may be excessive in patients with symptoms of peptic ulceration; calcium intake is correspondingly increased. If this is accompanied by excessive intake of alkali (e.g. NaHCO3), as an antacid, hypercalcaemia may develop. The alkali is thought to reduce urinary calcium excretion and to be important in the pathogenesis of the condition. The prevalence of this condition has been significantly decreased with the new medications used for the treatment of peptic ulcer but with the routine use of calcium supplements especially in postmenopausal women the condition recurs again, so, some authors prefer the use of the term calcium- alkali syndrome instead.

A simplified approach to patients with hypercalcemia



Hypocalcemia

Hypocalcemia is defined as ionized serum calcium below 4.65 mg/dL and total serum calcium below 8.5 mg/dL. Mild hypocalcemia is defined as total serum calcium of 8 to 8.5 mg/dL) and severe hypocalcemia as total serum calcium below 7.6 mg/dL.

Causes of hypocalcemia

A- Low parathyroid hormone levels (hypoparathyroidism)

Parathyroid destruction (Surgery, Radiation, Autoimmune)

B- high parathyroid hormone levels (secondary hyperparathyroidism)

C- Vitamin D deficiency

inadequate production of active vitamin D (1,25[OH]2D) as a result of chronic renal failure.

D- Drugs

Inhibitors of bone resorption (e.g., bisphosphonates, calcitonin)

Altered vitamin D metabolism (e.g., phenytoin, ketoconazole)

Miscellaneous (Acute pancreatitis, Massive tumor lysis, Hyperventilation)

In acute pancreatitis: hypocalcemia is caused by the precipitation of calcium soap in the abdominal cavity. Calcium soap represents a complex of free fatty acids and calcium which appears as white chalky deposits in the abdomen.

In massive tumor lysis, severe hyperphosphatemia will lead to the precipitation of calcium phosphate in the renal cortex leading to hypocalcemia and acute kidney injury.

Hyperventilation is associated with alkalosis which leads to increased calcium binding to albumin and low serum-free calcium level.

E- Massive blood transfusion

The citrate present in the transfused blood as an anticoagulant will make a complex with calcium leading to hypocalcemia. Furthermore, citrate itself is broken down into HCO3 leading to alkalosis which in turn results in low serum-free calcium levels.

F- 1α-hydroxylase deficiency

Specific deficiency of 1α-hydroxylase may be the cause of hypocalcaemia in vitamin D-resistant rickets, type I, a rare inherited disorder. In vitamin D-resistant rickets, type II, there is end-organ unresponsiveness to 1:25-DHCC.

The most common cause of hypocalcemia is calcitriol deficiency which could be caused by:
1- vitamin D deficiency
2- impaired activation of vitamin D as in chronic kidney disease.
The associated laboratory findings include:
Low Ca, high PTH, high ALP, low PO4 and extremely low urinary calcium.
Home work: how could you explain each of the laboratory findings?

Clinical features of hypocalcemia

The clinical presentation of hypocalcemia ranges from an asymptomatic biochemical abnormality to a severe, life-threatening condition. In mild hypocalcemia, patients may be asymptomatic. Those with more severe and long-term hypocalcemia may develop acute symptoms of neuromuscular irritability.

Common clinical features of hypocalcemia include:

1- Paresthesia, usually of fingers, toes, and circumoral regions.

2- Tetany, carpopedal spasm, muscle cramps.

3- Chvostek's sign (twitching of the circumoral muscles in response to gentle tapping of the facial nerve just anterior to the ear).

4- Trousseau's sign (carpal spasm elicited by inflation of a blood pressure cuff to 20 mm Hg above the patient's systolic blood pressure for 3 minutes).

5- Seizures of all types (i.e., focal or petit mal, grand mal, or syncope).

6- Prolonged QT interval on the electrocardiogram

7- Laryngospasm and Bronchospasm

Never forget to assess albumin level in patients with hypocalcemia to avoid artifactual hypocalcemia by applying the corrected calcium equation, or by direct measurement of ionized calcium.

Treatment

I- Acute Hypocalcemia

The preferred treatment for acute symptomatic hypocalcemia is calcium gluconate, 10 mL 10% w/v (2.26 mmol of calcium) intravenous, diluted in 50 mL of 5% dextrose or 0.9% sodium chloride and given by slow injection (>5 minutes); this can be repeated as required to control symptoms.

ll- Chronic Hypocalcemia

The two main agents available for the treatment of chronic (long-term) hypocalcemia are supplemental calcium and vitamin D preparations.

Hyperparathyroidism

Hyperparathyroidism is characterized by high concentrations of serum PTH and is generally classified into three types, referred to as primary, secondary, and tertiary.

Primary and tertiary hyperparathyroidism are associated with hypercalcemia, whereas secondary hyperparathyroidism is associated with hypocalcemia.

I- Primary Hyperparathyroidism

Most cases occur due to isolated adenoma of one of the 4 glands although adenocarcinoma may rarely present, which leads to hypercalcemia due to excessive secretion of PTH. It is three times more common in females than in males.

Clinical features

The same classical features of hypercalcemia.

Diagnosis

1- hypercalcemia

2- hypophosphatemia

3- high PTH

4- normal ALP

5- low HCO3 (metabolic acidosis may be present since PTH increases urinary HCO^{-3} losses)

Treatment

Parathyroidectomy

After parathyroidectomy, serum [calcium] falls rapidly, and it should be measured several times on the first postoperative day and at least daily for the next few days. If the serum [calcium] falls below normal, calcium gluconate should be given and treatment with vitamin D should be started.

II- Secondary Hyperparathyroidism

Hyperplasia of the parathyroid glands secondary to prolonged hypocalcemia. The most common cause of prolonged hypocalcemia is chronic kidney disease. Secondary hyperparathyroidism usually resolves with treatment of the underlying cause of hypocalcemia unless this hyperplasia may become permanent if the treatment is delayed leading to what is known as tertiary hyperparathyroidism where PTH level is persistently high even if the serum calcium level is normalized, which is treated with thyroidectomy.

Disorders and syndromes associated with hypercalcemia

I- Thyrotoxicosis

Mild hypercalcemia (<12 mg/dL) frequently accompanies thyrotoxicosis, which leads to increased bone turnover and resorption. Hypercalcemia may respond to treatment with B-adrenergic blockers.

II- Malignancy

Hypercalcemia may occur in 20 to 30% of patients with a malignancy, and this is usually due to increased bone resorption, which may either be directly due to skeletal metastases or indirectly due to tumor production of a humoral factor that stimulates osteoclastic bone resorption.

Hypoparathyroidism

In a patient with combined hypocalcemia and hyperphosphatemia, in the absence of renal disease, hypoparathyroidism is the most probable diagnosis. Serum PTH is extremely low or undetectable with normal ALP activity. Primary hypoparathyroidism is rare and the cause is usually secondary to surgical removal.

Pseudohypoparathyroidism is a rare but interesting condition, in which the end-organ receptors in the bone and kidneys fail to respond normally to PTH. Patients with pseudohypoparathyroidism have increased serum [PTH] in the presence of hypocalcemia and hyperphosphatemia.

The condition is treated with calcium and calcitriol supplements.

Metabolic bone disease

Because of the vital role of calcium in bone mineralization, disorders of calcium metabolism are regarded as common causes of metabolic bone disorders and at the top of these disorders lie osteomalacia and rickets in addition to osteoporosis.

Both osteomalacia and rickets represent a nearly similar condition but the age of presentation, hence, the clinical features differ although certain features are in common. The main defect is the failure of calcification of osteoid bone mostly due to vitamin D deficiency.

Clinical features include bone pain and tenderness with proximal myopathy.

Other causes of rickets or osteomalacia, unrelated to vitamin D deficiency or defects in its metabolism, have also been described. An inherited defect in the tubular reabsorption of phosphate, hypophosphataemic vitamin D-resistant rickets, leads to similar bone deformities, but without muscle weakness; there is a low serum [phosphate] and phosphaturia.

Pathological fractures are common in old age patients while bone deformity (bowing) and short stature are expected in untreated children. Furthermore, in females, pelvic distortion from rickets may cause problems with childbirth later in life.

Laboratory findings include low vitamin D, hypocalcemia and increased ALP with high PTH.

Treatment includes vitamin D and calcium supplements.

Osteoporosis

It is the most common metabolic bone disorder mainly affecting postmenopausal women although it is often overlooked and undertreated as it is usually asymptomatic and can initially present with a fracture. It is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility. It is classified into primary and secondary osteoporosis with further subtypes of the primary one according to etiology but the hallmark of the disease is accelerated bone loss. In postmenopausal women, low estrogen is the direct cause of osteoporosis.

Osteoporosis is characterized by decreased bone mass while osteomalacia is characterized by decreased bone minerals to bone matrix ration.
Osteoporosis is much difficult to be treated compared to osteomalacia.
Osteoporosis presents lately compared to osteomalacia.

Other causes include:

1- low testosterone in males.

2- hyperparathyroidism

3- Cushing's syndrome and prolonged steroid use

4- prolonged PPI use, heparin, certain antiepileptic drugs (phenytoin),

Diagnosis

Laboratory findings are normal

Measurement of bone mineral density is the investigation of choice by the use of DEXA scan (Dual X-ray AbsorptiometrY).

Outlines of treatment

1- Non-pharmacological therapy: exercise and cessation of smoking.
2- Recommended doses of vitamin D and calcium.
3- Bisphosphonates(alendronate , risedronate).
4- Denosumab (prolia): inhibits osteoclasts.
5- PTH

Treatment

Unfortunately, no treatment can completely reverse established osteoporosis, so prevention is of great importance. For secondary osteoporosis, treating the underlying cause can resolve the condition.

Bisphosphonate can be used for both the treatment and prevention of osteoporosis with a different dose of course.

****

Metabolic bone disease: biochemical investigations on blood specimens.

Diagnosis	Calcium	Phosphate (fasting)	PTH	Alkaline phosphatase	Ca^{2+}
Hyperparathyroidism					
Primary	↑ (or N)	↓ or N	↑ or N*	N or ↑	↑ (or N)
Secondary	↓ or N	↑ or N	↑	↑ or N	N
Tertiary	↑ or N	↑ or N	↑	↑ or N	↑
Rickets and osteomalacia					
Deficient intake	↓ or N	↓ or N	↑ (or N)	↑	N (or ↓)
Renal failure	↓ or N	↑ or N	↑	↑	N
Fanconi syndrome†	↓ or N	↓ or N	N	↑	N
Osteoporosis	N	N	N	N	N
Paget's disease	N (or ↑)	N	N	↑	N

==========

23-24

جامعة الفراهيدي – كلية الصيدلة

Tumor markers

Clinical Chemistry for 5th-year Pharmacy Students

ا.م.د. رائد ضياء هاشم

8

TUMOR MARKERS

Introduction

A tumor marker is a substance produced by a tumor or by the host in response to a tumor, which is used to differentiate a tumor from normal tissue, or to detect the presence of a tumor based on measurements in the blood or secretions. Such substances are found in cells, tissues, or body fluids and are measured qualitatively or quantitatively by chemical, immunologic, or molecular biological methods.

Morphologically, cancer tissue has been recognized by pathologists as resembling fetal tissue more than normal adult differentiated tissue. Tumors are graded according to their degree of differentiation as:
(1) well differentiated,
(2) poorly differentiated
(3) anaplastic (without form).

Tumor markers are the biochemical or immunologic counterparts of the differentiation state of the tumor. In general, some tumor markers represent the re-expression of substances produced normally by embryogenically closely related tissue.

Only a few tumor markers are specific to one type of cancer; others are seen in several cancer types. Many well-known markers are also seen in noncancerous conditions. Consequently, these tumor markers are not diagnostic for cancer. However, it is thought that the concentration of tumor markers in blood reflects tumor activity and volume.

From the clinical point of view, an ideal tumor marker should be both specific for a given type of cancer and sensitive enough to detect small tumors for early diagnosis or during screening. Unfortunately, few markers are specific for a single individual tumor (tumor-specific markers); most are found with different tumors of the same tissue type (tumor-associated markers).

In clinical practice, the most useful use of current tumor markers is in the evaluation of the progression of disease status after initial therapy and for monitoring subsequent treatment modalities.

Features of an ideal tumor marker

(1) specific production by premalignant or malignant tissue early in the progression of disease.
(2) produced at detectable levels in all patients with a specific malignancy.
(3) expression in an organ site-specific manner.
(4) evidence of presence in bodily fluids obtained noninvasively or in easily accessible tissue.
(5) levels related quantitatively to tumor volume, biological behavior, or disease progression.
(6) relatively short half-life, reflecting temporal changes in tumor burden and response to therapy.
(7) existence of a standardized, reproducible, and validated quantitative assay.

Cancer

A simple definition of cancer is the relatively autonomous growth of tissue. Understanding the cause of autonomous growth would clearly facilitate the search for a cure.
A carcinogen is an agent that causes cancer. A carcinogen may be:
1- physical (e.g., radiation)
2- chemical (e.g., a polycyclic hydrocarbon)
3- biological (e.g., a virus).

Exposure to such an agent may cause cancer by producing direct genotoxic effects on deoxyribonucleic acid (DNA) (e.g., as with radiation) or by increasing cell proliferation (e.g., by a hormone), or both (e.g., through the use of tobacco).

Prostate cancer is the leader among men, and breast cancer is the leader in women, followed by cancer of the lung, colon-rectum, and bladder (men) or uterine corpus (women).
Surprisingly, since the peak death rates from all cancers in 1990 for men and 1991 for women, death rates decreased significantly. This significant rate of decrease supports the conclusion that early detection and more effective treatment combined with prevention (e.g., decreasing smoking, and improving diet) could reduce the mortality rate of cancer in the future.

Advances in molecular genetics have resulted in a better understanding of the genesis of human cancer. The proliferation of normal cells is thought to be regulated by growth-promoting oncogenes and counterbalanced by growth-constraining tumor suppressor genes.

The development of cancer appears to involve the activation or the altered expression of oncogenes, or the loss or inactivation of a tumor suppressor gene.

The goal is to diagnose cancer when a tumor is still small enough to be completely removed surgically. Unfortunately, most cancers do not produce symptoms until the tumors are too large to be removed surgically, or until cancerous cells have already spread to other tissue (metastasized).

In general, tumor markers are classified into the following categories:

(1) enzymes
(2) hormones
(3) oncofetal antigens
(4) carbohydrate markers
(5) blood group antigens
(6) proteins
(7) receptors
(8) genes

In normal cells, genes regulate cell growth, maturation, and death. Genetic changes can manifest at various levels, encompassing the gain or loss of entire chromosomes or the occurrence of a single-point mutation that affects a solitary DNA nucleotide.

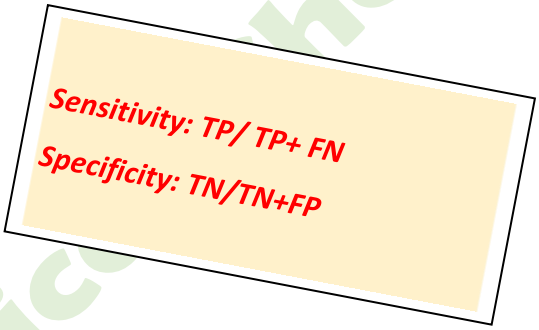
The two main genes involved in the development of cancer are:

Oncogenes: are responsible for normal cell division but when develop a mutation, they might lead to the development of cancer.

Tumor suppressor genes: these prevent cell division when there is damaged DNA, thus preventing the development of cancer.

Clinical applications of tumor markers

Despite the many clinical applications of tumor markers in the management of cancer, most of these applications are limited by the variable sensitivity and specificity of each tumor marker.



The two most reliable uses of tumor markers in clinical practice are:

1- Prediction of therapeutic response

Very few known markers have a powerful predicting response to specific therapies.

These include:

A- the steroid hormone receptors for predicting response to antiestrogens.

B- HERr-2/*neu* amplification for predicting response to Herceptin in breast cancer patients.

2- Monitoring the effectiveness of cancer therapy

For patients with advanced disease, who are treated with various modalities, it is important to know if therapy works. In this regard, biomarkers usually provide information that is readily interpretable, more economical, more sensitive, and safer than radiologic or invasive procedures.

In breast cancer, the concentration of markers, such as CA 15-3 or CA 27.29, changes with treatment and the clinical outcome of the patient.

Other limited uses include:

3- Screening for cancer

Except for PSA, most cancer markers are not specific to a particular tissue, and elevations may be due to diseases of other tissues, including benign and inflammatory diseases. Thus, diagnostic specificity may be low, leading to many false positives.

In screening, a confirmatory test is mandatory for a definitive diagnostic method that will separate true positives from false positives.

4- Diagnosing cancer

Surprisingly, tumor markers are of limited use in the diagnosis of cancers, again due to low specificity and sensitivity.

5- Evaluating cancer prognosis

Despite that many tumor markers have prognostic values; their accuracy is not accurate enough to warrant a specific mode of therapy.

7- Tumor staging

As the value of a tumor marker is not significantly related to the size of the tumor, its metastasis, lymph nodes state, or capsular penetration, tumor markers are of limited use in staging of cancer.

8- Localizing tumor and directing radiotherapeutic agents

Only a few tumor markers are applicable for this use.

Another important use of tumor markers but with controversy is:

9- Detecting tumor recurrence or remission

Despite the importance of using biomarkers to detect cancer relapse, current markers are limited by the following:

A- In certain groups of patients, biomarkers are not produced and do not detect relapses.

B- Therapies for treating recurrent diseases are not effective at present.

Sometimes biomarkers provide misleading information (e.g., clinical relapses occur without biomarker elevation, or biomarker is elevated nonspecifically, without progressive disease, leading to overtreatment or discontinuation of a current and successful treatment protocol).

"If no therapy is given, at least a linear increase in three consecutive samples (i.e., two-time intervals) on a log scale should be registered to establish a recurrence. Usual intervals could be three months but are clinically determined. After a first increase, next samples should be taken after 2 to 4 weeks, irrespective of the absolute concentration."

Groups of tumor markers

I- Enzymes

Enzymes are among the first groups of tumor markers that have been discovered. Only a few enzymes have enough sensitivity and specificity to be used as a tumor marker.

The most widely used tumor marker of this group is PSA (Prostate-specific antigen) is expressed by normal, benign, hyperplastic, and cancerous prostate glands and minimally by other tissue.

1- Alkaline phosphatase (ALP)

The alkaline phosphatase in the sera of normal adults comes primarily from the liver. As a tumor marker, ALP is elevated in the:

1- primary or secondary liver cancer

2- bony metastasis (prostate or breast cancer)

To determine the origin of elevated alkaline phosphatase, 5-nucleotidase or γ -glutamyltransferase can be measured simultaneously, their elevation suggests a hepatic origin of elevated ALP.

2- Lactate Dehydrogenase (LDH)

LDH is an intracellular enzyme released to the circulation in case of cell damage. Elevation of LDH in malignancy is rather nonspecific. It has been demonstrated in a variety of cancers, including liver cancer, non-Hodgkin's lymphoma, acute leukemia, nonseminomatous germ cell testicular cancer, seminoma, neuroblastoma, and other carcinomas, such as breast, colon, stomach, and lung cancer. Serum LDH has been shown to correlate with tumor mass in solid tumors and provides a prognostic indicator for disease progression.

3- PSA

Prostate cancer is the leading cancer in older men. It is potentially curable by radical prostatectomy when detected early (organ-confined). Therefore, early detection is important, and PSA is widely used for this purpose. It is considered one of the most promising tumor markers available.
PSA exists in two major forms in blood circulation, the free form, and the protein-bound form.

Clinical Applications of PSA

PSA is an extremely useful tumor marker and is used to detect and monitor treatment of prostate cancer.

A- Screening and Early Detection of Prostate Cancer

The use of PSA alone for screening and early detection of prostate cancer is of limited value because of the overlap between PSA concentration in patients with benign prostatic hyperplasia (BPH) and early prostate cancer, particularly between 4 and 10 μ g/L.

The use of serum PSA together with digital rectal examination (DRE) is more accurate and sensitive. Several approaches were used to improve the ability of PSA to detect early prostate cancer and/or spare unnecessary biopsies, the most important approach is the use of free PSA / total PSA ratio. Patients with prostate cancer have less free PSA / total PSA ratio than patients with BPH.

B- Monitoring Treatment

The greatest clinical use for PSA is in the monitoring of definitive treatment of prostate cancer. This treatment includes radical prostatectomy, radiation therapy, and antiandrogen therapy. As PSA is produced almost exclusively by prostatic tissue; thus, after radical prostatectomy, the PSA concentration should fall below the detection limit of the assay. This may require 2 to 3 weeks owing to the half-life of PSA.

If the half-life is longer than usual, it must be assumed that a residual tumor is present. Biochemical recurrence has been defined as two post-prostatectomy PSA concentrations ≥0.2 μ g/L. Increasing PSA after radical prostatectomy is a strong indication of disease recurrence.

II- Hormones

The production of hormones in cancer involves two separate routes.

First, the endocrine tissue that normally produces it can produce excess amounts of a hormone. Second, a hormone may be produced at a distant site by a nonendocrine tissue that normally does not produce the hormone. The latter condition is called *ectopic syndrome*.

Multiple endocrine neoplasias (MEN) syndromes (MEN-1, MEN-2A, and MEN-2B) are familial disorders inherited in an autosomal dominant fashion that is manifested by both benign and malignant tumors. Various polypeptide hormones, such as (1) ACTH, (2) calcitonin, (3) gastrin, (4) glucagon, (5) insulin, (6) secretin, and (7) vasoactive intestinal polypeptide might be found in this type of malignancy.

Examples of hormones used as tumor markers include:

1- Calcitonin

Calcitonin is a polypeptide with 32 amino acids produced by C cells of the thyroid. Normally, it secreted in response to increased serum calcium, inhibits the release of calcium from bone and thus lowers the serum calcium concentration.

An elevated concentration is usually associated with medullary carcinoma of the thyroid (MCT).

Calcitonin is used for screening, diagnosis, and monitoring therapy of MCT. Calcitonin concentrations appear to correlate with indicators of the extent of the disease, such as tumor volume and tumor involvement in local and distant metastases. Calcitonin is useful for monitoring treatment and detecting the recurrence of disease.

Calcitonin elevation has been reported in other nonmalignant conditions, such as:

(1) pulmonary disease (2) pancreatitis (3) hyperparathyroidism
(4) pernicious anemia (5) Paget's disease of bone (6) pregnancy

2- Human Chorionic Gonadotropin (hCG)

Basically, hCG is used for the diagnosis and monitoring of pregnancy. It is a useful tumor marker for tumors of the placenta (trophoblastic tumors) and for some tumors of the testes.

Clinical Applications

The upper reference limit in men and nonpregnant women is 5.0 IU/L.

-Patients with trophoblastic tumors typically have elevated concentrations of hCG (>1 million IU/L).

-It is also elevated in 70% of those with nonseminomatous testicular germ cell tumors, and less frequently in those with seminoma.

-Because hCG does not cross the blood-brain barrier, the normal cerebrospinal fluid-to-serum ratio is 1: 60. Higher concentrations in cerebrospinal fluid may indicate metastases to the brain.

-Furthermore, the response to therapy for patients with central nervous system metastasis may be observed by monitoring the CSF hCG concentration.

III- Oncofetal antigens

Oncofetal antigens are proteins produced during fetal life. These proteins are present in high concentration in the sera of fetuses and decrease to low concentration or disappear after birth.

In cancer patients, these proteins often reappear, revealing that certain genes are reactivated as the result of the malignant transformation of cells.

1- α-Fetoprotein (AFP)

AFP is a marker for hepatocellular and germ cell (nonseminoma) carcinoma.

Clinical Applications

- AFP is physiologically elevated during pregnancy, starting at the 12th week of gestation, and reaching its peak at the 14th week.
- Significant elevation of AFP occurs in hepatocellular carcinoma (HCC).
- Minimal elevation is seen in early HCC, hepatitis, or liver cirrhosis.
- AFP is also useful for determining prognosis and for monitoring therapy for hepatocellular carcinoma.
- The AFP concentration is a good indicator for monitoring therapy and the change in clinical status. Elevated AFP concentrations after surgery may indicate incomplete removal of the tumor or the presence of metastasis.
- The combination of AFP and hCG is useful in classifying and staging germ cell tumors.

2- Carcinoembryonic antigen (CEA)

CEA is a marker for colorectal, gastrointestinal, lung, and breast carcinoma, but because of significant false positive and false negative results, it is not used for screening. CEA testing may be useful as an adjunct to clinical staging. Persistently elevated concentrations that are 5 to 10 times the upper reference limit strongly suggest the presence of colon cancer.

After successful initial therapy, CEA concentrations decline. Rising CEA concentrations may indicate a recurrence of the disease. The lead time from CEA elevation to clinical recurrence is about 5 months.

IV- Carbohydrate markers

Carbohydrate-related tumor markers may be:

(1) antigens on the tumor cell surface.

(2) secreted by the tumor cells.

The most important examples of this group of tumor markers are:

1- CA 125 Ovarian, endometrial

2- CA 15-3 Breast, ovarian

3- CA 27.29

1- CA 15-3

In a healthy subject, CA 15-3 is present in trivial concentrations. Higher levels can be present in:

A- 5.5% of normal subjects

B- 23% of patients with primary breast cancer

C- 69% of those with metastatic breast cancer

Because of these relatively low rates, CA 15-3 should not be used to diagnose primary breast cancer, it is most useful in monitoring therapy and disease progression in metastatic breast cancer patients.

2- CA 27.29

CA 27.29 is a marker for breast carcinoma.

It is used for the detection of recurrent breast cancer in patients with stage II or stage III disease and for monitoring response to therapy in patients with stage IV (metastatic) disease.

3- CA 125

CA 125 is a marker for monitoring ovarian cancer.

The primary FDA-indicated use for CA 125 is to monitor response to therapy in patients with epithelial ovarian cancer.

The second FDA-indicated use is to detect residual or recurrent disease

CA 125 is also useful in differentiating benign from malignant disease in patients with palpable ovarian masses. This differentiation is important because surgical intervention for malignant ovarian masses is far more extensive than that for benign masses.

CA 125 is useful for detecting residual disease in cancer patients following initial therapy.

V- PROTEINS

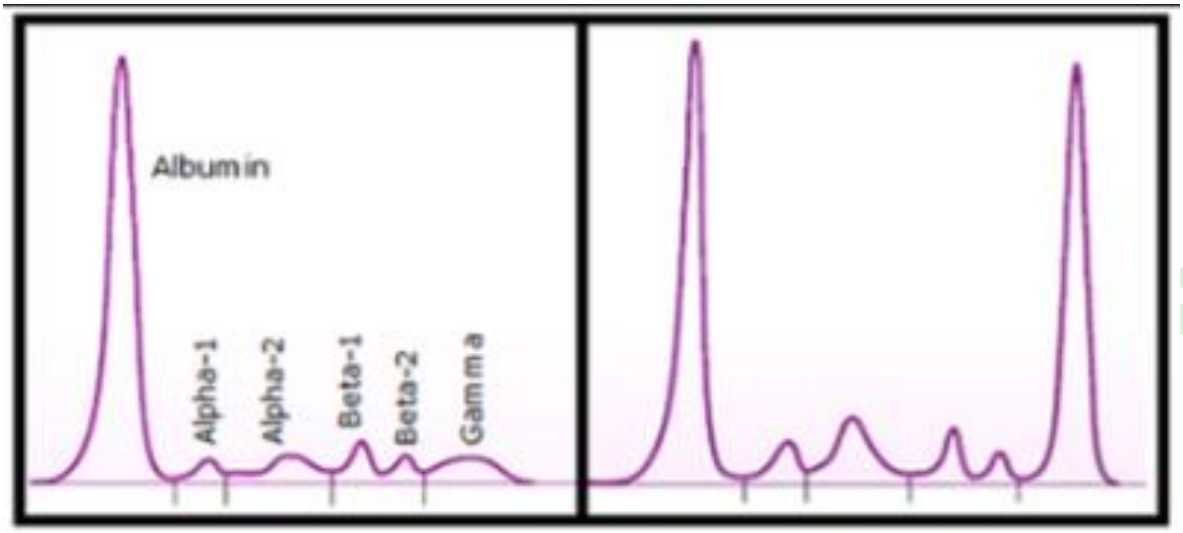
Several proteins, other than enzymes and hormones, can be used as tumor markers with various sensitivity and specificity.

1- Immunoglobulin

Monoclonal immunoglobulin has been used as a marker for multiple myeloma. Monoclonal paraproteins appear as sharp bands in the globulin region of serum electrophoretic patterns.

More than 95% of patients with multiple myeloma have such an electrophoretic pattern. Appearance of nonmalignant monoclonal immunoglobulins increases with age, reaching 5% in patients older than 75 years. Bence Jones protein is a free monoclonal immunoglobulin light chain in the urine.

The concentration of monoclonal immunoglobulin at initial diagnosis is a prognostic indicator of disease progression.



Protein categories in a normal serum electrophoresis trace (left) and abnormal monoclonal protein (right) seen in a Myeloma patient

Diagnostic criteria of multiple myeloma

- M-protein in serum: IgG ≥3 g/dL, IgA >1 g/dL ***or***
- Bence-Jones protein >1 g/24h ***and/or***
- Bone marrow clonal plasma cells ≥10%
- Signs of end-organ damage:

  Calcium >11.5 mg/dL (>2.65 mmol/L)
  Creatinine >2 mg/dL (177 µmol/L or more)
  Hemoglobin <10 g/dL or 2 g/dL <normal (<12.5 mmol/L<normal)
  Lytic or osteopenic bone disease

VI- Receptors

Estrogen and progesterone receptors

Estrogen and progesterone receptors are used in breast cancer as indicators for hormonal therapy. Patients with positive estrogen and progesterone receptors tend to respond to hormonal treatment. Those with negative receptors will be treated using other therapies, such as chemotherapy. Hormone receptors also serve as prognostic factors in breast cancer. Patients positive for hormone receptors tend to survive longer.

ERs and PRs are routinely measured in all newly diagnosed breast cancers. Of patients with carcinoma of the breast, 60% have tumors that are ER-positive. The greater the ER content of the tumor, the higher the response rate to endocrine therapy.

The PR assay is a useful adjunct to the assay of ERs. Indeed, metastatic breast cancer patients with both ER- and PR-positive tumors have a response rate of 75% to endocrine therapy, whereas those with ER-positive and PR-negative tumors have a 40% response rate.