MKU

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

DML 2212: CLINICAL CHEMISTRY II

UNIT TEACHING OUTLINE

WEEK	TOPIC
1 AND 2	LIPID METABOLISM, DISORDER AND ANALYSIS
3	CARDIAC FUNCTIONS ,DISORDERS AND ANALYSIS
4 AND 5	CARBOHYDRATE METABOLISM, DISORDERS AND ANALYSIS
6 AND 7	RENAL FUNCTIONS,DISORDERS AND ANALYSIS
8	LIVER FUNCTION ,DISORDERS AND ANALYSIS
9	ELECTROLYTES BALANCE ,TYPES DISORDERS AND DETERMINATION
10	BLOOD GASES AND ACID/BASE BALANCE, DISORDERS AND ANALYSIS
11	CEREBROSPINAL FLUID: COMPOSITION,COLLECTION,FUNCTIONS AND ANALYSIS
	STOOL ANALYSIS: COMPOSITION,COLLECTION, OCCULT BLOOD AND FAECCAL FAT ESTIMATION
12	Bone metabolism/endocrinology

LIPIDS DISORDERS AND LIPIDS ESTIMATIONS

LIPIDS (FATS) DISORDERS

-Lipids are insoluble (does not dissolve in water but are soluble (dissolves) in alcohol and other solvents. When diatary fats are digested and absorbed into the small intestine, they eventually re-form into triglycerides, which are then packaged into lipoproteins.

- Dietary fats, including cholesterol are absorbed from the small intestine and transported into the liver by lipoproteins called CHYLOMICRONS. Chylomicrons are large droplets of lipids with a thin shell of phospholipids, cholesterol, and protein. ***Once chylomicrons enter the bloodstream , an enzyme called lipoprotein lipase breaks down the tryglycerides into fatty acids and glycerol.***

-After 12-14 hours fast , chylomicrons are absent from the bloodstream. Thus, individuals who are having a lipid profile done should fast overnight to ensure that chylomicrons have been cleared.

LIPOPROTEINS

- Since the lipids(fats) are insoluble in body fluids, there movement within the blood circulation has to be facilitated by the combination with a compound which is soluble in the body fluids. Proteins in circulation have the characteristic of being soluble in the body fluids therefore combines with the lipids to form a compound referred to as LIPOPROTEIN.There are three types of lipoproteins in the blood namely:-High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C) and Very low density lipoprotein cholesterol (VLDL-C).
- *HDL-C and LDL-C are the major types of Cholesterol that circulate in the bloodstream and are the ones considered to be of clinical significance.VLDL-C is very low and has no diagnositic value. Therefore, a healthy person"s total cholesterol is mostly determined by the amount of the HDL-C and LDL-C in a measured sample of blood.(For example a total cholesterol level of 5.6 mmol/l could be represented by an HDL-C of 3 mmol/l and an LDL-C of 2.6 mmol/l or HDL-C of 2.6 mmol/l and an LDL-C of 3 mmol/l..*

- *From a clinical laboratory perspective, the levels of lipids is measured by carrying out a lipid profile estimation which takes into account total cholesterol (TC), HDL-C, LDL-C, the triglycerides and the ratio of TC/HDL-C.*

- *A health provider may order a lipid profile as part of annual exam or if there is specific concern about cardiovascular disease (CVD), especially coronary artery disease (CAD) also referred to as coronary heart disease(CHD)*

LIPIDS (FATS) DIORDERS

The lipids disorders forms part of the cardiovascular diseases which are coronary heart disease, hypertension, stroke, congenital heart disease and rheumatic heart disease. A person may have one of

these diseases or combination of forms at the same time. Each form exists in varying degrees of severity. All forms are capable of causing secondary damage to other body organs and body systems.

Among the above mentioned major CVD, coronary heart disease or coronary artery disease is the one directly related to the lipids disorder.

CORONARY HEART DISEASE OR CORONARY ARTERY DISEASE

-This form CVD involves damage to the vessels that supply blood to the heart muscles. The bulk of this blood is supplied by the coronary arteries. Any damage to these important vessels can cause a reduction of blood (and its vital oxygen and nutrients) to specific areas of heart muscles. The ultimate result of inadequate blood supply is a heart attack.

ATHEROSCLEROSIS

The principal cause for the development of coronary heart disease is atherosclerosis. Atherosclerosis produces a narrowing of the coronary arteries. This narrowing stems from the long term buildup of fatty deposits, called plaque, on the inner walls of the arteries. This buildup reduces the blood supply to specific portions of the heart. Some arteries of the heart can become so blocked (occluded) that all blood supply is stopped. Heart muscle begins to die when deprived of oxygen and nutrients. This damage is known as myocardial infarction (in lay terms, this event is called a heart attack).

LIPIDS AND ATHEROSCLEROSIS

-After much scientific studies, it has been determined that high levels of LDL-C, are a significant cause of atherosclerosis. This makes sense because LDL-C carry the greatest percentage of cholesterol in the bloodstream. LDL-C are more likely to deposit excess cholesterol into the artery walls. This contribute to plaque formation.For this reason, LDL-C, is often referred to as –THE BAD CHOLESTEROL---. High levels of LDL-C are partially related to inheritance, but they are clearly associated with smoking, poor dietary patterns, obesity and lack of exercise.

- On the other hand, high levels of HDL-C are related to decrease in the development of atherosclerosis. HDL-Care thought to transport cholesterol out of the bloodstream. Thus HDL-C, have been called the----GOOD CHOLESTEROL---. Certain lifestyle alteration such as quitting smoking, reducing obesity, increase physical activities, and decreasing dietary fats, help in increasing the levels of HDL-C.

- Reducing serum total cholesterol levels is a significant step in reducing the risk of death from coronary heart desease.

ANGINA PECTORIS

-When coronary arteries becomes narrowed, chest pain , or angina pectoris is often felt. This pain results from a reduction supply of oxygen to heart muscles. Usually angina pectoris is felt when the patient becomes stressed or exercises too strenuously. Angina pectoris reportedly can range from a feeling of mild indigestion to a severe viselike pressure in the chest. The pain may extend from the centre of the chest to the arm and even up to the jaw. Generally the more severe the blockage , the more pain is felt.

LIPID PROFILE ANALYSIS

-This is a panel of tests done in the investigation of fat metabolism disorders and its related complication such as diabetes mellitus, acute and chronic pancreatitis and some endocrine disorders.

- The tests under these profiles are: total cholesterol, triglycerides, high density lipoprotein cholesterol(HDL) and low density lipoprotein cholesterol (LDL)

TOTAL CHOLESTEROL(TC)

CHOLESTEROL OXIDASE /PEROXIDASE ENZYMATIC METHOD

Test principle

Cholesterol esters in the sample is hydrolyzed in the presence of cholesterol esterase to form cholesterol and fatty acids..The cholesterol is the oxidized in the presence of cholesterol oxidase to cholestene-3 – one and hydrogen peroxide.. the hydrogen peroxide formed reacts with 4 – aminoantipyrine and phenol in the presence of peroxidase to form a red dye quinoneimine and water. The red dye formed is directly proportional to the HDLC in the sample and it is measured photometrically at 500nm.

REFERENCE RANGES IN LITERATURE (3.6-7) MMOL/L

TRIGLYCERIDES (TG)

This is the most common complex lipid also referred to fatty acid derivative. This fatty acid derivative is covalently linked to an alcohol. The most common alcohol s found in human metabolism is glycerol-Esterification of fatty acids with glycerol produces GLYCERIDES (ACYLGLYCEROLS). TRIGLYCERIDES are the most prevalent dietary glycerol esters encountered.. they constitute 95% of the fat stored in the tissues and are the predorminant form of glycerol ester found in plasma.- Clinically , triglycerides assays are used to help classify various genetic and metabolic lipids disorders , and in the assessment of risk factors for atherosclerosis and coronary artery disease.. Triglyceride assays also are used in the diagnosis and treatment of patients with acute and chronic pancreatitis, DM , nephrosis and extrahepatic biliary obstruction.

Test principle

This is based on a series of coupled enzymatic reactions

-The triglyceride in the sample is hydrolyzed by lipase to give glycerol and fatty acids.

- The glycerol is phosphorylated by ATP in the presence of glycerol kinase(GK) and Mg^{2+} to produce glycerol-3-phosphate.

-Glycerol-3-phosphate is oxidized in the presence of glycerol phosphate oxidase(GPO) to produce hydrogen peroxide(H2O2) and dihydroxyacetone phosphate.

-The hydrogen peroxide formed reacts with 4- aminoantipyrine (4-AAP)(aminaphenazone) AND 3,5-dichloro-2-hydroxydenzeesulfonic acid (DHBS) in the presence of peroxidase to form a red quinoneimine dye. The red dye formed is directly proportional to the concentration of triglyceride concentration in the sample and its measured photometrically at 520nm

Nb/ if in the last step of reaction 3,5-dimethylaniline, disodium salt (MADS) is used instead of DHBS), a blue dye is formed and this is measured at 660 nm)

REFERENCE RANGES IN LITERATURE=(0.83-2.30MMOL/L

HIGH DENSITY LIPOPROTEIN –CHOLESTEROL (HDL-C)

-This is a part of lipid which is in circulation attached to a protein molecule (ie albumin), thus refered to as lipoprotein. It is produced in the liver and the intestines .

- HDL appears to be important in cholesterol efflux from the tissues , thereby reducing the amount of cholesterol stored there. Due to this action , HDL is referred to as GOOD CHOLESTEROL.

(A) PRECIPITATION METHOD

This method involve the estimation of plasma content of HDL-C , after selective precipitation of VLDL-C and LDL-C with various polyanions (eg heparin and manganese chloride). Centrifugation is carried out and the supernatant used to assay HDL-C using the reagents and apparatus for TOTAL CHOLESTEROL.

CHOLESTEROL OXIDASE /PEROXIDASE ENZYMATIC METHOD

Test principle

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-LDL-C can be fairly accurately be estimated using the FRIEDWALD formula:

LDL-C= TC- (TG/2.2+HDL-C)

NB/ ***This formula is not varied when the serum/plasma TG level is >4.5 mmol/l***

(B)LATEST METHODS CURRENTLY USED AS DIRECT ASSAYS HAVE INCORPORATED NON-HDL ANTI-B-LIPOPROTEIN IN THE FIRST STEP OF THE REACTION AS EXPLAINED BELOW.

Test principle

Anti human –B-lipoprotein antibody binds to lipoproteins other than HDL (LDL,VLDL. and chylomicrons).The antigen antibody complexes formed block enzyme reactions. HDLC is quantified by the presence of an enzyme chromogen system

REFERENCE RANGES IN LITERATURE=(>1.5MMOL/L

LDL CHOLESTEROL

This is the type of cholesterol that has been associated with causing the development of coronary heart disease(CHD), with numerous clinical and epidemiological studies demonstrating its atherogenic properties. High levels of LDLC- are associated with increased cardiovascular risk and familial hyperlipideamia. Reduced levels of LDL-C may be found in malabsorption and malnutrition.

Test principle

A protecting agent protects LDLC from enzymatic reactions. All non-LDLC lipoproteins(HDL,VLDL,CM) are broken down by reaction with cholesterol esterase(CHE) and cholesterol oxidase (CHO). H_2O_2 produced by this reaction is decomposed by catalase in the first step of the reaction.

In the second step of the reaction, the protecting agent is released from the LDLC and the catalase inactivated by sodium azide. LDLC can now be quantified by the CHO/POD system

REFERENCE RANGES IN LITERATURE=(<3.9Mmol/L)

SPECIMEN fasting specimen is recommended.. SERUM or PLASMA can be used

CARDIAC FUNCTION AND CARDIAC TEST PROFILE

INTRODUCTION

(1) NORMAL CARDIOVASCULAR FUNCTION

The cardiovascular or circulatory system uses a muscular pump to send a complex fluid on a continuous trip within a closed system of tubes. This pump is the heart, the fluid is the blood and the closed system of tubes is the network of blood vessels.

(1A) THE VASCULAR SYSTEM

The term vascular system refers to the body's blood vessels. The vessels includes:-

ARTERIES ----vessels that carry blood away from the heart and VEINS----vessels that carry blood toward the heart. Other vessels are arterioles, capillaries and venules.

(1B) THE HEART

The heart is a four chambered pump designed to create the pressure required to circulate blood throughout the body. It is made up of ***two upper chambers*** called **atria** and ***two lower chambers*** called **ventricles** . The thin walled atrial chambers are considered collecting chambers, whereas the thick-walled muscular ventricles are considered the pumping chambers. The right and left side of the heart are divided by a partition called **septum.**

For the heart muscle to function properly it must be supplied with adequate amounts of oxygen and this is accomplished by the ***two main coronary arteries*** (and their numerous branches).

heart stimulation

The heart contracts and relaxes through the delicate interplay of cardiac muscle tissue and cardiac electrical centers called ***nodes.*** Nodes tissues generate the electrical impulses necessary to contract heart muscles. The heart electrical activity is measured by an instrument called ***electrocardiograph*** (ECG or EKG) which produces a printout called **electrocardiogram** that can be evaluated to determine cardiac electrical functioning.

(1C) BLOOD

Blood accounts for **8% of the human body weight**, with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3.**The average adult has a blood volume of roughly 5 liters**, composed of plasma and several kinds of cells (occasionally called *corpuscles*); these formed elements of the blood are erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Blood's functions which are performed continuously, are quite similar to the overall functions of the circulatory system and includes the following:

- **Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)**
- **Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))**
- **Removal of waste such as carbon dioxide, urea, and lactic acid**

- **Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies**
- **Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)**
- **Messenger functions, including the transport of hormones and the signaling of tissue damage**
- **Regulation of body pH**
- **Regulation of core body temperature**
- **Hydraulic functions**

CARDIOVASCULAR DISEASE RISK FACTORS

The following are compounding risk factors that are associated with cardiovascular diseases. These risk factors includes those that cannot be changed, those that can be changed and those that are thought to be contributing to heart disease.

Risk factors that cannot be changed

(a) Increasing age----
Heart diseases tend to develop gradually over the course of one's life. Most of the consequences of heart disease are evident in old ages eg approximately 80% of people who die from heart attack are 65 years and above.

(b) Male gender----
Men are prone to heart disease than women. It is thought that women are some what more protected from heart disease than men because of their natural production of the hormone estrogen during their fertile years.

(c) Heredity----
Children born in families with history of heart disease are said to have a genetic predisposition to develop heart disease as they grow and develop throughout their lives

Risk factors that can be changed

There are four cardiovascular risk factors that that are influenced, in large part by our lifestyle choices.

(a) Cigarette or tobacco consumption
Smokers have a heart attack risk that is over twice that of nonsmokers. Smoking cigarettes is the major risk factor associated with sudden cardiac death. Cigarettes and tobacco smoke also adversely affects nonsmokers who are exposed to environmental tobacco smoke. Due to this risk, indoor smoking in public areas and business settings are increasing tremendously in most countries.

(b) Physical inactivity
Lack of exercise is a significant risk factor for heart disease. Regular exercise helps to strengthen the heart muscle, maintain healthy blood vessels, and improve the ability of the vascular system to transfer blood and oxygen to all parts of the body. Physical activities also helps to lower the overall blood cholesterol, encourages weight loss and retention of lean muscle mass and allows people to moderate stress in their lives.

(c) high blood lipid levels
The higher the blood cholesterol the higher the risk of heart disease. Specifically the high levels of low density lipoprotein cholesterol and triglycerides

(d) high blood pressure
High blood pressure can seriously damage a person's heart and blood vessels. High blood pressure causes the heart to work much harder, eventually causing the heart to enlarge and weaken. It increases the chances of stroke, heart attack, congestive heart failure and kidney disease.

Other risk factors that contribute to heart disease

(a) diabetes

Diabetes mellitus is a debilitating chronic disease that has a significant effect on the human body. In addition to increasing the risk of developing kidney disease, blindness, and nerve damage, DM increases the likelihood of developing heart and blood vessel disease. The cardiovascular damage is thought to occur when DM begins to alter normal cholesterol and blood fat levels.

(b) Obesity

Even without any other risk factor, odese people are more likely than nonobese people to develop heart disease and stroke. Obesity places considerable strain on the heart , and tends to influence both blood pressure and blood cholesterol levels.It also triggers diabetes in predisposed people.

(c) Individual response to stress

Unresolved stress over a long period may be a contributing factor to the development of heart disease. People who are unable to cope with stressful life experiences are more likely to develop negative dependence behaviors eg smoking, underactivity, poor dietary practice0 which can lead to cardiovascular problems.

FORMS OF CARDIOVASCULAR DISEASE

CORONARY HEART DISEASE (coronary artery disease)

Involves damage to the vessels that supply blood to the heart.The bulk of this blood is supplied by the coronary arteries. Any damage to these important vessels will cause a reduction of blood (and its vital oxygen and nutrients) to specific areas of the heart muscle. The ultimate result of inadequate blood supply is a heart attack.

Atherosclerosis is the principle cause of the development of coronary heart disease. It produces the narrowing of the coronary arteries which is due to long term buildup of fatty deposits called plaque on the inner walls of the arteries.

Hypertension

This is the consistent elevation of blood pressure. People with hypertension are often not aware that they have it. Hypertension is not thought as a curable disease , rather , it is controllable disease.

Stroke

Results due to blood vessel damage in the brain perhaps due to the blockage of a cerebral artery. Blood clot that forms within the artery (***thrombus***) or a clot that travels from another part of the body to the brain (***embolus***). The resultants accidents ie cerebral thrombosis or cerebral embolism causes between 70% to 80% of all strokes.

Congenital heart disease

This disease is present at birth. It is caused by valve damage, holes in the septum, blood vessel transposition and underdevelopment of the left side of the heart. Rubella has been known to cause this disease.

Rheumatic heart disease

This is the final stage in a series of complications started by streptococcal infections of the throat.

CARDIAC FUNCTION TESTS (CARDIAC PROFILE)

To investigate the above mention pathological complications of the cardiac system, the following parameters which makes cardiac profile are analyzed in the clinical laboratory:

(1) Creatine kinase or creatine phosphokinase(CK or CPK)

(2) Creatine kinase isoenzymes ie CK BB (CK-1), CK MB (CK-2) and CK MM (CK-3)

(3) Lactate dehydrogenase (LDH)

(4) Aspartate aminotransferase (AST)

(5) Hydroxybutyrate dehydrogenase (HBDH)

(6) Myoglobin (MYG)

(7) Troponin

Most routine laboratory are able to carry out analytes CPK,LDH, and AST. HBDH is analyzed in place of LDH. Creatine isoenzymes CK BB, CK MB and CK MM , MYOGLOBIN and TROPONIN are done as special chemistry.

CREATINE KINASE OR CREATINE PHOSPHOKINASE (CK OR CPK) AND ITS ISOENZYMES

CK catalyses the reversible phosphorylation of creatine by adenosine triphosphate(ATP) to form phosphocreatine(creatine phosphate) and adenosine diphosphate(ADP)

$$Cr + ATP \rightarrow ADP + PCr$$

CK activity is greatest in striated muscles, brain and heart tissue. CK is a dimer composed of two sub units ie B or Brain and M or muscle which are the product of two distinct genes. From these two sub units , three pairs of iso enzymes (isoforms) do exists ie CK BB, CK MB and CK MM. All these three iso-enzymes are found in the cell cytosol .

The CK BB isoform is predorminantly found in the brain, CK MB isoform predorminantly found in the heart tissue and CK MM predorminantly found in the skeletal muscles.

CLINICAL SIGNIFICANCE

Investigated in the diagnosis of diseases of the skeletal muscles (muscular dystrophy) especially in the Duchenne type

Investigated in the diagnosis of diseases of the heart. After myocardial infarction , CK levels in blood are invariably elevated.Also after a heart surgery the CK levels are elevated.

Diseases of the central nervous system will increase the activity of CK especially in cerebral ischemia, acute cerebralvascular disease and head injury.

Disease of thyroid------Serum CK activity demonstrate an inverse relationship with thyroid activity. About 60% of hypothyroid subjects usually have elevated CK levels. By contrast , in hyperthyroidism subjects, the serum CK activity tends to be at the low end of the reference ranges.

DETERMINATION OF CK ACTIVITY

This is an enzymatic kinetic UV method for quantitative determination of creatine kinase..

SPECIMEN OF CHOICE:serum, heparinized plasma

Reference range for the adult Kenyan population (26 – 262 U/L).

DETERMINATION OF CK ISOENZYMES (CK BB, CK MB AND CK MM)

The three techniques most commonly used for the determination of CK isoenzymes are electrophoresis, ion-exchange chromatography and immunological methods.Among these three methods , the routinely practiced method is the immunological technique based on antigen antibody reactions.

SPECIMEN OF CHOICE:serum, heparinized plasma

Reference range in literature of CK MB (0-5NG/ML).

MYOGLOBIN ESTIMATION

Myoglobin is a heme protein which reversibly binds oxygen, thus favouring its distribution in the muscle cells. Myoglobin is stored in the cytoplasm and this accounts for its rapid release from damaged muscle cells. The rapid increase of myoglobin concentration in blood makes it a useful, early maker of acute myocardial infarction. Since myoglobin is present in both cardiac and skeletal muscles, serum levels may increase in conditions relating not only to the heart (infarctus, heart surgery) but to the muscles (strenuous exercise, muscular dystrophy)

LAB INVESTIGATION

Uses immunological technique

SPECIMEN OF CHOICE:serum, heparinized plasma

Reference range in literature (10-46 µg/l).

Lactate Dehydrogenase (LDH)

LDH activity is present in all cells of the body and it is invariably found in the cytoplasm of the cell.LDH is a hydrogen transfer enzyme that catalyzes the oxidation of l-lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor. The reaction is reversible and the equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate to lactate.

LAB INVESTIGATION

This is an enzymatic kinetic UV test for the quantitative determination of LDH. In the reaction, LDH catalyzes the oxidation of lactate to pyruvate coupled with the reduction of NAD^+ to NADH. The increase of NADH is directly proportional to the LDH activity in the sample.

SPECIMEN OF CHOICE:serum, heparinized plasma

Reference range for the adult Kenyan population (FEMALE**:**86 – 532 U/L U/L, MALE: 216-466U/L)

ASPARTATE AMINOTRANSFERASE (AST)---

This is an enzyme found in the intracellular fliud of the cell and concerned with the transfer of aminoacids in an enzymatic reaction that leads to a metabolic activity that facilitates a physiological function.. AST is highly concentrated within the cell of many organs such as the hepatocytes,cardiac and some skeletal cells.

LAB INVESTIGATION

This is an enzymatic kinetic UV test for the quantitative determination of AST

SPECIMEN OF CHOICE:serum, heparinized plasma

Reference range for the adult Kenyan population (3-45 U/L)

TROPONIN

The troponins are a family of regulartory proteins found in skeletal and heart muscle (cardiac) fibers. There are three different types: troponin C (TnC), troponin T (TnT), and troponin I (TnI). Together, these three proteins regulate muscular contraction. The main difference is that the TnC subunit of troponin in skeletal muscle has four calcium ion binding sites, whereas (TnT), and (TnI) in cardiac muscle have only three calcium binding sites.

The other difference is that, ***TnC is predominantly found in the skeletal muscles*** where it exacts its regulatory function whereas ***Cardiac-specific troponins I and T (cTnI and cTnT) are troponins that are found only in the heart.*** Cardiac-specific troponins differ in their molecular

weights in that cTnI has a molecular weight of 23.9 kD whereas cTnT has a molecular weight of 39.7 kD.

All these types of troponin are normally present in very small to undetectable quantities in the blood.

Troponin tests (I and T) are primarily ordered to evaluate people who have chest pain to see if they have had a heart attack or other damage to their heart. Either a cardiac-specific troponin I or troponin T test can be performed; usually a laboratory will offer one test or the other. Troponin tests are sometimes ordered along with other cardiac biomarkers, such as AST,LDH,CPK, CK–MB, CK-MM or myoglobin. **However, troponins are the preferred tests for a suspected heart attack because they are more specific for heart injury than other tests (which may become elevated in skeletal muscle injury) and remain elevated for a longer period of time.**

LABORATORY INVESTIGATION
DETERMINATION OF TROPONIN I AND T

The most commonly used technique for the determination of CARDIAC TROPONINS is the immunological technique based on antigen antibody reactions.The two approaches of these two immunological techniques are (1)**enzyme linked fluorescent assay (ELFA**).
(2**)electrochemiluminescence immuno assay(ECLIA**)

Specimen of choice: serum or heperinized plasma

Normal Results as quoted in literature

Cardiac troponin levels are normally so low

- Troponin I : less than 10 µg/L
- Troponin T : 0–0.1 µg/L

Normal troponin levels 12 hours after chest pain has started mean a heart attack is unlikely.

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TOPIC THREE—CHO METABOLISM,DISORDERS AND ANALYSIS

Glucose is the end product of carbohydrate metabolism. It is a simple sugar that provides energy to all cells in human body (some cells, like brain cells and red blood cells, rely solely on glucose for fuel.

Human body tries to keep a constant supply of glucose for the cells by maintaining a constant concentration otherwise the cells would have more than enough glucose supply right after meal and starve in between meals and overnight.

The body responds to oversupply of glucose by storing the excess in the liver and the muscles in form of glycogen When glucose is in short supply, the body mobilizes glucose from stored glycogen and this is the key to maintain a constant blood-glucose level.

To maintain a constant blood-glucose level, the body relies on two hormones produced in the pancreas that have opposite actions: insulin and glucagon.

INSULIN

Insulin is made and secreted by the beta cells of the pancreatic islets (Islets of Langerhans), small islands of endocrine cells in the pancreas.

. It is required by almost all of the body's cells, since it ***enhances glucose entry into these cells****.*

GLUCAGON

Glucagon is another protein hormone that is made and secreted by the alpha cells of the pancreatic islets (Islets of Langerhans).

Glucagon acts on the same cells as insulin, but has the opposite effects: Stimulates the liver and muscles to break down stored glycogen (glycogenolysis) and release the glucose.

It stimulates gluconeogenesis in the liver. In contrast to insulin, glucagon mobilizes glucose from stores inside the body and increases the concentrations of glucose in the bloodstream. Insulin and glucagon have opposite effects on liver and other tissues for controlling blood-glucose level.

DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia (elevated blood glucose concentration) resulting from defects in insulin secretion, insulin action, or both.

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision.

CLASSIFICATION OF DIABETES MELLITUS

TYPE 1 DIABETES MELLITUS (JUVENILE DIABETES OR INSULIN-DEPENDENT DIABETES)

Diabetes is classified into three types: Type 1, Type 2 and gestational diabetes. Type 1 (juvenile diabetes or insulin-dependent diabetes) is caused by a lack of insulin. This type is found in five percent to 10 percent of diabetics and usually occurs in children or adolescents. Type 1 diabetics have an abnormal glucose-tolerance test and little or no insulin in their blood. In Type 1 diabetics, the beta cells of the pancreatic islets are destroyed, possibly by the person's own immune system, genetic or environmental factors

TYPE 2 DIABETES MELLITUS (ADULT-ONSET DIABETES OR NON-INSULIN-DEPENDENT DIABETES)

Type 2 (adult-onset diabetes or non-insulin-dependent diabetes) occurs when the body does not respond or can't use its own insulin (insulin resistance). Type 2 occurs in 90 percent to 95 percent of diabetics and usually occurs in adults over the age of 40 years, most often between the ages of 50years and 60 years.

About 55 percent of type 2 diabetes patients are obese at diagnosis).

However, environmental factors (almost certainly diet and weight) play a large part in the development of type 2 diabetes in addition to any genetic component.

Genes significantly associated with developing type 2 diabetes, include *TCF7L2*, *PPARG*, *FTO*, *KCNJ11*, *NOTCH2*, *WFS1*, *CDKAL1*, *IGF2BP2*, *SLC30A8*, *JAZF1*, and *HHEX*, *KCNJ11*

Type 2 diabetics have an abnormal glucose-tolerance test and higher than normal levels of insulin in their blood. In Type 2 diabetics, the insulin resistance is linked to obesity, but it is not know exactly how this occurs. Some studies suggest that the number of insulin receptors on liver, fat and muscle cells is reduced, while others suggest that the intracellular pathways activated by insulin in these cells are altered (Barrett, 2001).

GESTATIONAL DIABETES MELLITUS (GDM)

Gestational diabetes mellitus (GDM) can occur in some pregnant women and is similar to Type 2 diabetes mellitus*.GDM has also been defined as any degree of glucose intolerance with onset or first recognition during pregnancy.This broad definition of GDM therefore includes women whose glucose intolerance develops during pregnancy and those that had pre-existing diabetes which had not been diagnosed before pregnancy.*

Pregnancy is a diabetogenic condition characterized by insulin resistance .*Insulin resistance usually begins in the second trimester and progresses throughout the remainder of the pregnancy**. Insulin sensitivity is reduced by as much as 80% **(). Placental secretion of hormones, such as progesterone, cortisol, placental lactogen, prolactin, and growth hormone, is a major contributor to the insulin-resistant state seen in pregnancy***

GDM has long been known to raise the risk of macrosomia in the offspring, with the high glucose levels thought to cross the placenta and stimulate fetal insulin secretion which then acts as a growth factor (). Macrosomia brings its own difficulties such as increased risks of shoulder dystocia, brachial plexus injuries and clavicular fractures in natural deliveries leading to a higher requirement for birth by Caesarean section (). ***Other short-term risks associated with GDM in the mother are pregnancy-induced hypertension and pre-eclampsia, urinary tract infections, pyelonephritis and polyhydramosis (which itself leads to increased risk of abruption placentae). There is also increased risk of stillbirth and congenital abnormalities****. Management of GDM is by special diets and/or supplemental injections of insulin. It usually clears after the baby is delivered.*

IMPAIRED GLUCOSE TOLERANCE (IGT) AND IMPAIRED FASTING GLUCOSE (IFG)

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) is a category of hyperglycaemic disorder which identifies individuals whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal

PREVIOUS ABNORMALITY OF GLUCOSE TOLERANCE

*Previous abnormality of glucose tolerance is a category of hyperglycaemic disorder which identifies individuals known to have had an abnormality of glucose tolerance at one time but have been shown to have normal results on repeat testing. Someone who had Type 2 diabetes mellitus, was obese, not treated with insulin, and after weight reduction had a normal glucose tolerance test falls under this category****).***

POTENTIAL ABNORMALITY OF GLUCOSE TOLERANCE

Potential abnormality of glucose tolerance is a category of hyperglycaemic disorder which identifies individuals who are at greater than average theoretical risk for later development of diabetes mellitus. Identical twin of a patient with type 2 diabetes or a child of parents who each had type 2 diabetes falls under this category.

Regardless of the type of diabetes, diabetics exhibit several (but not necessarily all) of the following symptoms: Excessive thirst (polydipsia), frequent urination (polyuria), extreme hunger or constant eating (polyphagia), unexplained weight loss, presence of glucose in the urine (glycosuria), tiredness or fatigue, changes in vision, numbness or tingling in the extremities (hands, feet), slow-healing wounds or sores and abnormally high frequency of infection ().

HYPOGLYCÆMIA

*Hypoglycæmia is the medical term for a state produced by a lower than normal level of blood glucose. It can produce a variety of symptoms and effects but the principal problems arise from an inadequate supply of glucose to the brain, resulting in impairment of function (neuroglycopenia))****.*** *Effects can range from vaguely "feeling bad" to seizures, unconsciousness, and (rarely) permanent brain damage or death. The most common forms of hypoglycemia occur as a complication of treatment of diabetes mellitus with insulin or oral medications. Hypoglycemia is less common in non-diabetic persons, but can occur at*

any age, from many causes. Among the causes are excessive insulin produced in the body (hyperinsulinemia), inborn errors of metabolism, medications and poisons, alcohol, hormone deficiencies, prolonged starvation, alterations of metabolism associated with infection, and organ failure The precise level of glucose considered low enough to define hypoglycemia is dependent on:(i)the measurement method,(ii) the age of the person, (iii) presence or absence of effects, and(iv) purpose of the definition.

GLUCOSE TEST PROFILES

Clinical laboratory investigation plays a great role in the diagnosis of either hyperglycaemic or hypoglycaemic status of an individual. Laboratory diagnostic profile tools that forms part of ones glucose status diagnosis are:-fasting blood glucose (FBG), random blood glucose (RBG), oral glucose screening test (OGST) and oral glucose tolerance test (OGTT)

FASTING BLOOD GLUCOSE (FBG)

The success of FBG requires that an individual abstains or refrains from food of any kind overnight or any other period except water; and for purposes of confirming, fasting should take a minimum of 8 hours overnight. Ref values in literature 2.7-6.4 mmol/l

RANDOM BLOOD GLUCOSE (RBG)

There is no pre-preparation of the test .it can be done any time ie the client should not have fasted at all.Best test for carrying out population based studies. Ref values in literature 2.7-8.3 mmol/l

SCREENING GLUCOSE CHALLENGE TEST (SGCT)

*SGCT (sometimes called the O'Sullivan test) is a test performed under the two approaches recommended for the screening of GDM**.** Since there is no worldwide agreement on the best way to screen for GDM,universal and selective screening approaches have been adopte.*

Universal screening involves giving a drinking a solution containing 50 grams of glucose to non-fasting pregnant woman(nb/ random blood glucose is determined and should be within the glucose reference range) and blood glucose levels determined 1 hour later. Women with a 1-hour glucose level > 7.8 mmol/L are referred for a diagnostic OGTT.

Selective screeningwas developed by categorizing pregnant women into three groups ((low-, intermediate-, and high-risk) based on a complex scoring system using weighted risk factors: age, BMI before pregnancy, and race (Naylor et al., 1997).

-American Diabetic Association (ADA) recommends selective screening for GDM. –

-According to the ADA guidelines, patients should be screened for risk factors for GDM at their initial visit.

*A woman is considered **high risk** if she has one or more of the following: marked obesity, personal history of GDM, glucose intolerance or glycosuria, or a strong family history of type 2 diabetes.*

*A woman is considered **low risk** if she meets all of the following criteria: age < 25 years, normal pre-pregnancy weight, not a member of an ethnic/racial group with a high prevalence of diabetes no known diabetes in first-degree relatives, no history of abnormal glucose tolerance, and no history of a poor obstetric outcome.*

*A woman is considered **intermediate risk** if she does not fall into either the high- or low-risk category. If a woman is high risk, glucose testing should be done as soon as possible. (Brodyet al., 2003).*

ORAL GLUCOSE TOLERANCE TEST (OGTT)

This is the gold standard for the diagnosis diabetes, insulin resistance, and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism according to WHO and ADA.. OGTT has also been used to evaluate clinically useful measures of Beta-cell function to assess insulin secretion, in comparison with the gold standard method of the hyperglycemic clamp...

***Usually the OGTT is performed in the morning before 9am as glucose tolerance can exhibit a diurnal rhythm with a significant decrease in the afternoon (ADA, 2007).**At the same time the insulin concentration is at its optimal concentration.*

-Instructions are given to the client to avoid any other meal after super (OR/- The patient is instructed to fast (water is allowed) for 8–12 hours prior to the tests.)

-The procedure for oral glucose tolerance test involved testing the fasting blood glucose using capillary blood obtained by a finger prick.

-The client is requested to submit a random urine specimen, which is used to investigate any presence of glycosuria.

-Client to be considered to proceed with the test must have urine showing negative for glucosuria..

-Pure glucose depending on age and status of the client is given as follows:- children(1 gm per 1 kg body weight, adult men and adult non expectant women(75 gms) and expectant women(100gms).is weighed and dissolved in a glass of water and the subject consumed this glucose solution marking the start of the procedure.

-A period of 5 minutes is allowed for the consumption of the glucose solution.

-Citric acid is provided to prevent nausea.

-Blood glucose level is tested by using capillary blood obtained through a finger prick after every 30 minutes for two hours and a half client is required to void urine after every one hour during the period of the procedure.

-The results are recorded in OGTT chart.

-During the three previous days the subject must have an unrestricted diet and unlimited physical activity.

-The subject should remain seated during the test and should not smoke throughout the test.

--Fasting blood glucose level of the subject is tested and should be within the laboratory FBG reference range.

*-The test involves drinking a solution containing a certain amount of glucose (pregnant women (100gm), adults (75 gm) and children (1 gm/1kg body weight) within five minutes and drawing blood to measure glucose levels at the start and on set time intervals thereafter ie 0.5 hrs, 1hr,1.5hrs and 2hrs. (Eschwegeet al., 2000**)**.*

-Current studies have established that a single OGTT can over estimate the diagnosis of diabetes and impaired glucose tolerance.

-Therefore it is important to carryout at least two OGTT to make proper diagnosis (Zhanget al., 2010).

POSTPRANDIAL GLUCOSE TEST (PPGT)

Two hours postprandial blood sugar measures blood glucose exactly 2 hours after eating a meal which acts as a glucose challenge to the body's metabolism. By this point blood sugar

has usually gone back down in healthy people, but it may still be elevated in people with diabetes (Goldsteinet al., 2004). Thus, it serves as a test of whether a person may have diabetes, or of whether a person who has diabetes is successfully controlling their blood sugar. A fasting blood sugar (FBS) is done before the 2 hr PPG. It is an easily performed screening test for diabetes mellitus. In healthy, non-diabetic subjects in American population, 2-hr postprandial blood glucose levels are usually <6.7 and rarely >7.8 mmol/L (ADA, 2001).This test has been used in large, randomized, prospective trials that showed reductions in hyperglycemia and management of diabetes-related risk factors significantly reduce microvascular and macrovascular complications in patients with either type 1 or type 2 diabetes (Stratton et al., 2000). PPGT has been found to be a more useful diagnostic parameter than FBG and HbA1c especially in the investigations of diabetes and cardiovascular related complications (Temelkova-Kurktschievet al., 2000)

GLYCOSYLATED HAEMOGLOBIN TEST (GLYCATED HAEMOGLOBIN, HBA1c)

INTRODUCTION

Glycosylated hemoglobin (haemoglobin A1c, HbA1c, A1C, or Hb1c; sometimes also HbA1c) is a form of hemoglobin that is measured primarily to identify the average plasmaglucoseconcentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Glycosylated hemoglobin measures the percentage of hemoglobin bound to glucose. Hemoglobin is a protein found in every red blood cell. As hemoglobin and glucose are together in the red blood cell, the glucose gradually binds to the A1c form of hemoglobin in a process called glycosylation. The amount bound reflects how much glucose has been in the blood during the past average 120-day lifespan of red cells.

Normal levels of glucose produce a normal amount of glycosolated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycosolated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement.

In the normal 120-day lifespan of the red blood cell, glucose molecules react with hemoglobin, forming glycosolated hemoglobin. In individuals with poorly controlled diabetes, the quantities of these glycosolated hemoglobins are much higher than in healthy people.

Once a hemoglobin molecule is glycosolated, it remains that way. A buildup of glycosolated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle.

Measuring glycosolated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA_{1c} level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is related to a rather shorter period of two to four weeks.

Purpose

HBA1c test is therefore a test that measures the amount of hemoglobin bound to glucose. It is a measure of how much glucose has been in the blood during the past two to four months. As a person's blood sugar becomes higher, more of the person's hemoglobin becomes glycosylated. The glucose remains attached to the hemoglobin for the life of the red blood cell, or about 2 to 3 months.

HBA1c measures the percentage of hemoglobin bound to glucose. Hemoglobin is a protein found in every red blood cell. As hemoglobin and glucose are together in the red blood cell, the glucose gradually binds to the A1c form of hemoglobin in a process called glycosylation. The amount bound reflects how much glucose has been in the blood during the past average 120-day lifespan of red cells.

This information helps a physician evaluate how well a person is responding to diabetes treatment and to determine how long sugar levels have been high in a person newly diagnosed with diabetes.

Indications and use

Glycosolated hemoglobin testing is recommended for both:

*(**a) checking blood sugar control in people who might be pre-diabetic and***

(b) monitoring blood sugar control in patients having diabetes mellitus.

RECOMMENDATION SCHEDULE FOR THE TEST

It is recommended that glycosolated hemoglobin test be performed during a person's first diabetes evaluation, again after treatment is begun and sugar levels are stabilized, then repeated at least semiannually. If the person does not meet treatment goals or sugar levels have not stabilized, the test should be repeated quarterly.

***GLYCOSOLATED HEMOGLOBIN MEASUREMENT IS NOT APPROPRIATE** :*

(a) where there has been a change in diet or treatment within 6 weeks.
(b) To people with recent blood loss, hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle-cell disease and other conditions, as well as those that have donated blood recently.

Reference ranges in literature

Diabetes treatment should achieve glycosylated hemoglobin levels of less than 7.0%. Normal value for a non-diabetic person is 4.0-6.0%..Because laboratories use different methods, results from different laboratories can not always be compared.

Methodology Options

There are currently four main techniques for determining glycated hemoglobins:

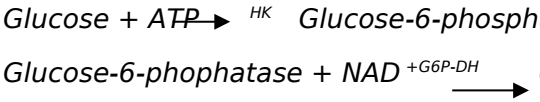
1. *Calorimetric end point reaction assay*
2. *Immunoassay - based on binding to specific antibodies.*

Specimen of choice: sequestrenated blood(blood collected in an EDTA vacuitainer) or capillary blood

GLUCOSE LABORATORY ANALYSIS

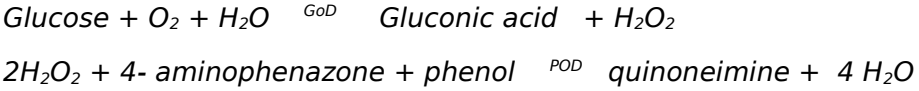
(i) Hexokinase method

Glucose reagent is used to measure the concentration of glucose by a timed end point reaction method. In the reaction, hexokinase catalysed the transfer of phosphate group from adenosine triphosphate (ATP) to glucose to form adenosine diphosphate (ADP) and glucose-6-phosphate. The glucose-6-phosphate was then oxidized to 6-phosphogluconate with the concomitant reduction of ß-nicotinamide adenine dinucleotide (NAD) to reduced ß-nicotinamide adenine dinucleotide (ß-NADH) by the catalytic action of glucose-6-phospate dehydrogenase (G6PDH).



(ii) Glucoose oxidase method

Glucose reagent is used to measure the concentration of glucose by a timed end point reaction method. In the reaction, glucose oxidase (GoD) catalyzed the oxidation of glucose to gluconic acid with the formation of hydrogen peroxide (H_2O_2). The H_2O_2 formed reacted with phenol and 4- aminophenazone under the caltlysis of peroxidase (POD) to form a red–violet quinoneimine which was directly proportional to the concentration of glycose in the sample.



Specimen of choice:*whole blood, heperinized plasma or serum*

Reference ranges:*FBG(2.7-6.4), RBG(2.7-8.3) mmol/L*

***Units of measure**: The commonly used units of measure are either mmol/L or mg/dl(mg%). A blood glucose report can be changed from one unit to another to suite the requesting health provider or for uniformity purposes.*

Example: Given a blood glucose report as 198 mg/dl convert this to mmol/L

STEP ONE:

Mwt of glucose($C_6H_{12}O_6$)=180gms

180 gms=180,000mgs

STEP TWO:

One molar glucose solution is made by dissolving one mole(180,000mgs)glucose in one litre of water. Therefore:

180,000mgs in 1L =1M OR 180000mg in 1000mls=1M

1M = 1000mmols

1000mmols=180000 mgs /L

1 mmol = 1mmol/1000mmmol x 180000mgs/L

1mmol=180 mgs/L

STEP THREE:

1mmol=180 mgs/L

1L=10 dL

Therefore 180 mgs are in 10 dl , how many mgs are in a dl?

180 mgs=10dl

1dl=1/10x180mgs=18mgs

1 dl has 18 mgs= 18mgs/dl

Then 1 mmol/L=18 mgs/dl

Therefore 198 mgs/dl=?mmol/l

1 mmol/l=18 mgs/dl

198mgs/dl=198mgs/dl/18 mgs/dl X 1mmmol/l =11 mmol/l

QUANTITATIVE ANALYSIS OF KETONES IN THE MANAGEMENT OF DIABETES MELLITUS

Introduction

- *Energy required for metabolic activities is delivered from glucose*
- *Insulin and glucagon are important hormones that control blood glucose.*
- *Made and secreted by the pancrease but have opposite roles*
- *Insulin enhances entry of glucose into the cells*
- *Insulin insufficiency, insensitivity and deficiency results causes diabetes mellitus(DM)*
- ***Types of DM of great medical importance*** *–*
- *IDDM(Type 1 DM),NIDDM(Type 2DM) and gestational DM*
- *Insulin inactivity causes the body to source energy from fats resulting with formation of ketones*
- ***Three types of ketones---acetone, acetoactate and β-hydroxybutyrate***
- *Under normal metabolic conditions ketones do not accumulate in blood therefore undetectable*
- *During starvation, after exercise or at night there is presence of ketones in the body*
- ***In DM ketones level rises due to:-***
- *(i)rise in blood glucose levels due to insulin inactivity----No entry of glucose into ICF*
- *(ii)body cells burning fat for energy production*
- *(iii) continuous accumulation of ketones as fat is used as source of energy*
- ***Diabetic ketoacidosis (DKA) is a complication which results from accumulation of ketones in the circulation of a diabetic patient***
- *β-hydroxybutyrate is the ketone related to the development of DKA*
- *DKA is more common in IDDM than in NIDDM and DKA is a potentially lethal in either case of DM.*

ANALYSIS OF KETONES

- *Urine(qualitatively) and Blood(quantitatively)*

Urine(qualitatively)

- ***Until*** *recently has been the only method of ketones analysis*
- ***Limitation s:***

(i) could only reveal the presences of ketones BUT NOT the levels

(ii)detects acetone and acetoactate BUT NOT β-hydroxybutyrate (main cause of DKA)

(iii)Urine analysis for ketones is retrospective

(iv)Resolution of ketoacidotic episode is difficult to monitor

(v)Urine specimens not easily obtained in the prsence of dehydration and altered consciousness

Quantitative analysis

- *This is the analysis of β-hydroxybutyrate (β –HB)using blood specimen*
  - *BENEFITS OF QUANTITATIVE ANALYSIS*
  - *(i)gives the actual levels of β –HB in blood*
  - *(ii)gives earlywarning of impending DKA*
  - *(iii) the specimen is easily available*
  - *(iv) testing is done prospectively*
  - *(v) iv)Resolution of ketoacidotic episode is easy to monitor*

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

RENAL FUNCTION

(**A) THE KIDNEY**

-A normal human being has two pair of kidneys which are located just below the level of the ribs at the back of the body,(retroperitoneal space) one on either side of the spine where they are protected from damage by the rib cage.

- Each kidney weigh (adult kidney) about 150 g and measures approximately 12 cms in length. And it is bean shaped.

B) THE FUNCTIONS OF THE KIDNEY

-The functions of the kidney may be characterized as EXCRETORY, REGULATORY and ENDOCRINE.

1) EXCRETORY FUNCTION

-Serves to rid the body of most of the undesirable end products of metabolism as well as any excess of inorganic substances ingested in the diet. The metabolic waste products include the non-protein nitrogenous compounds (NPN) eg urea, creatinine, and uric acid, a number of other organic acids including amino acids are excreted in small quantities.

- - In order to achieve the excretion of the waste metabolic products and the ingested surpluses without disrupting homoestasis (this is the maintenance of the optimal chemical composition of the intra- and extra-cellular fluids through out the body), the kidney must exercise both its excretory and regulatory function.

2) REGULATORY FUNCTION

- The regulatory function of the kidney plays a major role in homoestasis (this is the maintenance of the optimal chemical composition of the intra- and extra-cellular fluids through out the body). The mechanism of differential re-absorption and secretion located in the tubules of the nephron are effectors of regulation.(*NB will be discussed in details in the formation of urine.}*

3) ENDOCRINE FUNCTION

- The endocrine function of the kidney can be regarded as either primary or secondary. Primary because the kidney is an endocrine organ producing hormones eg **Renin**, **Prostagladins** and **Erythropoeitin**.

THE NEPHRON

-This is the functional unit of the kidney. Each kidney has approximately **1 million** nephrons. The nephron consists structurally of -:1) the glomerulus (which is a spherical epitherial space invaginated by a capillary network that connects the afferent and efferent arterioles and 2) a tubule of epithelial cells continuous with the glomerular epithelial space and ultimately leading to collecting ducts that empty into the renal pelvis

- Three distinct processes take place in each nephron:-

1) The blood flowing through the capillaries of the glomerulus is filtered , the filtrate passing into the tubules through the glomerular membrane =this process is called ***GLOMERULAR FILTRATION***. The glomerular filtrate contains most of the constituents of the blood with the exception of the protein which does not pass through the normal glomerular membrane in any appreciable quantity.

2) As the glomerular filtrate passes through the walls of the tubule, more solutes are added by secretion through the walls of the tubules from the adjoining blood vessels= This process is called ***TUBULAR SECRETION***.

3) Solutes and water pass back into the blood from the tubules through the walls of the tubules= This process is called ***TUBULAR REABSORPTION***

The end product of these three processes passes through larger collecting tubules into the ureters which carry it into the bladder , from where it is excreted through the urethra as **urine**.The constituent of urine are the products of glomerular filtration plus the products of tubular secretion minus constituents returned into the blood through tubular reaabsorption.

FACTORS THAT AFFECT THE OPTIMAL FUNCTION OF THE KIDNEY

-The normal rate of urine formation requires presence of properly functioning kidneys. These kidneys should receive adequate blood supply at a sufficient high pressure.There should be no obstruction to urine out flow. Malfunction in the formation and elimination of urine may be due to :- a) pre-renal causes, 2) renal causes and 3) post-renal causes.

-**PRE-RENAL CAUSES**

-prerenal causes include those factors affecting blood pressure, blood volume and blood flow eg shock, haemorhage, dehydration, prolonged diarrhaea and cardiac failure.

-**RENAL CAUSES**

-these are factors that will affect directly the GFR, various tubular activities and renal blood vessels

-**POST RENAL CAUSES**

- These are factors that will decrease renal function and those that obstruct urine flow . They include :-

(1) renal calculi

(2) carcinoma or tumors that compress the ureter, urethra or bladder opening

(3) enlarged prostrate glands occludes(blocks) the bladder wholly or partially.

Nb/ Chemistry alone cannot differentiate between the three prime causes of renal dysfunction (pre renal, renal and post renal) hence clinical history and physical examination of the patient is very important.

-Several diseases have bee found to cause renal malfunction and they include:-

-(a) glomerulonephritis----this is a non pus forming inflammation of the glomeruli

(b) nephrotic syndrome---this is a condition characterized by reduction in blood plasma albumin , albuminuria

(c) pyelonephritis---a type of renal infection spreading from the pelvis to the cortex of the kidney, The origin of the infection is usually from the ureter and below or from from the blood stream.

FORMATION OF URINE

-The ***FORMATION OF THE KIDNEY IS THE KEY ROLE OF THE KIDNEY***. The urine formed by the kidney constitutes most of the undesirable end products of metabolism as well as any excess of inorganic substances ingested in the diet.

-The undesirable waste products of metabolism includes non-protein nitrogenous compounds such as urea, creatinine,uric acid . A number of organic acids including amino acids are excreted in small quantities. Excess inorganic substances excreted by the kidney includes sodium, potassium, chloride, calcium, phosphates, magnesium, sulphates and bicarbonates.

- In the formation of urine there are several activities involved which includes:

1) Filtration

2) Re-absorption and secretion (tubular function)

3) Hormonal function

1) FILTRATION PROCESS

-The first step in urine formation is filtration at the glomerulus of plasma water driven by a net filtration pressure of about 15mmhg.

-The filtrate is referred to as ULTRAFILTRATE because its composition is essentially the same as that of that plasma, with notable absence of molecules of mwt above approximately 15,000 daltons.

- Each kidney contains about 1 million nephrons and about 170-200 litres of ultraflitrate pass through the glomeruli in 24 hours.nb/ In the passage of the glomeruli filtrate through the tubules, re-absorption of solutes and water in various regions of the tubules reduces the total volume to 0.4-2 litres per day.

- The filtration of blood at the bowmans capsule is a passive process, whereby the flow of fluid from the blood depends on the fact that hydrostatic pressure in the capillaries is greater than that in the tubules lumen.

- Any factor that reduces the pressure gradient will lower the filtration rate. An imbalance in the hydrostatic pressure 2^0 to vascular collapse , renal arterial occlusive disease , inflammatory disease, or obstruction of urinary flow system results in a marked decrease in glomeruli filtration resulting with the formation of scanty urine (oliguria) or no formation of urine at all (anuria). The maintenance of glomeruli filtration is dependent on an adequate renal blood flow rate as well as adequate perfusion pressure. The normal renal blood supply is well designed to meet these needs.

2) RE-ABSORPTION AND SECRETION (TUBULAR FUNCTION)

- In the tubules the solute composition of the ultrafiltrate is altered by the processes of reabsorption and secretion so that the urine excreted may have a completely different composition from that of the original filtered fluid.

- Different regions of the tubule have been shown to specialize in certain functions.

a) In the proximal tubules , 60-80 % of the ultrafiltrate is reabsorbed. This activity is a passive process.

b) Threshold substance ie glucose is also reabsorbed in the proximal tubule.

-In the loop of henle and collecting duct there two mechanisms which are responsible for the nature of urine produced.. These are ***COUNTER CURRENT MULTIPLICATION and COUNTER CURRENT EXCHANGE..***

****COUNTER CURRENT MULTIPLICATION**

-This is an active process

- It is responsible for the removal of solutes and reabsorption of water from the loop of henle and collecting duct

- This process occurs in the absence of ADH.

- It creates a urine low osmolality (diluted urine).

****COUNTER CURRENT EXCHANGE**..

- This is a passive process.

- It is responsible for differential reabsorption of water without solutes from the loop of henle and collecting duct

- This process occurs in the presence of ADH.

- It creates a urine high osmolality (concentrated urine)

ANTI DIURETIC HORMONE MODE OF ACTION

-ADH determines the [ ] of urine produced.

- It is produced in the hypothalamus and stored in the posterior pituitary

- The amount of ADH produced depends on the osmoreceptors present in the hypothalamus which are stimulated by the high plasma osmolality or low plasma volume (hypovolumea).

- ADH causes the pores in the collecting duct to increase in diameter permitting an increase amount of water to be osmoticaly reabsorbed into the renal medulla . The resulting urine osmolality becomes x 5 that of the plasma.

- In the absence of ADH, eg in diabetic inspidus or a non response of the collecting tubules to ADH, large volumes of dilute urine results.-

However , at the distal collecting ducts ADH, not only increases the permeability of the cells to water but also to urea. . This is the main reason fro the rise in urinary osmolality.

RENAL DISORDERS

INTRODUCTION- There are several renal disorders which are classified according to the clinical presentation and the kidney (nephron) structural change. Consequently, many names for renal diseases actually represent clinical syndromes and their structural basis as revealed by a renal biobsy.

- An additional difficulty in classifying renal diseases is the tendency of distinct primary disease to emerge with time into a single clinical entity, regardless of their initial etiology or anatomic region of injury. ***This is contributed by the fact that there is a great***

relationship between the anatomy and function of renal structures and their blood supply. For example any decrease in the normal cardiac output may impair all the functions of the nephron and produce the clinical syndrome of pre-renal azotomia (increased blood urea).If the decrease in the cardiac output is very severe it may lead to injury of the renal tubules and the syndrome of acute renal failure .***The injury of the renal tubules triggers the release of renin from the kidney resulting with hypertension. Hypertension is in fact a complication of many renal diseases of varied causes. Additionally severe hypertension itself can damage renal arterioles, further compromising nephron function.***

- Irrespective of the initial cause of the injury whether immunological, injury of the glomerulus or toxic injury to the tubular cells, the ultimate result is the loss of complete nephron.

- Most of the renal disorders will start to be noticed when the nephrons have lost 75 % of their functioning capacity. The loss of >90% of the nephrons function resulting to end stage renal disease produces remarkably consistent clinical and laboratory picture. Once the renal disease has reached this stage, it may be impossible to determine the site and nature of the initial injury.

-Some of the renal disorders to be discussed includes: end stage renal failure/disease (ESRD), acute renal failure (ARF), chronic renal failure (CRF), glomerular diseases (GD) (for example-acute glomerulonephritis (GN) and nephrotic syndrome (NS))

END STAGE RENAL FAILURE (ESRF/ESRD)

-This is the stage where the kidney has lost entirely (>90%) its noble functions that is has failed to maintain adequate excretory, regulatory and endocrine function.

- This stage is characterized by the constellation of symptoms, physical signs and abnormal diagnostic studies. These characters can also be referred to as ***uremic syndrome.*** The classic signs of uremia includes:- progressive weakness and easy fatigability, hypertension, loss of appetite, nausea and vomiting, muscle wasting, tremors, abnormal mental function, frequent but shallow respiration and metabolic acidosis.. This syndrome progress to produce stupor, coma, and ultimately death unless support is provided by haemodialysis or successive renal transplantation..

ACUTE RENAL FAILURE (ARF)

-This clinical syndrome occurs when there has been a rapid and severe reduction of GFR OF < 10ML/MIN.

- Patients with ARF are oliguric (reduced urine output) for example will produce < 400mls of urine after fluid challage.

-During this oliguric state the patient is unable to excrete water and sodium, leading to expansion of ECF which results to oedema, hypertension and congestive heart failure.

- Another feature of ARF is that water is retained in excess of salt producing ***hyponatreamia(low levels of sodium in the blood***). If this hyponatreamia is very severe, central nervous system symptoms results (for example: seizures and coma).

- Another feature encountered in ARF is inability of the nephron to excreate potassium resulting with life threatening ***hyperkaleamia (high levels of potassium in the blood).***

- Other features seen in ARF are: retention of phosphate leading to ***hyperphosphotaemia .and hypocalcaemia (inadequate mineralization).***

- Accumulation of urea and creatinine is evident. When ARF is due to under perfusion (lesser blood supply to the kidney) of the kidney, BUN will accumulate in excess of creatinine (high bun/creat ratio).The rise in serum creatinine and less often the increase in BUN is used to monitor the degree of renal impairment.

- Patients with ARF develop all symptoms of uremic syndrome. However anaemia and metabolic bone disease seen in CRF are not very prominent.

- Patients with ARF are at risk of getting gastrointestinal haemorrhage and systemic infection which are the major cause of death.

- Some causes of ARF includes:- crush (accident) syndrome, toxic injury to the kidney (drug toxicity), hypertension and malaria.

- ARF is manageable by the control of the primary cause for example loss of blood (give blood transfusion) , drug toxicity(withdrawal of the drug or giving an anti dose), hypertension (control with hypertensive drugs).

- Recovery from ARF can be achieved by subjecting the patient to haemodialysis.

LABORATORY INVESTIGATIONS

-serial monitoring of creatinine, GFR,(creatinine clearance), serum eletrolytes, urine volume, urinalysis and serum and urine osmolality.

CHRONIC RENAL FAILURE (CRF)

-Pts who do not recover from ARF may develop CRF if early death does not occur.- CRF is the end result of a variety of many conditions such as: chronic glomerulonephritis, chronic obstructive uropathy, polycystic kidney disease, renal artery blockage and any other condition that causes tubular dysfucntion.

- ***As ARF progresses without any kind of treatment, more nephrons get damaged and the oliguric nature of the patient changes into anuric nature (total suppression of urine output) due to the fact that all the nephrons have been damaged resulting to no more urine output. The patient is said to be in a total renal shutdown***.

-All the symptoms of uremia syndrome are very prominent in patients having CRF.

- Inability to excrete potassium resulting with hyperkalaemia is very evident as in the case of ARF. Hypernatreamia, hperphosphotaemia and hypocalcaemia are also evident.

- ***If not managed , this condition is often terminal with exceedingly high rise in urea, creatinine, potassium and marked severe acidosis.***

- The life of CRF pt can be sustained by haemodialysis or a successive kidney transplant.

LABORATORY INVESTIGATIONS

-serial monitoring of creatinine, GFR,(creatinine clearance), serum eletrolytes, uric acid, urine volume, urinalysis and serum and urine osmolality.

GLOMERULAR DISEASES

-These are pathological factors that cause direct injury to the renal glomerulus while preserving tubular functions and may resemble pre-renal factors.

- Eventually, chronic progressive glomerular disease will affect blood supply to the tubules and this will result in the loss of complete nephrons producing CRF and the uremic syndrome.

- The two most common glomerular diseases are : acute glomerulonephritis and the nephritic syndrome.

ACUTE GLOMERULONEPHRITIS

-This pathological process is related to recent group A beta – haemolytic streptococcal infection. Other causes includes :- reactions to drugs, acute infection of the kidney, systemic disease with immune complexes deposition in the kidney such as systemic lupus erythematosus (SLE), bacterial endocarditis and recent viral infection.

- This condition is characterized by the rapid onset of haematuria, proteinuria, reduced GFR, sodium and water retention resulting to oedema, hypertension and congestive heart failure. Oliguria may also be evident.

- In pts suspected of acute post streptococcal glomerulonephritis, evidence of recent infection may be found in increased titres of antibodies to streptococcal extracellular products ie Antistreptolysin O (ASO).

NEPHROTIC SYNDROME

- This pathological process is related any interference with the normal glomerular filtration processes.

- The glomelular capillary wall is a special membrane which is uniquely designed to support rapid utrafiltration and to retain nearly all proteins with in the plasma..

- The glomerulus capillary wall is composed of three structural elements:-

(i) a layer of endothelial ells

(ii) the glomerular basement membrane that separate the endothelial cells from epithelial cells.

(iii) The outer epithelial cell layer that is attached to the basement membrane by the discrete cytoplasmic extentions.

-These glomerular structures allows for the utrafiltration at a rate that removes approximately 20 % of the plasma water (with small molecular weight solutes) during transit of plasma through the glomerular capillaries but that restricts passage of protein molecules with mw of > 15000 daltons eg (albumin).

- Apart from the high molecular weight of solutes, molecular shape and charge also affect glomerular flitration eg globular proteins will be less cleared than those similar sized molecules which are linear in shape.

- Molecules that are highly negatively charged exhibit much lower glomerular permeability than do neutral molecules of the same size.This is a strong direct evidence that the filtering membrane has a negative charge that tends to repel negatively charged molecules and thus decrease the effective radius of pores in the membrane as molecules with like charges approach. The glycosialoproteins structures in the membrane provides the negative charge and any factor that may injure , decrease or alter the glycosialoprotein content of the basement membrane will be the cause of nephrotic syndrome. Circulating polyanions eg albumin are thought to be repelled by the rich distribution of the inner barriers of the negatively charged glycosialoproteins coat.

- An established case of nephrotic syndrome is characterized by proteinuria (massive proteinuria of > 3.5 g/dl), hypoalbuminaemia, oedema and hyperlipidaemia.

- GFR is usually depressed.

- The loss of albumin in urine overwhelmes hepatic synthesis capacity and hypoalbuminaemia results (<3 g/dl).

- Loss of intravascular onchotic pressure results in oedema which is enhanced by sodium and water retention.

- The genesis of hyperlipaedemia is not het clear.

CAUSE OF N/S

(i) primary renal disease---most types of glomerulonephritis usually due to deposition of immune complexes (SLE) on the glomeruli. This accounts to 80% of all n/s cases.

(ii) secondary renal diseases eg diabetic mellitus, amyloidosis(bilateral destruction of adrenal glands by an autoimmune disease), SLE, malaria and renal vein thrombosis.

(iii) other causes are drugs and toxins.

LABORATORY INVESTIGATIONS

(i) urinalysis---to detect proteinuria

(ii) total serum/plasma protein and albumin estimation-------to detect hypoproteinaemia and hypoalbuminaemia

(iii) serum protein electrophoresis-------alpha 2 and beta globulins are usually increased.

(iv) serum IgG -----usually decreased while the other are normal.

(v) serum binding proteins------eg transferring, transcortin and thyroxine binding globulin are usually decreased.Also decreased are total serum iron, t3(triiodothyronine) and t4 (thyroxine

(vi) lipid profile----total cholesterol and triglycerides will be elevated.

RENAL CALCULI

Renal calculi are usually composed of products of metabolism present in normal glomerular filtrate often at concentration near their maximum solubility. Minor changes in urinary composition may cause precipitation of such constituents in the kidney or in the urinary tract.

CONDITION FAVOURING CALCULI FORMATION

(1) A high urinary concentration of one or more constituents of the glomerular filtration due to :-

(a) low urine volume with normal renal function due to restricted fluid intake or excessive fluid loss over a long period of time.

(b) a high rate of excretion of the metabolic product forming stone , due to high plasma levels than the filtrate levels or impairment of tubular re-absorption from the filtrate.

(c) change in ph of the urine often due to bacterial infection which favours precipitation of different salts at different hydrogen ion concentration (ph).

(d) urinary stagnation due to obstruction to urinary flow .

(e) lack of normal inhibitors:- eg pyrophosphate and glycoprotein which prevents the formation of calcium phosphate and calcium oxalate crystals respectively.

- Renal calculi (urinary calculi) constitute the following:-

(a) calcium containing salts----between 70-80 % of all renal stones contain calcium.

Precipitation is favoured by hypercalciuria. Alkaline conditions favouring calcium phosphate precipitation and stone formation are usually present in pts with CRF due to urease spliting organisms such as proteus valgaris. These stones are usually hard, white and radiopaque.They are also small in size and are usually lodged in the ureters.

The management of these stones involves;-

** treatment of the primary cause eg urinary infection

** treatment of hypercalcaemia (by reducing dietary intake).

** maintain a high fluid intake both day and night

** use of thiazide diuretics to reduce urinary calcium excretion.

(b) **Uric acid stones**-------This constitutes 10% of all renal stones. They are associated with hyperuricaemia with or without clinical gout. Precipitation is favoured by acidic urine. The stones are usually small, yellowish brown.

The management of these stones involves:-

** treatment of hyperuricaemia by reducing purine intake.

** increase renal excretion of urates by taking uricosuric drugs(probenecid and salicylates)

** a lot of fluid intake is encouraged both day and night.

(C) **Cystine**--- these stones are very rare and when present they resemble uric acid stones.

(d) **xanthine stones** – these are very uncommon and when present may be due to rare inborn error called xanthinuria. They have been reported during treatment with xanthine oxidase inhibitors. Such as allopurinol, which impair the conversion of xanthine to uric acid.

MANAGEMENT OF RENAL DISORDERS

(1) DIALYSIS

-Dialysis helps to remove urea and other toxic substances from plasma and collects electrolytes balance by dialyzing the patient's blood against fluid containing no urea and appropriate concentration of electrolytes , free ionized calcium and other plasma constituents.

-The principle forms of dialysis includes:-

(a)HAEMODIALYSIS

- In haemodialysis, blood is passed through an artificial kidney(DIALYZER) . This dialyzer contains a fliud (dialyzing fluid or dialysate) which consists mainly of water . The water that make this dialysate is highly purified . The dialysate does not contain any of the substances intended to be cleared from the blood eg urea and creatinine. It does contain some of the substances the body needs such as calcium and biocarbonates. The following are the function of the dialysis machine:-

(a) it pumps blood from the patient via the patients access (which can be either catheter (blood lines),fistula(an artery joined to a vein) or a graft (placed between an artery and a vein)., around the machine and back to the patient.

(b)it mixes and warms the dialysate which passes through the artificial kidney.

(c) it monitors what is in the dialysate to make sure the right amount of the right substance s are there throughout the dialysis session.

(d) it checks the blood for air so that none gets into the patients bloodstream. (prevents air embolism)

(e) it monitors the pressure of the patients blood in the access .

- NOTE / during dialysis , heparin is added into the blood circulating in the dialyzer to be prevent any formation of clots.

-The number of sessions and the dialyis period depends on :

(a) the amount of waste in the blood (urea and creatinine levels)

(b) the body size of the patient

(b) HAEMODIAFILTRATION

-This is an improved form of haemodialysis where large volumes of water and solutes can be removed through a highly permeable membrane.

(c) CONTINOUS AMBULATORY PERITONEAL DIALYSIS (CAPD)

- The folds of peritoneum are used as the dialyzing membranewith capillaries on one side and appropriate fluid of high osmolality infused into the peritoneum on the other side.

- After a suitable time to allow for equilibrium of diffusible solutes depending on the type of peritoneal dialysis, the peritoneum cavity is drained and cycle repeated.

THE FOLLOWING CAN BE NOTED ON DIALYSIS

(1) Dialysis is used in cases of ARF until renal function improves OR as a regularly repeated procedure in suitable cases of CRF.

(2) Dialysis may also be used to prepare a patient for kidney transplantation and maintain him/her until the transplant function adequately.

(3) dialysis will not cure the patient and will not get rid of the renal disorder symptoms. It only control the symptoms and prevent the patient from dying. The renal function achievement of either PD and HD is only 10 % of the function the two normal kidneys. Comparatively the achievement of HD over that of PD is 15-20 %. PD is slow in removing potassium, hence when hyperkalemia is the prime indication for dialysis , HD is the preffered and more efficient means of treatment unless contraindicated.

- Even though dialysis technology has its limitations, it stands today as one of the management of renal failure and it is advisable to start this treatment as early as possible.

(2) KIDNEY TRANSPLANTATION

- This is a procedure whereby a renal patient receives a suitable kidney from a suitable donor. Almost anyone can donate a kidney. However, there are some situations where live kidney donation would not be possible. These includes people with the following conditions: (i) HIV or AIDS- related infection, hepatitis b,c and e infection (ii) major heart or breathing problems (iii) diabetes (either type) (iv) significant kidney disease (v) cancers (vi) hypertention (vii) intravenous drug abuse (viii) pregnancy (ix) having one kidney (x) < 18 years and > 65 years (xi) psychiatric disorders.

-Donors and recipient must be compatible ie same blood group and their tissues should match. The tissue typing (genetic make-up) should score > 65% match.

- Transplantation of kidney into immunologically suitable recipient corrects many of the abnormalities of CRF that HD only partially corrects.

- The only major problem encountered by the recipient is the rejection of the transplanted kidney .the medication given to the recipient to prevent rejection causes the body immunity to go down since the drugs are immunosuppresants. (eg cyclosporine).The most immediate danger is the infection by opportunistic organisms such as fungi, protozoa, and certain bacteria.

- Biochemical monitoring of renal transplant is designed to detect deterioration of function of the transplanted kidney especially as it relates to delayed rejection of the graft.. Several monitoring of creatinine is very important in early recognition of decline in GFR.

LIVING KIDNEY TRANSPLANTS

This involves the transplantation of a kidney to a recipient whereby the kidney is either :-

(i) from a living donor who is related to the recipient by blood eg a brother, sister, mother, father,
uncle , aunt, first cousin. This type of transplantation will require the donor and the recipient to tissue match >65%for any transplantation to commence.

(ii) From a living donor who is not related to the recipient by blood.This type of donors donate one of
their kidneys to the kidney bank where tissue typing is done. The prospective recipient must tissue match >65% with the non- related donor for transplantation to commence.

CADAVERIC KIDNEY TRANSPLANT

-This involves the transplantation of a kidney to a recipient whereby the kidney is from a kidney bank. The donor of the kidney may be dead (cadaveric). Cadaveric are people who have been declared brain stem dead in hospital intensive care units. Brain stem death means the donor brain is completely dead, with no chance of recovery. However in this situation many organs (including the kidneys) are still functioning. The most common cause of the brain death is a head injury resulting from a road traffic accident or a brain haemorrhage.

-- A tissue match is done and the cadaveric kidney musthave a good tissue match(65 %) with that of the cadaveric donor.

RENAL FUNCTION TESTS

These are a panel of tests carried nout in the clinical chemistry laboratory to investigate the functioning of the kidney. The panel has the following analytes:-blood urea nitrogen, electrolytes, creatinine, uric acid and creatinine clearance test.

BLOOD UREA NITROGEN(BUN)

Urea is the major nitrogen containing metabolic product of protein metabolism in human.

METHODS FOR THE DETERMINATION OF UREA*.*

There are two methods ie indirect and direct.

INDIRECT METHOD

-These methods are based on preliminary hydrolysis of urea with urease followed by some process that quantities the ammonium ion.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

$2NH_4^+ + CO3^{2-}$

NB/spectrophotometric approach to ammonium quantitation include the Berthelot Reaction and the enzymatic assay with glutamate dehydrogenase.

(1)Berthelot Reaction

in the first step of reaction, urea was hydrolyzed with urease to form ammonia and carbondioxide. In the second step of the reaction, ammonia was reacted with phenol and hypochlorite in alkaline medium to form a green indophenol complex which was directly proportional to the concentration of urea in the sample.

$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$

$NH_4^+ + 5\ NAOCL + 2\ phenol \xrightarrow{OH^-} Indophenol + 5\ NACL + 5H_2O$

NB/ -nitroprusside is used as a catalyst

-absorbance of dissociated indophenol, a blue chromophore is measured spectrophotometrically at 560 nm

ENZYMATIC ASSAY OF UREA USING GLUTAMATE DEHYDROGENASE (GLUDH)

(2) Glutamate Dehydrogenase (GLUDH)

BUN reagent is used to measure the concentration of urea by an enzymatic rate method. In the reaction urea is hydorlyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalysed the condensation of ammonia and –Ketoglutarate to glutamate with the concomitant oxidation of reduced B-nicotinamide adenine dinuelotide (NADH) to B-incotinamide adenine dinucleotide (NAD).

PRINCIPLE……

$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$

$NH_3 + Ketoglutarate + NADH + H^+ \xrightarrow{GLDH} glutamate + NAD^+ + H_2O$

NB/(1) For serum or plasma assays , the reaction system for both approaches is usually formulated with urease so that the addition of the sample containing urea starts the reaction.

(2)in the enzymatic assay using glutamate dehydrogenase , the decrease in absorbance is monitored at 340 nm in either endpoint or kinetic mode. This approach has been adopted in most of the automated clinical chemistry analyzers.

DIRECT METHOD OF UREA ESTIMATION-----this method is now obselete

CONVERSION OF UREA TO BLOOD UREA NITROGEN

-.Currently clinicians have adopted the terminology of requesting urea as blood urea nitrogen (BUN).

-. The recommended [ ] unit for reporting BUN is mmol/L. Incase a urea report is given in mg/dl and the clinician want the blood urea nitrogen, the following conversion is done:-

Mwt of urea= 60gms

Urea has 28gms of nitrogen,

Therefore 28/60=0.467.

- So to convert urea (either when given as g/l or mg/dl) to BUN the report is multiplied by 0.467.

-. Reports given in mmol/l for urea are given the same for BUN, since the nitrogen in urea is part of what makes the mole of urea.

Eg (15-39) mg/dl urea=(7-18) mg/dl BUN OR (2.5-6.4) mmol/l

SPECIMEN: serum or urine is suitable for assay. plasma is also suitable provided that the anticoagulant used contains neither ammmonium nor flouride salts. flouride inhibits urease since it is an enzyme poison. the best anticoagulant is heparin. Urea is stable in serum for up to 24 hrs at room temperature , for at least a week at (4-8) ^{0}c. and for several months when frozen at -70 ^{0}c.

RREFERENCE RANGES:- (2.7-8.3) mmol/L as quoted in literature. It is recommended that each laboratory or laboratories in the same geographical location should establish their own ref.ranges based on the population in that region.

Nb/ Neonates have lowe r urea levels than adults due to the kidney size whilst old persons above 70 years have higher levels due to the deterioration rate of the kidney functions.

CREATININE

-This is the spontaneous degradation product of creatine in the muscles. The formation of creatinine is reasonably constant and has a direct relationship to the body muscle mass.

- Creatinine is freely filtered by the glomerulus but it is not reabsorded to any appreciable extent if at all under normal circumstance. A small but significant amount of creatinine is also excreted by active tubular secretion , which increases with increasing plasma creatinine [ ].

- The formation and excretion of creatinine which is usually constant , makes it one of the best renal fuction test.

- By virtual of its relative independence of such factors as diet ,hydration and protein metabolism, plasma creatinine is significantly a more reliable screening test for renal function than blood urea nitrogen.

- in relation to sex, men have a higher normal creatinine than women since males have a greater muscle mass than females , whilst children due to their small body mass have lower normal creatinine.

DETERMINATION OF CREATININE BY JAFFE REACTION METHOD

PRINCIPLE:-

Creatinine reacts with picrate in an alkaline medium solution to form a creatinine-picrate complexone (red colour) whose intensity is directly proportional to the amount of creatinine in the sample. The intensity of the colour complexone is measure at 510nm

Creatinine+ picric acid $OH^- \rightarrow$ creatinine+picrate complexone(red colour)

Specimen =(serum, plasma and urine)

Ref. Range in literature for adults, Men (60-120): Women (55-115)

KENYAN BASED REFERENCE RANGES:MEN= (68-128)UMOL/L*:*WOMEN: = (60-122) UMOL/L

SARCOSINE ENZYMATIC METHOD

This is an alternative method for creatinine determination

This enzymatic method for creatinine utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase is used to

convert creatinine to creatine.

Creatine is broken down to sarcosine and urea by creatine amidinohydrolase. Further enzyme linked steps with sarcosine oxidase and peroxidase yield a colored

chromogen read at 545nm.

Creatinine measurements are used in the diagnosis and treatment of kidney diseases, in the monitoring of dialysis patients, and as an integral part of diagnostic equations to measure renal function.

CREATININE CLEARANCE(CrCL)

This is so far one of the best clearance test to determine the clearance capability of the kidney. In essence it estimates the glomerular filtration rate (GFR) Creatinine is released into the circulation at a relatively constant rate and has a stable plasma concentration. Creatinine is freely filtered across the glomerulus and is neither reabsorbed nor metabolized by the kidney.

The CrCL as a measure of GFR has several unique advantages over the clearance of inulin or other exogenous substances. Not only are plasma and urine creatinine easily measured, but extensive data are available for reference ranges of both plasma creatinine and CrCL in individuals of all age groups. Therefore CrCL remains the most practicableand popular method for estimating GFR in most hospitals. Linear decrease of CrCL over time , as renal function fails has been thoroughlydocumented in cases such as diabetic nephropathy and glomerulonephritis. - In the management of renal diseases and monitoring of chemotherapy treatment for cancer patients , creatinine clearance is the first choice test.

LABORATORY DIAGNOSIS OF CrCL

-urine is collected over a period of 24 hrs and preserved in a container with 10 mls conc hcl, thymol or toluene whereby the first urine during this time is discarded and the last one included.NB/ if no preservative is available, the urine can be refrigerated all throughout the collection period

- Blood specimen is collected within this 24 hrs due to its constant nature in the plasma.

- creatinine estimation is done using the standard method of creatinine estimation.

- the following formula reffered to as standard formula is used for the calculation of CrCL for a an average adult

$$\frac{\text{24hrs Urine volume(mls) x urine creatinine (umol/l}}{\text{Serum creatinie (umol/l) x 1440 min}}$$

To calculate the CrCL for children and adults with low body weight, the above formula is multiplied by the factor 1.73/A (this factor normalizes clearance for average body surface , correcting as it does for variation in creatinine excretion relative to lean body (body) mass..

A is calculated as follows:

Log A = (0.425 log weight in kg) + (0.725 log height in cm)- 2.144.

NB/ (1)FALSELY low values will be obtained if the collected urine is not refrigerated and / or a bacterial inhibitor (thymol, conc hcl or toloune)is not added to the urine container.

(2) the urine creatinine concentration should be determined no later than 24 hrs after collection . Acidity or alkalinity of the urine (as a result of bacterial metabolism)promotes the conversion of creatinine to creatine , which is not measured directly by Jaffe reaction method . Bacteria in addition to causing a change in PH of the urine also produces creatinase which destroy creatinine.

REFERENCE RANGES OF CrCL(as quated in literature)MEN= (85-125)ML/MIN: WOMEN: = (75-115) ML/MIN.

KENYAN BASED REFERENCE RANGES:MEN= (52-110)ML/MIN: ***WOMEN: = (50-92) ML/MIN***

NB/ *the difference in the reference ranges is due to the differences in gfr and also to the fact that males have more muscles than females.*

ESTIMATED CREATININE CLEARANCE (Ecrcl)

This is achieved by the use of COCKCROFT AND GAULT formula which use the following characters (factors):

(a) age(yrs) (b) weight(kgs) (c) serum creatinine (d) constants ie male(1.23, female(1.04)

FORMULA:

[(140-AGE) X WEIGHT(KG) X CONSTANT(MALE:1.23,FEMALE:1.04)] DIVIDE BY SERUM CREATININE(UMOL/L)

KENYAN BASED REFERENCE RANGES:MEN= (54-118)ML/MIN: ***WOMEN: = (58-106) ML/MIN***

ELECTROLYTES

The electrolytes to be covered under renal function tests are:- sodium, potassium and chlorides .

(1) SODIUM -

This is the major cation of the extracellular fliud, representing approximately 90% of the 154 mmol/l of inorganic cations per liter of plasma water.. Thus sodium ions are responsible for almost half the osmolality of the plasma and play a central role in maintaining the normal distribution of water and osmotic pressure in the extracellular fluid compartment.

- The average daily diet contains 130-260 mmol(8-15g) of sodium chloride, which is nearly completely absorbed from the GIT . Since the body requirement is only 1-2 mmol/d the excess is excreted in the urine. The kidneys are the ultimate regulators of the amount of sodium and potassium in the body.

- Sodium is initially filtered by the glomeruli , but approximately 90% of the filtered load is reabsorbed in the renal tubules along with bicarbonates , chloride and water.

- HYPONATREMIA (decreased plasma sodium cocentration) is a predictable consequence of decreased intake of sodium , particularly when low intake is complicated by unusual losses.. **Depletional hyponatremia** may be the result of excessive sweating, prolonged vomiting, persistent diarrhea, or salt losing enteropathies (ie conditions in which sodium loss is greater than water loss). The dilutional type of hyponatremia is secondary to excessive retention of water. This is seen in conditions such as edema, ascites in chronic cardiac failure, uncontrolled diabetes, hepatic cirrhosis , nephritic syndrome and malnutrition.

- HYPERNATREMIA(increased plasma sodium concentration) is often attributable to excessive loss of sodium –poor body fluids ie to water loss disproportionate to sodium loss as may occur in profuse sweating ,vomiting and diarrhea or polyuria. Other causes are decreased production of ADH, decreased tubular sensitivity to the hormone. and osmotic diuresis.

- other causes are due to accumulation of sodium as in inappropriate forms of parenteral therapy with saline solution or high salt intake without corresponding intake of water.

DETERMINATION OF SODIUM

(A)SPECIMENS---Serum, heparinized, plasma, sweat, urine, feces or gastrointestinal fluid .IF plasma is used for assay, the lithium or ammonium saltof heparin should be used as anticoagulant. urine should be collected without any addition of preservative.

(B)METHODS OF ANALYSIS AVAILABLE

- There are three widely used methods of estimation of sodium namely:- A)ion selective electrode and B) colorimetric method

A) ION SELECTIVE ELECTRODE ANALYTICAL METHOD

ION SELECTIVE ELECTRODES (ISE)

-These electrodes are made up of membranes which are permeable to selected anions (-ve) and cation (+ve).-For analytical application, the membranes are required that possesses a selective permeability to a single ion species. Eg In the estimation of sodium, the ISE used only selects the sodium ions in a forest of other ions. The same will occur with the other ions.

B) COLORIMETRIC ANALYTICAL METHODS FOR SODIUM

- This technique involves the use of a single to use liquid reagent that contains a sodium selective macrocyclic compound known as a CHROMOGENIC CRYPTAHEMISPHERAND. This chromogenic cryptahemispherand compound is composed of highly selective sodium ionophorethat is covalently linked to an ionizable chromophoric group. At the constant PH (alkalinic)maintained by the buffered reagent, this chromophoric group exists in an equilibrium of its protonated and unprotonated forms which have distinctly different absorption spectra. Complexation of sodium ion by the chromogenic ionophore induces a change in the basicity of the chromophoric group and hence a change in the ration of its two spectrally different forms. Acolor change is observed and measured at 500nm, the rate of change in absorbance being proportional to the sodium concentration in the sample.

REFERENCE RANGES AS QUOTED IN LITERATURE FOR SODIUM= 136-148 MMOL/L.NB/ NO DIFFERENCE BETWEEN CHILDREN AND ADULTS.

POTASSIUM

Potassium is a major intracellular, cation. In pathological conditions it is either reduced [hypokalemia]or elevated [hyperkalemia,]. The pathological conditions associated with hypokalemia includes, vomiting, diarrhoea, and renal diseases. While the pathological condition associated with hyperkalemia includes; acute /chronic renal failure renal tubular acidosis. Hypo-and hyper- kalemia may have profound adverse effects on the neuromuscular system and especially on the myocardium and if not treated causes cardiac arrest .

DETERMINATION OF POTASSIUM

(A) SPECIMENS---Serum, heparinized , plasma, sweat, urine, feces or gastrointestinal fluid .IF plasma is used for assay, the lithium or ammonium salt of heparin should be used as anticoagulant. urine should be collected without any addition of preservative.

(B)METHODS OF ANALYSIS AVAILABLE

- There are three widely used methods of estimation of potassuim namely :- A)ion selective electrode and B colorimetric method

A) ION SELECTIVE ELECTRODE ANALYTICAL METHOD--- THE PRINCIPLE IS SAME AS THAT OF SODIUM

B) COLORIMETRIC ANALYTICAL METHODS

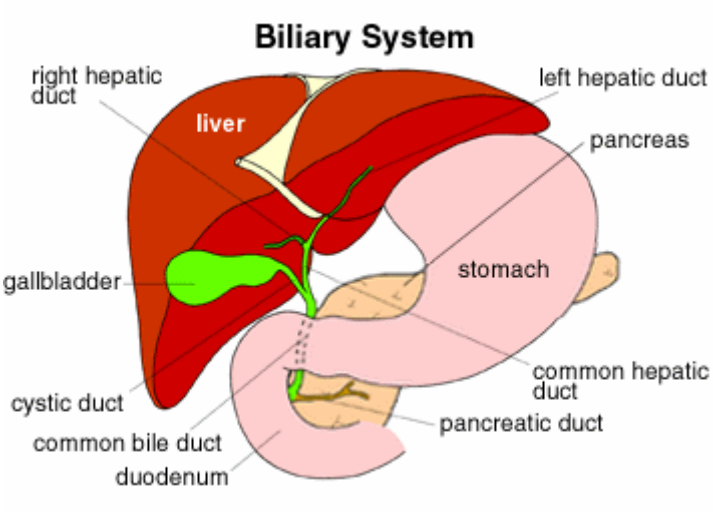
PRINCIPLE---potassium ions in a protein – free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium

tetraphenylboron complexion. The turbidity produced is directly proportional to the potassium concentration in the sample..the absorbance is measured at 578nm

REFERENCE RANGES AS QUOTED IN LITERATURE FOR SODIUM= 3.6-4.8 MMOL/L..NB/ NEONATES HAVE HIGHER POTASSIUM LEVELS THAN THE ADULTS

LIVER, LIVER DISODERS AND LIVER FUNCTION TESTS PROFILE

The liver is the largest organ inside the body and the most metabolically active. In an adult, it is about the size of a football and weighs close to three pounds. It is located behind the ribs in the upper right-hand portion of the abdomen. Shaped like a triangle, the liver is dark reddish-brown and consists of two main lobes. There are over 300 billion specialized cells in the liver that are connected by a well organized system of bile ducts and blood vessels called the biliary system (Perez and Lima, 2007).



This complex organ performs multiple interrelated functions that are essential for life, including:

1) uptake, storage, metabolism, and release of nutrients (e.g., amino acids, carbohydrates, lipids, vitamins, and minerals);

2) synthesis of bile salts from cholesterol and their secretion to assist with fat absorption and digestion;

3) synthesis and secretion of plasma proteins necessary for blood clotting and transport of molecules through the circulation;

4) detoxification of drugs, hormones, and the endproducts of metabolism and distribution to the bile or urine for excretion; and

5) removal of bacteria and dying red blood cells from the circulation.

This functional complexity is attested by the fact that reconstitution of the organ in the form of an artificial or bioartificial liver capable of supporting patients with liver failure has yet to be achieved.

The liver is subject to damage by a wide variety of diseases. Viruses, bacterial infections, toxins, and several forms of cancer are a few of the possible causes of liver disease. Some diseases cause very sudden and severe liver damage and others are slow and insidious. If the disease progresses slowly, symptoms of disease may not appear for many months, but the liver may be slowly destroyed. A normal liver has a lot of excess capacity. By the time signs appear in some slow moving diseases, 3/4 of the liver may already have been destroyed. On the other hand, many liver problems are mild or short lived, and the liver is very good at healing and regenerating itself once the cause of the disease is under control.

Liver disease (also called hepatic disease) is a broad term describing any single number of diseases affecting the liver. Many are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the hemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile. Some of the liver diseases includes; hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, cirrhosis,cystic Fibrosis, cancer, wilson's disease, gilbert's disease

HEPATITIS

Hepatitis is an inflammation of the liver characterized by the presence of inflammatorycells in the tissue of the organ. The condition can be self-limiting (healing on its own) or can progress to fibrosis (scarring) and cirrhosis. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite) and malaise. Hepatitis is ***acute*** *when it lasts less than six months and chronic when it persists longer. A group of viruses known as the hepatitis viruses (hepatitis A,B,C and E) cause most cases of hepatitis worldwide, but it can also be due to toxins (notably alcohol, certain medications, some industrial organic solvent and plants), other infections and autoimmune diseases .*

ALCOHOLIC LIVER DISEASE

Research established a firm connection between heavy alcohol consumption and liver disease. Studies show that the amount of alcohol consumed and the duration of that consumption are closely associated with cirrhosis. The most prevalent types of alcoholic liver disease are fatty liver, alcoholic hepatitis, and cirrhosis. Often, as people continue to

drink heavily, they progress from fatty liver to hepatitis to cirrhosis. The disorders can also occur together, however, and liver biopsies can show signs of all three in some people. About 10 percent to 15 percent of people with alcoholism develop cirrhosis, but many survive it. Many are unaware that they have it, and about 30 percent to 40 percent of cirrhosis cases are discovered at autopsy.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

NAFLD refers to a group of conditions where there is accumulation of excess fat in the liver of people who drink little or no alcohol. This is a chronic liver disease that affects a high proportion of the world's population.

LIVER CIRRHOSIS

Cirrhosis is a potentially life-threatening condition of the liver that refers to scarring of the liver which results in abnormal liver function as a consequence of chronic (long-term) liver injury. This scarring replaces healthy tissue and prevents the liver from working normally. Cirrhosis usually develops after years of liver inflammation.

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CYSTIC FIBROSIS (CF)

Evidence of chronic liver disease is found in 25% of patients with cystic fibrosis (CF) and is the cause of liver decompensation in 2–3%. Studies suggest that approximately 20–25% of CF patients will develop liver disease but only 6–8% of these will have established cirrhosis, majority of which will present in the first 20 years of life

LIVERCANCER

Liver cancer can refer to a variety of malignant diseases that are either primary or secondary to the liver. Primary liver cancers, which originate in the liver, include hepatocellular carcinoma (HCC), cholangiocarcinoma, hepatoblastoma, fibrolamellar carcinoma, angiosarcoma, and several other rarer forms. The liver is also a frequent site for secondary tumors that have metastasized, most frequently from colorectal, breast, or gastric cancers (Llovet et al., 2005).

WILSON'S DISEASE

Wilson's disease is an autosomal-recessive disorder caused by mutation in the ATP7B gene, with resultant impairment of biliary excretion of copper. Subsequent copper accumulation, first in the liver but ultimately in the brain and other tissues, produces classical clinical manifestations that may include hepatic, neurological, psychiatric, ophthalmological, and other derangements

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LIVER FUNCTION TESTS

Liver function tests is a profile that is composed of the following analytes:-total protein(TP), albumin(ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase(AST),Gamma glutamyl transferase (GGT or γ GT) , Total bilirubin (T BILI) and Direct bilirubin (D BILI) .This profiles investigates the health status of the liver thereby diagnizing the pathological disorders affecting the liver.

Total protein (TP)

Total protein reagent was used to measure the concentration of total protein by a timed endpoint biuret method. In the reaction, the peptide bonds in the protein sample binds to cupric ions in an alkaline medium to form a peptide/copper complex. 6µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 560nm. This change was directly proportional to the concentration of TP in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for four minutes.

Principle of the reaction

protein + cu^{2+} $\overset{oh^-}{\rightleftharpoons}$ protein-copper complex.

reference range in literature (62-83G/L)

Albumin (ALB)

Albumin reagent was used to measure albumin concentration by a timed endpoint method. Albumin combined with bromocresol green to form a coloured product. 3 µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 600nm.This change was directly proportional to the concentration of ALB in the sample and was used to calculate and express concentration in g/l. The reaction took place at 37°C for one and half minutes.

Principle of the reaction

Albumin +BCG $\longrightarrow$ Albumin / BCG complex

reference range in literature (30-52G/L)

Alkaline phosphatase (ALP)

Alkaline phosphatase reagent was used to measure alkaline phosphatase activity by a kinetic UV method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction alkaline phosphatase catalyzed the hydrolysis of the colorless organic phosphate ester substrate, p-nitrophenylphosphate to the yellow colored product, p-nitrophenol and phosphate. The reaction occurred at an alkaline pH of 10.3. 5µl of the sample was reacted with 250 µl of the reagent. The change in absorbance was monitored at 410 nm and this change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principle of the reaction

p-Nitrophenylphosphate + H_2O $\xrightarrow{PH\ 10.3,\ Mg}$ p-nitrophenol + Phosphate (colorless) (yellow)

***reference range in literature (98-279U/L)**

Alanine aminotransferase (ALT)

The ALT reagent was used to measure alanine aminotransferase in the sample by an enzymatic kinetic UV rate method. In the assay reaction, the ALT catalyzed the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamine.The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinuleotide (NAD). Pyridoxal-5-phosphate was required in this reaction as a cofactor that was required for transaminase activity by binding to the enzyme using Schiff-base linkage. 10µl of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of ALT.The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principle of the reaction

L-Alanine + α-Ketoglutarate $\xrightarrow{ALT}$ Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ Lactate + NAD^+

reference range in literature (<42U/L)

Aspartate aminotransferase (AST)

AST reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method. In the reaction aspartate aminotransferase catalysed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate.The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the

concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD). 10µl of the sample was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of AST.The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principle.of the reaction

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ *Oxaloacetate + L-Glutamate*

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ *Malate +* NAD^+

****reference range in literature (<39 U/L)***

Gamma glutamyl transferase (GGT)

GGT reagent was used to measure γ-glutamyl transferase activity by an enzymatic kinetic UV rate method. In the reaction, γ-glutamyl transferase catalysed the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. 5µl of the sample was reacted with 200µl of the reagent. The rate of formation of 5-amino-2-nitrobenzoate was proportional to the activity of GGT present in the sample and was measured kinetically at 405nm. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

Principleof the reaction

L-γ–glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{GGT}$ *L- γ – glutamylglycylglycine + 5-amino-2-nitrobenzoate*

****reference range in literature (Male:5-45U/L, female: 5-35U/L***

Total bilirubin (T BILI)

A stabilized diazonium salt (3, 5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacted with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an accelerator (caffeine) to form azobilirubin (purple). 8 µl of sample was reacted with 280 µl of reagent and the change in absorbance was monitored at 578nm.This change was directly proportional to the concentration of T BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37°C for two minutes.

Nb/ A separate sample blank were performed (set) to reduce endogenous serum interference.

Principle.of the reaction

Bilirubin + DPD $\xrightarrow{caffeine}$ *Azobilirubin*

reference range in literature (<23umol/L)

Direct bilirubin (D BILI)

A stabilized diazonium salt (3,5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacts with conjugated bilirubin directly in an acidic medium to form azobilirubin (purple). 5 µl of sample was reacted with 160 µl of reagent and the change in absorbance was monitored at 546nm.This change was directly proportional to the concentration of D BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37°C for two minutes.

Principleof the reaction

Bilirubin + DPD $\xrightarrow{H+}$ *Azobilirubin (purple colour))*

reference range in literature (<4.3umol/L)

ENZYMATIC REACTIONS

Introduction------Clinical enzymology is the application of the science of enzymes to the diagnosis and treatment of pathological disorders. Enzymes are proteins with catalytic properties due to their powers of specific activation of their substrates. This definition indicates the characteristic properties of enzymes which in turn govern the principles of methods of enzymes analysis.

Enzyme nomenclature---Due to the number and different types of enzymatic reactions available, it became necessary to have a definitive and standardized system of identifying enzymes.The responsibility of naming enzymes has been achieved by the Enzyme Commission (EC) of the International Union of Biochemistry(IUB)

Two names are given to each enzyme: (1)—systematic name that clearly describes the nature of reaction catalyzed, (2)—practical name which is suitable for everyday use.

The unique numerical designation for each enzyme consists of four numbers separated by periods. The number is prefixed by the letters ***EC,*** denoting Enzyme Commission eg EC 2.2.8.11.The first three numbers indicates the class, subclass, and sub-subclass to which the

enzyme has been assigned. The last number is the specific serial number given to each enzyme within its sub-class.

It is a common and convenient practice in clinical chemistry to use capital letter abbreviations for the names of certain enzymes, such as ALT for alanine aminotransferase (EC 2.61.2). Example of others, AST for aspartate aminotransferase, LD for lactatedehydrogenase and CK for creatine kinase.

---DEFINATION OF AN ENZYME-----

Enzymes are catalysts in nature that is control a reaction by increasing the rate of a particular reaction without itself being consumed or permanently altered; at the end of a catalysed reaction the catalyst appears unchanged in form and in quantity, whereas the main reaction materials have undergone transformation into new products.

Enzymes are protein catalysts of biologicalorigin and virtually all the chemical reactions that take place in living matter are catalysed by specific enzymes.

Enzyme act through the formation of an enzyme-substrate complex, ***ES,*** in which a molecule of substrate is bound to the specialized substate –binding region (the active centre) of the enzyme molecule. The binding process transforms the substrate molecule to an activated state, and the energy required for this transformation is provided by free energy of binding of ***S*** and ***E***. At the end of the reaction, the ***ES***complexone breaks down to give the reaction products*(P*) and free enzyme(***E***).

E + S = ES→P +E

The rate of enzymatic catalysed reactions are governed by :-

(a) **Enzyme concentration----**ie the rate of reaction is generally proportional to the concentration of enzyme present in the system and this is the basis for the quantitative determination of enzymes by measurement of reaction rates

(b) **Substrate concentration-**---If the enzyme concentration is fixed and the substrate concentration is varied, the rate of reaction is almost directly proportional to the concentration of substrate at low values of the latter ie the reaction is essentially first order with respect to substrate concentration.

(c) **Ph -**-----the rate of enzyme catalysed reactions typically shows a marked dependence on Ph. Many of the enzyme in blood plasma show maximum activity in vitro somewhere in the ph range from 7 to 8 (nb/ in vivo the ph of blood is 7-35-7.45). The pronounced effects of ph on enzymes reactions emphasize the need always to control this variable by means of adequate buffer solutions.

(d) **Temperature—**Therate of an enzymatic reaction increases as the temperature at which the reaction is taking place increases. The choice of temperature for the assay of enzymes of clinical importance is (30-37) degree centigrades. During the enzymatic reactions the changes should be +/- 0.1 degree centigrade.Otherwise high temperatures are known to denature the enzyme molecules.

COENZYMES----these are smaller molecules than enzymes proteins molecules. Co-enzymes, act as specific substrates in two substrate reaction and their effect on therate of reaction follows Michaelis-Menten pattern of dependence on substrate concentration. Example NAD^+ , NADH, $NADP^+$.These co-enzymes are bound to the enzymes during a enzymatic reactions and the reaction would not take place in their absence.

Progress Of Enzymatic Reactions And The Measurements Of Reaction Rates.

-Since the rate of an enzyme-catalyzed reaction is diretly proportional to the amount of active enzyme present in the system, determination of the rate of reaction under defined and controlled conditions provides a very sensitive and specific method for the measurement of enzyme in specimens such as serum.

The progress of convertion of the substrate into products in the presence of an enzyme can be followed by measuring the decreasing concentration of substrate or the increasing concentration of products. These principles are found among assays of clinically important enzymes.

-All determinations of reaction rate involve the measurement of the amount of change produced in a defined time interval ie all measurements of reaction rate are kinetic measurements.

-At the moment when the enzyme and substrate are mixed , the rate of the reaction is zero. Typically, the rate then rises rapidly to a maximum value , which remains constantfor a period of time. **During the period of constant reaction rate, which may short or long in different circumstances, the rate depends only on the enzyme concentration and is completely independent of substrate concentration. The reaction is said to follow zero-order kinetics ie the rate is proportional to the zero power of substrate concentration**. *As more and more substrate is consumed, the reaction rate declines and enters a phase of first –order dependence on substrate concentration.* Other factors that contribute to the decline in the reaction include accumulation of products that may be inhibitory, the growing importance of the reverse reaction, and even enzyme denaturation.

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BODY FLUIDS AND ELECTROLYTES BALANCE

--The main fluid in the body is water. Total body **water is 60% of body weight** whilst the other **40% is made up of fats, protein, carbohydrates and minerals**.

--The distribution of the body water is as follows:-

*Intracellular fluid which makes up 40% or (2/3) of the body water.

*** The extracellular fluid makes up the remainder 20% or (1/3) of the body water and consists of: Interstitial fluid – (fluid between the cells),-Plasma and lymph –(the intra-vascular fluid, - Cerebrospinal fluid (found in ventricles of brain and surrounding the brain and spinal cord), - G.I. tract fluids (gastric, pancreatic and intestinal juices), - Synovial fluid (found inside synovial joint capsules), - Eye and ear fluids (aqueous and vitreous humours, perilymph and endolymph.), - Pleural, pericardial and peritoneal fluids and Glomerular filtrate.**

-- Water moves from one compartment to another due to osmosis. Osmosis is chiefly influenced by dissolved solutes in fluids. Selectively permeable membranes separate body fluids into distinct compartments. Plasma membranes of individual cells separate ICF from ECF and blood vessel walls separate blood plasma from interstitial fluid.

--The major components of these fluids include water and solutes. The solute is mostly comprised of electrolytes: inorganic compounds that dissociate into ions. Electrolytes include cations (positively charged atoms – sodium, potassium, calcium, magnesium, carbonate) and anions (negatively charged atoms – chloride, sulphide, phosphate, bicarbonate).

SOURCES OF BODY FLUIDS

1. Preformed water represents about 2,300 ml/day of our daily intake. This is the water found in our food and drink.

2. Metabolic water is produced through the catabolic breakdown of nutrients occurring during cellular respiration, This amounts to about 200 ml/day.

3. Combining preformed and metabolic water gives us a total daily intake of 2,500 ml.

FLUID BALANCE

Fluid Balance - When each compartment of the body contains the appropriate concentration of water and electrolytes, the body is said to be in fluid balance. Normally, there is a balance achieved between our total daily intake and output of water.

Regulation of Fluid Gain

The term *fluid balance* defines the state where a body's required amount of water is present and proportioned *normally* among the various compartments; this state is inseparable from *electrolyte balance.*

Under normal conditions water loss equals water gain and a body's water volume remains constant. Avenues for water loss include the kidneys, skin, lungs, feces, and menstruation.

Water is sourced mostly from dietary intake; this is called *preformed water*. However, metabolic processes such as cellular respiration and dehydration synthesis reactions

generate a small component. Approximately 1.6 litres per day is sourced from ingested water, 0.7 litres per day from ingested food, and 0.2 litres from metabolic processes.

Water is not produced by the body to maintain homeostasis; metabolic water production is simply a by-product of cellular respiration. The body regulates water intake via the thirst reflex which stimulates us to drink.

When water loss is greater than water gain the body reaches a state of dehydration, and dehydration stimulates the thirst reflex in three ways:

1. **the level of saliva drops resulting in a dry mucosa in the mouth and pharynx;**
2. **there is an increase in blood osmotic pressure which stimulates osmoreceptors in the hypothalamus;**
3. **there is a drop in blood volume, which leads to the renin/angiotensinII pathway stimulating the thirst centre in the hypothalamus.**

All three mechanisms stimulate the *thirst centre* in the hypothalamus resulting in the sensation of thirst and causing us to drink to increase fluid volume. Drinking inhibits the thirst centre by stretching the stomach and intestines and reducing the osmotic pressure of the blood [1].

Typically, if the thirst centre is activated dehydration has already occurred to some extent, and it is noted that this reflex is not always reliable in young children, the elderly, or those in a confused mental state [1].

Regulation of Fluid Loss

If fluid loss regulation is considered as the maintenance of the volume of fluid already present in the body, then three hormones play a key role: **Antidiuretic Hormone (ADH), Aldosterone, and Atrial Natriuretic Peptide (ANP).**

A drop in body fluid results in an increase in blood tonicity and a decrease in blood volume which in turn causes the release of *renin* in the kidneys and stimulation of osmoreceptors in the hypothalamus. The hypothalamus causes ADH to be released from the posterior pituitary gland. ADH targets the kidneys, and sudoriferous glands reducing fluid loss through each, and it also causes arterioles to constrict. Renin is released by the juxtaglomerular cells in the kidneys, it acts on angiotensinogen a plasma protein produced in the liver to form angiotensin I. Angiotensin I is converted in the lungs to the active hormone angiotensin II by the imaginatively named angiotensin converting enzyme (ACE). Angiotensin II causes: vasoconstriction of the arterioles; stimulation of the release of aldosterone by the adrenal cortex; stimulation of the thirst centre in the hypothalamus; and stimulation of the release of more ADH. Aldosterone increases the retention of sodium and chloride ions as well as water by the kidneys. These factors combined result in an increase in the level of body fluid and an increase in blood pressure.

On the other hand an increase in body fluid results in an increase in blood volume. This causes stretching of the right atrium, which stimulates the release of ANP a hormone that increases the loss of fluid in the urine. At the same time the level of ADH and renin is decreased under negative feedback.

Other factors that control fluid loss include:

- **severe dehydration which results in a decrease in blood pressure and a decrease in the glomerular filtration rate with a resultant decrease in the loss of water in urine;**
- **water overload will increase the blood pressure and the glomerular filtration rate resulting in an increased loss of water in the urine;**
- **hyperventilation increases fluid loss via water vapour in the lungs;**
- **vomiting and diarrhea increase fluid loss via the GIT;**
- **fever, heavy perspiration, and skin loss (burns) increase fluid loss via the skin.**

FLUID IMBALANCES

(**1**) **fluid volume deficit (FVD) ----**deficiency in both the amount of water and electrolytes in the ECF where water and electrolyte proportions remain near normal **(**commonly known as hypovolemia). Occurs as a result of: **-(A)** abnormal losses through the skin, gastrointestinal tract, or kidney (B) decreased intake of fluid (C) bleeding (D)a shift of fluid into a third space ie the shift of fluid from the intravascular space into an area where it is not readily accessible as ECF, e.g.: sequestered in the bowel ,in the interstitial spaces as edema, in inflamed tissue,in potential spaces such as the peritoneal or pleural cavities .Nb/the patient with a shift of fluid into a third space may not manifest signs/symptoms of fluid volume deficit

(**2**) **fluid volume excess (fve)** ----excessive retention of water and sodium in similar proportions to normal ECF (commonly known as hypervolemia). Occurs as a result of: (A) excessive intake of sodium chloride (B) administering sodium-containing infusions too rapidly, particularly to patients with impaired regulatory mechanisms (C) disease processes that alter regulatory mechanisms, such as congestive heart failure, renal failure, cirrhosis of the liver, and Cushing's syndrome.

* In FVE, both the intravascular and interstitial spaces have an increased water and sodium chloride content . Excess interstitial fluid is known as edema . Edema can be found around the: eyes, fingers and ankles. Edema may result in a weight gain in excess of 5%

(3)Dehydration

Dehydration is a deficit of body water that results when the output of water exceeds intake. Dehydration stimulates the thirst mechanism, instigating water consumption. Sweating and the output of urine both decrease. If water intake continues to fall short of water loss, dehydration worsens.

Causes of dehydration may include:--vomiting, diarrhea, diuretics, excessive heat, excessive sweating, fever and decreased water intake

Dehydration induces water to move from the reservoir inside cells into the blood. If dehydration progresses, body tissues begin to dry out and the cells start to shrivel and malfunction. The most susceptible cells to dehydration are the brain cells. Mental confusion, one of the most common signs of severe dehydration, may result and can lead to **coma**. Dehydration can occur when excessive water is lost with such diseases as **diabetes mellitus**, diabetes insipidus, and Addison's disease.

Dehydration is often accompanied by a deficiency of electrolytes, sodium and potassium in particular. Water does not move as rapidly from the reservoir inside of the cells into the blood when electrolyte concentration is decreased. Blood pressure can decline due to a lower volume of water circulating in the bloodstream. A drop in blood pressure can cause lightheadedness, or a feeling of impending blackout, especially upon standing (orthostatic hypotension). Continued fluid and electrolyte imbalance may further reduce blood pressure, causing **shock** and damage to many internal organs including the brain, kidneys, and **liver**.

treatment of dehydration.

Consumption of plain water is usually sufficient for mild dehydration, although when both water and electrolyte losses have occurred after vigorous **exercise**, electrolytes must be replaced; sodium and potassium in particular. Adding a little salt to drinking water or consuming such drinks as Gatorade during or following exercise can replace lost fluids. Individuals with **heart** or kidney problems should consult a physician regarding the replacement of fluids after exercise.

Sodium chloride may be administered intravenously by medical personnel if blood pressure decreases enough to induce shock or risk of shock. The underlying cause of dehydration must be addressed along with treatment to replace fluids. For example, if the cause of dehydration is due to diarrhea, then drugs may be given to alleviate diarrhea as well. Following treatment of the cause, individuals may be monitored to ensure that oral fluid intake is adequate to maintain fluid hydration.

Overhydration

Overhydration is an excess of body water that results when water intake exceeds output. Drinking large amounts of water does not typically lead to overhydration if the kidneys, heart, and pituitary gland are functioning properly. An adult would have to drink more than 2 US gallons per day (7.6 L per day) to exceed the body's ability to excrete water. Excessive body water causes electrolytes in the blood, including sodium, to become overly diluted. Overhydration occurs in individuals whose kidneys do not function normally, primarily in kidney, heart, or liver disease. People with these conditions may have to limit their water and dietary salt intake. Similar to dehydration, the brain is the most sensitive organ to overhydration. The brain cells can adapt to increased fluid volume when overhydration increases slowly; however, when it occurs rapidly, mental confusion, seizures, and coma can result.

Overhydration can occur alone or in conjunction with excess blood volume. Distinguishing between the two conditions may be quite complicated. Overhydration induces water accumulation within and around the cells but does not typically show symptoms of fluid accumulation. On the other hand, with excess blood volume, there is an accumulation of

sodium and the body cannot transfer water into the reservoir within cells. Conditions such as **heart failure** and liver cirrhosis may induce volume overload, whereby fluid accumulates around cells in the abdomen, chest, and lower legs.

treatment of overhydration. Treatment of overhydration depends somewhat upon the cause, although whatever the underlying condition, fluids must be limited. Drinking less than about 1 US quart (1 L) of fluid a day usually improves overhydration over several days. Fluids should only be limited at a physician's request. A diuretic may be prescribed to increase water output by the kidneys. Diuretics are particularly beneficial for treatment of excess blood volume where overhydration is accompanied by excess blood volume.

ELECTROLYTES AND ELECTROLYTES BALANCE

*Electrolytes are anions (negatively charged particles) or cations (positively charged particles) depending on whether they move in an electrical field towards the anode (positively charged side) or towards the cathode (negatively charged side) that is whether they have a neative or positive charge.

** Electrolytes are essential components of all living matter and include the major electrolytes: sodium, potassium, chloride, magnesium, calcium, bicardonate, phosphate lactate and sulphate.as well as trace elements.Although amino acids and proteins in solution also carry an electrical charge, in clinical chemistry they are usually considered separately from electrolyes.

*** The major electrolytes occur primarily as free ions; the trace metals occurs primarily in combination with proteins..

**** The diatary requirements for electrolytes vary widely; most need to be consumed only in small amounts or at rare intervals and are retained when in short supply.Some like calcium and phosphorus, are continuously excreted and must be ingested regularly in order to prevent deficiency.

ROLE OF ELECTROLYTES

*The role of electrolytes is manifold.There are almost no metabolic processes that are not dependent on or affected by electrolytes. Among functions of electrolytes are:-

(a) maintenance of osmotic pressure and water distribution in various body fluid compartments.

(b) maintenance of proper body ph

(c) regulation of proper heart and other muscle functions

(d) involvement in oxidation-reduction (electron transfer) reactions

(e) participation in catalysis as cofactors for enzymes

* Therefore abnormal levels of electrolytes may be either the cause or the consequences of a variety of disorders, and determination of electrolytes is one of the most importantfunctions of clinical chemistry laboratory.

* Sodium, potassium, chloride and bicarbonate in body fluids are commonly grouped in the familiar test order for an electrolyte profile.

REGULATION OF ELECTROLYTES

(A) SODIUM (Na^+)

-Chief electrolyte in the ECF representing 90% of the total cations per liter of plasma water.Thus sodium ions are responsible for almost half the osmolality of the plasma.

*Average daily requirement is approximately 1-2 mmol/day and the intake is approximately 130-260 mmol (8-15 g0 of sodium chloride.This daily intake is nearly completely absorbed from the gastrointestinal tract.The excess sodium is excreted in the urine, the kidneys are the ultimate regulators of the amountof sodium in the body. small amounts are lost in the feces and perspiration. Aldosterone increases Na+ reabsorption in the collecting ducts of the tubules. *Sodium-rich foods: bacon ,ham , sausage , mustard , processed cheese , vegetables , bread cereal , salted snack foods and table salt (about 46% sodium),

-Functions:

(a) regulating ECF volume and distribution

(b) maintaining blood volume

(c) transmitting nerve impulses and contracting muscles

DETERMINATION OF SODIUM IN BODY FLUIDS: Is estimated through flame photometry, ion selective electrodes(ISE) or colorometric method. *Referencerange for serum sodium as quoted in literature =136-148 mmol/l.

(B)POTASSIUM (K+)

* This is the chief electrolyte in the intracellular fluid. In tissue cell its average concentration is 150 mmol/l. In the erythrocytes the concentration is 105 mmol/l, approximately 23 times that of the plasma..High intracellular concentration are maintained because potassium difuses only slowlyoutward through the cell membrane whereas the sodium- potassium –ATP pump continually transports potassiuminto the cell.

*Excreted primarily by the kidneys .Kidneys have no effective means of conserving potassium Potassium deficits develop rapidly if it is excreted in excess without being replaced simultaneously .It can also be lost through gastrointestinal tract and some through perspiration and saliva .Potassium regulation is achieved through:-renal excretion and conservation - aldosterone increases K+ excretion - movement into and out of cells ,-insulin helps K+ move into cells and tissue damage and acidosis shifts K+ out of cells into the ECF .

*foods rich in potassium: bananas, dates, oranges, melons, potatoes, prunes and figs

* ***Functions:***

(a) maintaining ICF osmolality

(b) transmitting nerve and other chemical impulses

(c) regulating cardiac impulse transmission and muscle contraction

(d) skeletal and smooth muscle function

(e) regulating acid-base balance

DETERMINATION OF POTASSIUM IN BODY FLUIDS: Is estimated through flame photometry , ion selective electrodes(ISE) or colorometric method. *Referencerange for serum sodium as quoted in literature =3.5-5.2 mmol/l.

(C) CHLORIDE (CL-)

***This is the major extracellular anion** and represents the largest fraction of the plasma total anion concentration of approximately154 mmol/l. It is present in the blood, interstitial fluid and in minute amount in the ICF.Chloride ions in food are almost completely absorbed from the intestinal tract but the average dialy reduirement is not known..They are filtered along the glomeruli and passively reabsorbed along with Na+ in the proximal tubules.In the loop of henle, chloride is actively reabsorbed by the so called CHLORIDE PUMP whose action promotes reabsorption of Na+ as well.Surplus chloride is excreted in the urine, excessive losses in the sweat as can occur in hot weather is minimized by the secretion and action of aldosterone hormone which causes the sweat glands to reabsorb more Na+ and Cl-.

* foods rich in chloride are: foods rich in sodium, dairy products and meat.

Functions:

(a)hydrochloric acid (HCL) production

(b)regulating ECF balance and vascular volume

(c) regulating acid-base balance

(d)buffer in oxygen-carbon dioxide exchange in red blood cells (RBCs)

* DETERMINATION OF CHLORIDE IN SERUM:, ion selective electrodes(ISE) or colorometric method. *Referencerange for serum sodium as quoted in literature =95-105 mmol/l.

(**D)BICARBONATE ($HC0_3$-)**

*Chief chemical base buffer within the body present in both the ECF and ICF. Average daily requirement is not known. Unlike other electrolytes that must be consumed in the diet, adequate amounts of bicarbonate are produced through metabolic processes to meet the body's needs *Bicarbonates are excreted by the kidneys and its regulation is through excretion and and reabsorption by the kidneys

***Functions:**

(a)chief chemical base buffer involved in acid-base balance

(b)essential component of the carbonic acid-bicarbonate buffering system

* DETERMINATION OF bicarbonate IN SERUM: colorometric method. *Reference range for serum bicarbonate as quoted in literature =23-29 MMOL/L

(b)metabolizing carbohydrate, protein, and fat

(c) cellular metabolism; producing ATP and DNA

(d) muscle, nerve, and RBC function

(e) buffer in the oxygen - carbon dioxide exchange in RBCs

** DETERMINATION of PHOSPHORUS in SERUM: colorometric method.(molybdate methods *Referencerange for serum bicarbonate as quoted in literature =0.83-2.3 mmol/l.

FLUID AND ELECTROLYTE MOVEMENT

There are four ways in which fluid and electrolytes move in the body:-osmosis, diffusion, active transport and filtration.

(1) OSMOSIS

***T**his is the **movement of a solvent across** a selectively permeable cell membrane from an **area of higher concentration of solutes** to an **area of lower concentration of solutes** until equilibrium is established . Water is the primary solvent that holds solutes (substances) in solution in the body.The concentration of these solutes in the body fluids is osmolality, whichis determined by the total solute concentrationwithin a a fluid compartment. Osmolality is measured as parts of solute per kilogram of water and is reported as milliosmols per lkg(mOsmol/L). The osmolality of plasma is 275-295 mOsmol/L. The greatest determinant of osmolality within a fluid compartment is sodium concentration.

*Tonicity---may be used to refer to the osmolality of a solution. ***Isotonic solutions*** have the same osmolality as body fluidseg 0.9% normal saline. With isotonic solutions, water remains in the intravascular compartment without any net flow across selective membranes

* hypertonic solutios—have a higher osmolality than body fluids eg 3% saline solution.With a hypertonic solution in the intravascular compartment, water moves out of the intracellular compartment(inside the cell) and into intravascular compartment that is hypertonic causing the cell to shrink

*hypotonic solutions have a lower osmolality than the body fluids eg 0.45% saline. With hypotonic solutions water moves out of the intravascular compartment into intracellular compartment(inside the cells)that is hypetonic causing the cell to burst.

(2) **DIFFUSION**

* This is the **movement of solutes across** a selectively permeable cell membrane from an area of higher concentration of solutes to an area of lower concentration of solutes until equilibrium is established . "***Coasting downhill***"--- diffusion is affected by:

(A) the size of the solutes:-. Larger solutes move less quickly and, consequently, have a lower rate of diffusion WHILST smaller solutes move more quickly and, consequently, have a higher rate of diffusion

(B) the concentration of the solutes :-solutes move from an area of higher concentration of solutes to an area of lower concentration of solutes

(C) the temperature of the solutes:- increases in temperature increase the rate of motion of solutes and, consequently, leads to a higher rate of diffusion WHILST decreases in temperature decrease the rate of motion of solutes and, consequently, leads to a lower rate of diffusion

(3) **ACTIVE TRANSPORT** * Movement of **solutes across a selectively permeable cell membrane,** usually against a pressure gradient and with the expenditure of metabolic energy(ATP) , from an area of higher concentration of solutes to an area of lower concentration of solutes until equilibrium is established ."Pumping uphill" in active transport: a solute combines with a carrier on the outside surface of a cell membrane , the solute and carrier move to the inside surface of the cell membrane .Once on the inside surface of the cell membrane, the solute and carrier separate and the solute is released to the inside of the cell. Eg the sodium-potassium pump is an important active transport mechanism: Under normal conditions: sodium concentrations are higher in the ECF and potassium concentrations are higher in the ICF.So to maintain these conditions, the sodium-potassium pump continually pumps sodium out of the cells into the ECF and pumps potassium into the cells into the ICF .

(4)**FILTRATION** * Movement of **solutes and solvent across a permeable cell membrane** from an area of higher concentration of solutes to an area of lower concentration of solutes until equilibrium is established . This is influenced by two pressures ie colloid osmotic, (or oncotic pressure) and the hydrostatic pressure. Oncotic pressure is that pressure that prevents water from moving out from the intravascular to extravascular compartments to maintain vascular volume whilst hydrostatic pressure is that pressure that moves water outward from the intravascular to extravascular compartments

ELECTROLYTE IMBALANCES

Electrolyte imbalance ----This occurs when the levels of the body electrolytes are either elevated (increased) or reduced (decreased) in the ECF. The term of reference is the reference value of the electrolyte used in a given population. The term used to describe elevated values of a certain electrolyte is hyper- and that for reduced value is hypo-.

(1) **HYPONATREMIA** ---sodium deficit or (reduced levels of sodium) in the ECF, or serum sodium level less than 135 mmol/l (reference range lower limit). Risk factors that can lead to loss of sodium, includes:- (a)loss os GIT fluids, (b)use of diuretics (c)adrenal insufficiency (d) gains of water, e.g.: (excessive administration of IV fluids , disease states associated with SIADH (syndrome of inappropriate anti diuretic hormone secretion), pharmacologic agents than may impair water excretion .

*__Signs/symptoms__ :- anorexia, nausea and vomiting , lethargy, confusion , muscle cramps muscular twitching, seizures and coma

(2) **HYPERNATREMIA** --sodium excess (or elevated levels of sodium) in the ECF, or serum sodium level greater than 145 mmol/l (reference range upper limit). Risk factors that can lead to hypernatremia includes:- water deprivation, increased sensible and insensible water loss , ingestion of a large amount of salt , excessive parenteral administration of sodium-containing solutions, profuse sweating and diabetes insipidus

*__Signs/symptoms__ :-thirst , elevated body temperature, tongue dry and swollen , sticky mucus membranes AND in severe hypernatremia: the symptom/signs are:- disorientation ,hallucinations , lethargy when undisturbed , irritable and hyperactive , focal or grand mal seizures

(2)**HYPOKALEMIA ----**potassium deficit (reduced)in the ECF, or serum potassium level less than 3.5 mmol/l (reference range lower limit). Risk factors that can lead to hypokalemia includes:-diarrhea, vomiting or gastric suction , potassium-wasting diuretics , steriod administration and certain antibiotics , poor intake as in anorexia nervosa, alcoholism, potassium-free parenteral fluids and polyruia

* **Signs/symptoms** :-fatigue, anorexia, nausea, and vomiting , muscle weakness , decreased bowel motility , cardiac arrythmias , increased sensitivity to digitalis , polyuria, nocturia, dilute urine , postural hypotension , ECG changes , paresthesias or tender muscles

(3**) HYPERKALEMIA** ---potassium excess (elevateLevels) in the ECF, or serum potassium level greater than 5.2 mmol/L. Risk factors that can lead to hyperkalemia includes:- decreased potassium excretion, e.g.: oliguric renal failure, potassium-sparing diuretics , hypoaldosteronism ,high potassium intake, especially in the presence of renal insufficiency , shift of potassium out of cells, e.g. acidosis, tissue trauma, malignant cell lysis

*__Signs/symptoms__ ----vague muscle weakness, cardiac arrythmias , paresthesias of the face, tongue, feet, and hands , flaccid muscle paralysis, GIT symptoms such as nausea, intermittent intestinal colic, or diarrhea may occur

(4) **HYPOCHLOREMIA AND HYPERCHLOREMIA** are very rare conditions since most of the foodsconsumed has cholide and the body is able to control any deficiency or excesses of this ion successively.

ELECTROLYTES ANALYSIS

(SODIUM AND POTASSIUM ALREADY DISCUSSED)

Bicarbonate (HCO_3^-)

Bicarbonate reagent is used to measure the concentration of bicarbonate in the sample by an enzymatic rate method. In the assay reaction, HCO_3^- reacts with phosphoenolpyruvate (PEP) a reaction catalyzed by Phosphoenolpyruvate Carboxylase (PEPC) to form oxaloacetate and hydrogen phosphate ($H_2PO_4^-$). The oxaloacetate is then reduced to malate in the presence of Malate dehydrogenase (MDH) with the concurrent oxidation of β-Nicotinamide

Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinuleotide (oxidized form) (NAD). Magnesium ions were required in this reaction to act as a co-factor. Sample is reacted with reagent and the change in absorbance is monitored at 340nm, due to reduction of NAD. This change is directly proportional to the concentration of HCO_3^- in the sample and is used to calculate and express concentration in mmol/L. The reaction takes place at 37°C

$$\text{Phosphoenolpyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

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BLOOD GASES AND ACID/BASE BALANCE AND ACID/BASE DISORDERS

INTRODUCTION

-Metabolic processes of the body results with the production of large amounts of carbonic acids (H_2CO_3), sulfuric, phosphoric and other acids. These products of metabolism are transported to the excretory organs (lungs and kidney) via the ECF and the blood with minimal change in the plasma PH. This is accomplished by the combined functions of the buffer systems of the blood and the mechanisms of respiratory and renal systems.

- Clinical management of respiratory and metabolic disorders of patients undergoing surgical correction of cardio-pulmonary disorders has been greatly improved by the development of instrumentations for rapid accurate measurement of oxygen and carbon dioxide in blood and expired air.

- The determination of blood gases and ph also plays an important role in the detection of acid/base imbalance and in following the effect of therapy.

- The measurement of blood gases, acid/base balance and ph of blood is very important in the management of patients in HDU(high dependency unit) and in ICU(INTENSIVE **CARE UNIT). These are patients who can not breath normally especially those in a coma.**

- The gases commonly measured are O_2 and CO_2 . Although small amounts of these gases exist as dissolved gases in the blood , they are for the most part , chemically combined with other substances. CO_2 exist chiefly as HCO_3^- while most of O_2 is bound to haemoglobin to form oxyhaemoglobin. The PH of blood is dependent on the relative amounts of **HCO_3^- and carbonic acid (HCO_3) which are present in the blood.**

RESPIRATION

- Respiration is responsible for the supply of oxygen needed by the tissue cells for their metabolic activities , and for removing carbon dioxide produced by these metabolic activities.There are two types of respiration ie EXTERNAL AND INTERNAL RESPIRATION.

- EXTERNAL RESPIRATION---This is the term given for the exchange of oxygen and carbon dioxide in the lungs between alveolar air and blood whilst INTERNAL O_2 and CO_2 RESPIRATION the oxygen in the blood is exchanged with the carbon dioxide in the cells. Regulation of respiration is mediated by neuro-chemical centres in the brain.

O_2 AND CO_2 IN THE BLOOD

Carbon dioxide is transported in the blood in several forms and the three most abundant and important are :- a)bicarbonate ---in plasma and rbcs

b) carbamino haemoglobin-----in rbcs

c) dissolved carbondioxide(d CO_2) ---in fluid of rbcs and plasma

- oxygen is transported to the tissues in two forms:

a) reversibly bound to haemoglobin in rbcs(HB O_2)

b)) dissolved oxygen(d O_2) ---in fluid of rbcs and plasma

-The various forms of carbon dioxide deserves special notice since it is on understanding of these forms that comprehension of acid/base regulation is based. In plasma , a small portion of dissolved carbon dioxide reacts with plasma water to form carbonic acid and bicardonate

$$\mathbf{CO_2 + H_2O} \xrightarrow{\text{CD}} H_2CO_3$$

$$H_2CO_3 \text{ ---------- } H+ \quad + \quad HCO_3^-$$

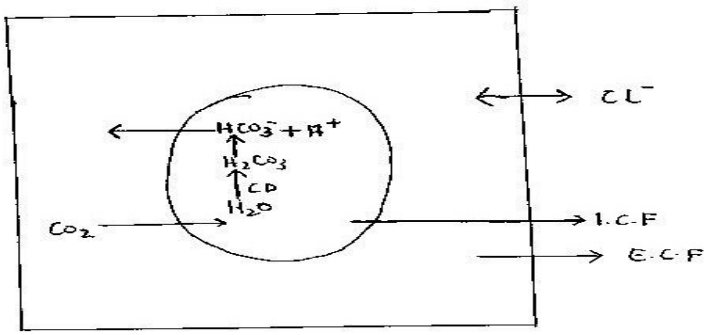
(CD=carbonate dehydratase)

Nb/ (1) the kidney plays an important role in maintaining the circulating HCO_3^-

And eliminating the circulating H+ from circulation

(2) The CD (CARBONATE DEHYDRATASE)is present in high concentration in rbcs and renal tubular cells therefore the organs concerned with acid/base balance are rbcs and tubular cells. At the same time not only do rbcs have high concentration of CD BUT it also have the means of removing one of the products ie hydrogen ions

BICARBONATE GENERATION BY RBCS



WHAT IS ACID/BASE BALANCE?

In physiology, a component is said to be in balance if the rates of outputs and inputs are equal for a given time interval.

-A positive balance indicates that there is a net gain the component over a given period of time whereas a negative balance indicates there is a net loss of the component over a given period of time. Evaluation of a net balance takes into account all inputs (intakes plus production) and all outputs (excretion plus metabolic conversion) over a period of given time interval.

- Therefore acid base balance involves CO_2 balance as well as non-carbonic acid and base and the effect this has on the acid/base status of titratable acids or base and other acid/base variables.

- The PH of plasma is a function of two independent variables. ie The PCO_2

Which is regulated by respiratory mechanisms and the concentration of titratable base, which is regulated by the renal mechanism. Plasma bicarbonate is always taken as a measure of the base in plasma and ECF. The hydrogen ion balance is closely associated with

the balance of other electrolytes because hydrogen ion cannot be introduced into the ECF without concomitent anion eg CL or in exchange for a cation (Na +)

THE RELATIONSHIP BETWEEN PH, HCO_3^- AND H_2CO_3

This relationship is explained by the HENDERSON-HASSELBALCH equation.

The equation is derived starting with the following equilibrium

$H_2CO_3 \cdots\cdots H^+ + HCO_3^-$

Let K be the equilibrium constant

$$K = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

Make [H+] the subject

$$[H^+] = K \times \frac{[H_2CO_3]}{[HCO_3^-]}$$

Take the log of both sides

$$\text{Log}[H^+] = \text{Log } K + \text{Log}\frac{[H_2CO_3]}{[HCO_3^-]}$$

Multiply both sides by -1

$$-\text{Log}[H^+] = -\text{Log } K - \text{Log}[H_2CO_3]/[HCO_3^-]$$

By definition $-\text{Log}[H+] = PH$ and $-\text{Log } K = pK$ therefore

$$pH = pK - [\text{Log}[H_2CO_3] - \log[HCO_3^-]$$

$$pH = pK - \log[H_2CO_3] + \log[HCO_3^-]$$

$$pH = pK + \text{Log}\,[HCO_3^-]/[H_2CO_3]$$

Nb/ The Pk of H_2CO_3 at 37°C is 6.1 thus 6.1 + Log + Log $[\underline{HCO_3^-}]/ [H_2CO_3]$

Nb/the H_2CO_3 includes the actual H_2CO_3 which is present along with dissolved CO_2 .

-Normally the HCO_{3-} in blood is approximately 26 mmol/l while the $[H_2CO_3]$ is approximately 1.3 mmol/l. . The blood PH can be calculated by inserting these values into the equation

$$pH = 6.1 + \text{Log } \frac{26}{1.3}$$

pH = 6.1 + Log 20

pH = 6.1 + 1.3= 7.4 (normal blood pH)

ACID/ BASE DISORDERS

-Many pathological conditions are accompanied by or caused by disturbances of acid-base balance and electrolyte composition of the body. These changes are usually reflected in the acid /base and anion/ cation patterns of the ECF.

- Since blood is the only sample of ECF that can be obtained, acid/ base status is evaluated from measurement of blood pH , PCO_2 and HCO_{3-} or [TCO_2] . It is important to note that the results obtained in blood or plasma may not always be the reflection of the acid/ base status of ICF.

- Acid / base balances disorders can be classified into four groups ie METABOLIC ACIDOSIS and ALKALOSIS and RESPIRATORY ACIDOSIS and ALKALOSIS.

METABOLIC ACIDOSIS(PRIMARY BICARBONATE DEFICIT)

- Caused by -:

(a) production of organic acids that exceeds the rate of elimination

(b) reduced excretion of acids in renal failure

(c) excessive loss of bicarbonates (base) due to excessive loss of duodenal fluids as in diarrhaea

In these conditions the ratio $[\frac{[HCO_3^-]}{[H_2CO_3]}$ is decreased because of the primary decrease in

Bicarbonates ([[HCO_3-]). In such situation, the body will try to compensate for the bicarbonate deficit inorder to restore the acid/ base balance ie to restore the pH between (7.35- 7.45)

$$6.1 + \text{Log} + \text{Log} \frac{[HCO_3^-]}{[H_2CO_3]}$$

Pathological conditions associated with metabolic acidosis are renal failure, ketoacidosis and salicylate intoxication.

Laboratory finding------plasma bicarbonate, total carbondioxide and partial pressure of carbondioxide and pH are all low.

METABOLIC ALKALOSIS (PRIMARY BICARBONATE EXCESS)

Caused by :-

(a) excess administration of alkali eg NaHCO3, Citrate (as in transfused blood) or some anti acids.

(b) excess lossof HCL from the stomach after prolonged vomiting and after gastric suction.

(c) renal bicarbonate retention

(d) prolonged administration of certain diuretics

(d) prolonged administration of laxatives.

- In these conditions, the ratio[$\frac{[HCO_3^-]}{[H_2CO_3]}$ is increased because of the primary increase

of bicarbonate.

Laboratory finding------plasma bicarbonate, dissolved carbon dioxide and partial pressure of carbondioxide , and pH are all high.

RESPIRATORY ACIDOSIS (PRIMARY DISSOLVED CARBONIC DIOXIDE EXCESS)

- Any condition that decreases elimination of carbondioxide through the lungs results in an increase of partial pressure of carbondioxide(hypercapnia) and respiratory acidosis. Increase in carbonic acid will results with the decrease in the ratio $\frac{[HCO_3^-]}{[H_2CO_3]}$

Cause :-

(a) any factor that directly depress the respiratory centre eg CNS trauma, infections or mechanical obstruction of the air way

(b) cardiac disease may cause respiratory acidosis.

Laboratory findings:- low pH, High [H_2CO_3]

RESPIRATORY ALKALOSIS (PRIMARY DISSOLVED CARBONIC DIOXIDE DEFICIT)

-A decrease in partial pressure of carbon dioxide (hypocapnia) caused by an increase in the rate of or depth of respiration or both. Exces elimination of carbon dioxide which causes an increase in [$\frac{HCO_3^-}{H_2CO_3}$] ratio and an increase in pH.

Causes :- hysteria, fever, CNS infections pneumonia , asthma , pulmonary embolism and CHF.

Lab findings- pH high, [$\frac{HCO_3^-}{H_2CO_3}$] is high,

LABORATORY INVESTIGATION

An **arterial blood gas** (**ABG**) is a blood test that is performed using blood from an artery. It involves puncturing an artery with a thin needle and syringe and drawing a small volume of blood. The most common puncture site is the radial artery at the wrist, but sometimes the femoral artery in the groin or other sites are used. The blood can also be drawn from an arterial catheter.

The test is used to determine the pH of the blood, the partial pressure of carbon dioxide and oxygen, and the bicarbonate level. Many blood gas analyzers will also report concentrations of lactate, hemoglobin, several electrolytes, oxyhemoglobin, carboxyhemoglobin and methemoglobin. ABG testing is mainly used in pulmonology, to determine gas exchange levels in the blood related to lung function, but has a variety of applications in other areas of medicine.

TEST PRINCIPLES/CLINICAL SIGNIFICANCE

Blood gas analysis is performed to assess the acid-base balance and evaluate the respiratory

oxygenation status of the patient.

pH, pCO_2, and pO_2 are measured directly from the specimen by using electrodes. The pH and pCO_2 electrodes are potentiometric where the voltage produced across a semi-permeable membrane to hydrogen ions or CO_2 gas is proportional to concentration. pO_2 is measured similarly, but using an amperometric (current producing) electrode.

The bicarbonate ($cHCO_3^-$ $(P)_c$) , total CO_2 ($ctCO_2(P)_c$) and arterial base excess (ABE_c) are calculated according to the following formulae:

H_2CO_3 (carbonic acid) = $pCO_2 \times 0.0301$
HCO_3^- (bicarbonate) = inv. log (pH - pK_a) × H_2CO_3
tCO_2 (total CO_2) = H_2CO_3 + HCO_3^-

SPECIMEN TYPE

Heparinized Arterial blood is the specimen of choice. Capillary blood collected in heparinized tubes can also be used especially when dealing with neonates. Specimens must be placed on wet ice analyzed within 10 minutes of collection if held at room temperature or within one hour if kept at 4-8°C.

REFERENCE INTERVALS QOUTED IN LITERATURE

The following ranges are used for interpretation of results:

	Arterial		
pH	7.35-7.45		
pCO_2	35-45 mmHg		
pO_2	68-85 mmHg		
HCO_3^-	22-26 mmol/L		
tCO_2	23-27 mmol/L		
sO_2	92-95 %		

ANION GAP

-This is a calculation used to detect altered concentration of ions other than (sodium, potassium, chloride and bicarbonates.

- The law of electro neutrality demands that the number positive change (sodium, potassium calcium, magnesium and traces of other cations) in plasma exactly equal the number of negative change (chloride, bicarbonate, sulphate, phosphates , protein anions organic acids and traces of other anions).

-Sodium and potassium ion provide over 90% of plasma cations concentrationin a normal subject. The balance includes low concentrations of calcium and magnesium which vary very

little even in disease. Over 80% of plasma anions is accounted for by chloride and bicarbonates. The remaining 20% or so is referred to as unmeasured anions (made up of proteins, urates, phosphates, sulphates and other organic anions)

Therefore the term anion gap refers to the differences in charge concentration between unmeasured anions (UA-) and unmeasured cations (UC+). The anion gap can be calculated using the follwing formula where "A" represent the anion gap

"A"= ($Na^+ + K^+$) – ($CL^- + HCO_3^-$]

($Na^+ + K^+$) = "A" + ($CL^- + HCO_3^-$]

REFERENCE RANGE IN LITERATURE OF ANION GAP (15-20) mmol/L

BONE METABOLISM, DISORDERS AND LABORATORY INVESTGATIONS

CALCIUM (CAL) METABOLISM AND RELATED DISORDERS

Calcium is a mineral that helps build strong bones and teeth. Calcium and phosphorus in the mineral phase of the hard tissue i.e. bone and teeth co-exist in a relatively fixed proportions. The three main regulators of calcium and phosphorus homeostasis are parathyroid hormone,vitamin D and calcitonin each exert independent effects on both ions. Intracellularly, calcium is the prime inorganic messenger for regulation of cell functions. Calcium is needed throughout an individual's life and the amount required changes over time. A lot of calcium is required during childhood years to build strong bone, a bit less during the middle years to keep bones strong, and much later in life to prevent bone loss.

Pathological conditions associated with calcium levels in blood are hypercalcaemia and hypocalcaemia. These are determined in relation to the reference ranges of calcium in a particular population.

Hypercalcemia is a condition of elevated levels of calcium in plasma and it is a common metabolic abnormality frequently related to primary hyperparathyroidism and cancer. Hypercalcaemia affects 8 in 100,000 individuals and it common between the ages 40-50 years. In relation to gender, females are more affected than males. Studies have identified hypercalcaemia as a

complication in critically ill patient with adrenal insufficiency. Hypercalcemia has been reported to occur not only with primary hypoadrenalism but also with secondary hypoadrenalism .

Hypocalcaemia is a condition of low calcium levels in plasma. It manifests as a symptom of. Hypocalcemia is a common condition characterized by a net loss of calcium from extracellular fluid in greater quantities than can be replaced by the intestine or bone. Hypocalcemia is caused by a number of clinical entities eg a parathyroid hormone [PTH] deficiency/malfunction, a Vitamin D deficiency, or unusually high magnesium levels hypermagnesaemia, or low magnesium levels hypomagnesaemiaand often presents with signs of generalized neuromuscular irritability including paresthesia, muscle cramps, laryngospasm, tetany, and seizures. Osteomalacia (adults) and rickets (children) are disease conditions encountered in hypocalcaemia and similar to hypophosphotaemia.

PHOSPHORUS (PHOS) METABOLISM AND RELATED DISORDERS

Phosphorus is also an abundant element in the body. At least 85% is found in the bone whilst the other 15% is found mostly combined with lipids proteins, carbohydrates and other organic substances. Most phosphorus in the extracellular fluid is inorganic. Major organs involved in phosphorus homeostasis are small intestines, kidneys and the skeleton, which acts as a storage reservoir. Phosphorus is present virtually in all foods. Consequently dietary deficiencies do not occur. Dietary intake is between 800-1000mg for adults and is mostly from milk and other dairy product.

Pathological conditions related to phosphorus are hypophosphotaemia (reduced phosphorus levels) and hyperphosphotaemia (elevated phosphorus levels). Hypophosphotaemia is a common condition is critically ill patient and its aetiology multifactorial. Phosphate plays an important role in the glycolysis and the production of high energy compounds such as Adenosine tri-phosphate (ATP). Evidence of its depletion in critically ill patients may manifest itself as impairment in respiratory, cardiovascular, neuromuscular and haematological function.

Osteomalacia (adults) and rickets (children) are caused by an inadequate supply of vitamin D leading to inadequate mineralisation of bone matrix. Vitamin D deficiency is most often caused by nutritional deficiency but can be secondary to a wide range of other underlying causes, such as disorders of the gut, pancreas, liver and kidney. Normal bone mineralisation depends on adequate calcium and phosphate and this is maintained by vitamin D. Vitamin D is supplied either in the diet or produced from a precursor in the skin following exposure to ultraviolet light. 90% of our daily vitamin D requirement is usually obtained by the action of sunlight on the skin and 10% is obtained from the diet .

HORMONES THAT REGULATE CALCIUM AND PHOPHORUS CONCENTRATION IN THE CIRCULATION

The three primary regulators of calcium and phosphorus are parathyroid hormone (PTH), calcitonin(CT) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D

PARATHYROID HORMONE (PTH)

-Synthesized by the parathyroid glands located on the posteriorsurface of the thyroid gland.

-Its primary role is the regulation of ECF calcium concentration. Synthesis and secretion of PTH is under precise feedback regulation by the concentration of ionized calcium in the circulation.PTH synthesis and secretion are stimulated within a few minutes by low concentration of calcium whilst high concentration of calcium suppresses secretion.

-although PTH affects the concentration of phosphorus in the circulation, high or low levels of phosphorus does not have a direct effect on the secretion of PTH.

-It stimulates osteoclasts to mobilize calcium from bones

- it increases renal tubular reabsorption of calcium

It inhibits proximal renal tubular reabsorption of phophorusit promotes bone formation in certain circumstances after PTH binds to membrane receptors of target organs

THE NET EFFECTS OF PTH ARE INCREASES IN PLASMA CALCIUM AND A DECREASE IN PHOSPHORUS CONCENTRATION.

CALCITONIN

-Produced by C cells of the thyroid gland.

- it role is to decrease resorption of calcium and phosphorus from bone by inhibiting the action of osteoclasts leading to lower plasma levels of calcium and phosphorus.

- CT has its greatest physiological effect in the fetus and young children, probablybecause bone calcium turn over is very rapid at this stage of development.

-plasma CT levels are are higher in neonate and in pregnant and lactating womenthan in normal adults. CT may have a function in promoting deposition of calcium in the skeleton of the human fetus and may be a causative factor in neonatal hypocalcemia.

VITAMIN D AND ITS METABOLITES

There are two families: VIT D_3 (cholecalciferol)—is the natural form produced by the skin from the action of sunlight. VIT D_2 (Ergocalciferol)—manufactured commercially from precursors of plant origin.

1,25-$(OH)_2$D(vit D) has the following biological actions:

1. **Stimulates calcium absorption by the small intestine**
2. **Stimulates phosphorus absorption by the small intestine**
3. **Stimulates calcium resorption from bones, in this action,PTH acts synergistically**

(4) It increases calcium reabsorption by the distal renal tubules.

In summary, the net effect of 1,25-$(OH)_2$D, is an increase in plasma calcium and phosphate concentrations.

REGULATION OF CALCIUM AND PHOPHORUS METABOLISM

-The concentration of calcium and phosphorus in plasma are determined by the balance between absorption and excretion in the kidneys, the intestine and to a small extent the skin, and by exchanges between plasma, ECF and tissues including bone.

- These dynamic processes are determined by many factors , principally parathyroid hormone (PTH),calcitonin (CT) and vitamin D. The actions of these three hormones results in a remarkably precise control of the plasma calcium concentration in healthy individuals.

-In the case of plasma phosphorus concentration regulation, PTH exerts a particularly important effect by inhibiting phosphate reabsorption by the kidney tubules. Therefore PTH lowers the plasma phosphorus concentration.

CALCIUM, PHOSPHORUS RELATED DISODERS

Rickets (AND Osteomalacia) ----- is a softening of bones in children due to deficiency or impaired metabolism of vitamin D,[1] phosphorus or calcium,[2] potentially leading to fractures and deformity. Rickets is among the most frequent childhood diseases in many developing countries. The predominant cause is a vitamin D deficiency, but lack of adequate calcium in the diet may also lead to rickets (cases of severe diarrhea and vomiting may be the cause of the deficiency). Although it can occur in adults, the majority of cases occur in children suffering from severe malnutrition, usually resulting from famine or starvation during the early stages of childhood.Osteomalacia is a similar condition occurring in adults, generally due to a deficiency of vitamin D.

(1)OSTEOPOROSIS----describes a group of diseases of diverse causes that are characterized by the reduction of bone mass that is required for normal mechanical function even though the ration of mineral to the organic matter is normal.

-It can be secondary to well defined condations such as cushings disease, glucorticoid administration and chronic immobilization. Most often it is primary and not associated to any definite disease.

-Idiopathic osteoporosis is the most common skeletal abnormality in post menopause women and it is frequently seen in aged individuals.

LAB FINDINGS----- Normal calcium and phosphorus. Raised calcium when the pt is immobilized and raised ALP in a pt that has a fractured bone.

DISORDERS OF CALCUIM AND PHOSPHORUS

-The disorders are hypercalcaemia, hyperphosphataemia, hypocalcaemia ,and hypophosphotaemia.

-Hypercalcaemia is associated with hyperparathyroidism. Most patients with primary hyperparathyroidism are either asymptomatic or have fatigue, weakness, anorexia, nausea and vomitingwhich are present if hypercalcaemia is protracted and severe.

-HYPERCALCAEMIA AND MALIGNANCY

High levels of calcium occurs in 10-20 %of patients with malignant diseases. Malignant associated with hypercalcaemia are classified in three categories ie either SOLID, TUMORS (secretes a humorol factor with PTH like activity), SOLID NON SECRETING TUMORS withskeletal metastasis, HAEMATOLOGIC MALIGANCY(multiple myeloma, lymphoma and leukemia are most common neoplasia.

DISEASES ASSOCIATED WITH HYPOCALCAEMIA.

-Hypoparathyroidism---due to deficient secretion of PTH. Causes could be due to advertent removal or damage during thyriodectomy or an autoimmune destruction of the glands.

- low calcium could be due to vit d deficiency.

-Heriditary vit d dependency rickets type 1 and 2.

-Intestinal malabsorption could lead to rickets(children) or oesteomalacia(adults)

-HYPERPHOSPHATAEMIA.

-RENAL OSTEODYSTROPHY—This refers to skeletal lesions that occur in CRF. Rickets, osteomalacia, hyperparathyroidism, osteosceleria, and osteoporosis are found in varying degrees.

-In CRF the ability of the kidney to excrete inorganic phosphorus is greatly impaired and hyperphosphataemia results.

HYPOPHOSPHATAEMIA

-Rickets and osteomalacia may occur in chronic hypophosphataemia..

BONE DISEASE WITH NORMAL CALCIUM AND INORGANIC PHOSPHORUS.

OSTEOPOROSIS===This is a term used to describe a group of diseases of diverse causes that are characterized by reduction of bone mass that is required for normal mechanical function . Osteoporosis could be secondary to conditions such as Cushings disease , glucocorticiod administration and chronic immobilization.. THIS CONDITION IS VERY COMMON IN OLD AGE..

PAGETS DISEASE OF BONE

-This is a non metabolic bone disease characterized by excessive bone resorption and repair , increased turn over of bone minerals and matrix and formation of abnormal bones .Evidence of viral origin has recently been presented..

LABORATORY ANALYTICAL METHODS

CALCIUM (CAL)

Calcium has two methods of analysis namely (i) o-Cresolphthalein (oCPC) and (ii) Arsenazo III (2,2'-[1,8-Dihydroxy-3,6- disulphonaphthylene-2,7-bisazo] bisbenzenearsonic acid) which are used widely in diagnostic laboratory.

(i) o - Cresolphthalein method

This is an end point reaction for calcium estimation. Calcium ions reacted with o-CPC-complexone in an alkaline medium in the presence of hydroxyquinoline to form a purple coloured complexone which was directly proportional to the calcium concentration in the sample. 3µl of sample is reacted with 100 µl of reagent and the change in absorbance is monitored at 575nm. This change is directly proportional to the concentration of Calcium in the sample and is used to calculate and express concentration in mmol/L. The reaction takes place at 37°C.

Ca^{2+} + o-CPC $\xrightarrow{\text{8-Hydroxyquinoline}}$ Ca- o-CPC complex^{2+} (purple)

(ii) Arseazo III method

This is an end point reaction for calcium estimation. Calcium ions reacted with Arseazo III in an acidic medium to form a purple coloured complex which is directly proportional to calcium concentration in the sample. 1µl of sample was reacted with 100 µl of reagent and the change in absorbance was monitored at 650nm. This change is directly proportional to the concentration of Calcium in the sample and is used to calculate and express concentration in mmol/L. The reaction takes place at 37°C.

Ca^{2+} + Arseazo III $\xrightarrow{\text{pH 6.5}}$ Ca^{2+} - Arseazo III complex (purple)

SPECIMENS OF CHOICE ; Heparinized plasma, serum

Nb/EDTA *(ethylenediamine tetra-acetic acid)* plasma should not be used since the anti coagulant (EDTA) chelating affect of binding calcium will clear calcium from the solution therefore no reaction will take place.

Reference range in literature (2.02-2.6 mmol/L)

PHOSPHORUS (PHOS)

Phosphorus reagent is used to measure the phosphorus concentration by a timed end point method. In the reaction, inorganic phosphorus reacts with ammonium molybdate in an acidic solution to form a coloured phosphomolybdate complex. 4µl of sample is reacted with 268 µl of reagent and the change in absorbance is monitored at 340nm. This change is directly proportional to the concentration of PHOS in the sample and is used to calculate and express concentration in mmol/L. The reaction takes place at 37°C.

Principle of the reaction

Phosphorus + molybdate $\xrightarrow{H_2SO_4}$ phosphomolybdate-complex

SPECIMENS OF CHOICE ; Heparinized plasma, serum

Reference range in literature (1.03-2.3 mmol/L)

MAGNESIUM (MG) METABOLISM AND RELATED DISORDERS

Magnesium is one of the major intracellular cation. It is an essential factor in many important enzymatic reactions, either as an integral part of a metal-coenzyme or as an activator. The best defined manifestation of magnesium deficiency is impairment of neuromuscular function e.g., hyperirritability, tetany, convulsions and electrocardiographic changes. Conditions associated with hypomagnesaemia (reduced magnesium blood levels) include chronic alcoholism, childhood malnutrition, lactation, malabsorption, acute pancreatitis, hypoparathyroidism, aldosteronism and prolonged intravenous feeding (Lu and Nightingale, 2000). Increased levels of magnesium (hypermagnesaemia) have been observed in dehydration, severe diabetic acidosis and Addison's disease. Conditions that interfere with glomerular filtration .e.g., renal failure, result in retention of magnesium and hence elevation of plasma levels.

MAGNESIUM ANALYSIS

This is an end point reaction method for the determination of MG. In the reaction, MG formed a coloured complex with xylidyl blue in a strong alkaline medium. 2µl of sample is reacted with 200 µl of reagent and the change in absorbance is monitored at 546nm. This change is directly proportional to the concentration of MG in the sample and is used to calculate and express concentration in mmol/L. The reaction takes place at 37°C. Purple colour produced is directly proportional to the concentration of MG in the sample.

$$MG^{2+} + \text{Xylidyl blue} \xrightarrow{pH=11.4} \text{Purple colour}$$

SPECIMENS OF CHOICE ; Heparinized plasma, serum

Reference range in literature (0.9-1.1 mmol/L

CSF

- **Total volume in adults=150 mls and in neonates=10-60mls.**
- **Normal colour is clear with viscosity comparable to water.**
- **Abnormal csf may appear cloudy, smoky,hazy, turbid or grossly haemolysed**
- **It is considered the most precious specimen in the laboratory due to the process one undergoes to remove it.The process is not comfortable at all. It**

is Obtained from the puncture of the lumber region. Lumber puncture are done by the physicians.

- it is requested where there is suspicion of a cerebro-vascular accident, meningitis, demyelinating dse, or menengial involvement in malignant dse.
- The csf specimen is collected in fluoride bottles for the analysis of micro protein and glucose.nb/the raise in microprotein and fall in glucose levels signifies that there is bacterial infection.

STOOL CHEMISTRY

Stool is the waste product of digestion excreted by the bowels. Composition.

Smell: Has fowl smell

Color: Depend on diet, age disease and drugs.

Collection: Patient should be warned against contaminating the stool specimen with urine since among other things urine has a negative effect on protozoa.Random specimen in polypots with screw caps since these ones are odor free, leak proof and easy to transport

QUANTITY: Normally 100 to 200 gms of stool is passed per day.

72 HOURS STOOL SPECIMEN

72 hrs stool specimen is collected for the estimation of faecal fat in patient suspected to suffer from malabsorption of fat at the small intestines.No preservatives added

Storage: $4^{o}C \leq 24$ hrs

(a) Substances of chemical importance include.

- Muscle fibre indicate pancreatic insufficiency
- Starch granules indicate starch indigestion.
- Cellulose structure not indicative
- Crystals

(b) Charcot lyden crystals indicate parasitic infection.

Blood in Stool

. For occult blood test, the patient is instructed to abstain from meat diet for three consecutive days prior to the day of stool collection.Note that there is no preservative added.

It can be visible or invisible when it's present but invisible it's referred to as occult blood in stool. It can be caused by parasitic infection, ulcers, cancers etc. It can be detected by:

(a) Microscopic method

(b) Chemical method

The chemical methods:

(i) O – tolidine (ortho-otolidine)

(ii) Haematest

(iii) Ocultest

(iv) Phenolpthalein method – Not carcinogenic.

(v) Guiac test – Not carcinogenic

The principles of all these are similar. Blood contain Hb and if the stool contain blood the Hb in the Rbc's breakdown the H_2O_2 incorparated in the reagent into H_2O and O_2. The H_2O_2 can also be broken down by Fe derivatives in the specimens or in the reagent.

$$H_2O_2(l) \xrightarrow[\text{Reduced}]{\text{Hb or Fe}} H_2O\ (l) + O_2\ (g)$$

The O_2 oxidizes the reduced chromogen system incorporated in the reagent forming a coloured compound. O_2 + reduced Chromogen system coloured compound.

The color produced depend on the chromogen system used.

O – tolidine (ortho-tolidine) + oxygen blue/green compound.

Haematest O-tolidine + O_2 blue/green compound.

Guiac test + O_2 blue colour

Phenolpthalein

Phenolpthalein is an indicator which is weakly acidic, colourless in acid media and red in alkaline solution. In the phenolphthalein test for the detection of occult blood in stool, phenolphthalein reagent is oxidized to form a red color.

Explanation of principle (phenolphthalein test)

During preparation, phenolphthalein is reduced to phenolpthalin which is colourless at alkaline pH (but phenolphthalein the indicator is red in alkaline pH). After the H_2O_2 is broken down into O_2 and H_2O by the Hb or any Fe derivatives, then the O_2 produced oxidzes phenolphthalin to phenolphthalein which at alkaline ph (due to the presence of NaOH) gives a red colour.

$$H_2O_2 \xrightarrow[\text{Fe derivatives}]{\text{Hb}} H_2O + O_2$$

$$O_2(g) + \text{Phenolpthalin (colourless)} \xrightarrow{\text{-OH}} \text{Phenolpthalein (red)}$$

ENDOCRINOLOGY

- **Endocrinology** is a branch of biology and medicine dealing with the endocrine system, its diseases, and its specific secretions called hormones,
- The endocrine system consists of several glands, all in different parts of the body, that secrete hormones directly into the blood rather than into a duct system.
- Hormones have many different functions and modes of action; one hormone may have several effects on different target organs, and, conversely, one target organ may be affected by more than one hormone.

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TYPES OF HORMONES

Amines eg Amines, such as norepinephrine, epinephrine, and dopamine. Thyroid hormones eg triiodothyronine (T3) and 3,5,3’,5’-tetraiodothyronine (thyroxine, T4).

Peptide and protein eg thyrotropin-releasing hormone), follicle-stimulating hormone), All hormones secreted by the pituitary gland are peptide hormones(TSH,PRL,FSH,LH,GH,ACTH,OXY,ADH and insulin from the pancreas.

Steroid eg glucocorticoids, mineralocorticoids, androgens (testosterone), estrogens, and progestogens and vitamin D

AN OVERVIEW OF HYPOTHALMUS ,PITUITARY AND TARGET GLAND HORMONAL PRODUCTION AND ACTION

- The pituitary gland is often portrayed as the "master gland" of the body. Such praise is justified in the sense that the anterior and posterior pituitary secrete a battery of hormones that collectively influence all cells and affect virtually all physiologic processes.

- *The pituitary gland may be king, but the power behind the throne is clearly the hypothalamus. - hypothalamus* secrete hormones that strictly control secretion of hormones from the anterior pituitary. The hypothalamic hormones are referred to as **releasing hormones** and **inhibiting hormones**, reflecting their influence on anterior pituitary hormones. Specific hypothalamic hormones bind to receptors on specific pituitary cells, modulating the release of the hormone they produce.

- As an example, thyroid-releasing hormone from the hypothalamus binds to receptors on anterior pituitary cells called thyrotrophs, stimulating them to secrete thyroid-stimulating hormone or TSH. The anterior pituitary hormones enter the systemic circulation and bind to their receptors on other target organs. In the case of TSH, the target organ is the thyroid gland.
- *Clearly, robust control systems must be in place to prevent over or under-secretion of hypothalamic and pituitary hormones.* A prominent mechanism for control of the releasing and inhibiting hormones is negative and positive feedback mechanisms.

The following table summarizes the major hormones synthesized and secreted by the pituitary gland, along with summary statements about their major target organs and physiologic effects

Pituitary Gland Lobe	**Hormone**	**Major Target Organ**	**Physiologic Effects**
*Anterior Pituitary **(adenohypophysis)***	Growth hormone	Liver, adipose tissue	Promotes growth (indirectly), control of protein, lipid and carbohydrate metabolism
	Thyroid-stimulating hormone	Thyroid gland	Stimulates secretion of thyroid hormones
	Adrenocorticotropic hormone	Adrenal gland (cortex section of the kidney)	Stimulates secretion of glucocorticoids
	Prolactin	Mammary gland	Milk production
	Luteinizing hormone	Ovary and testis	Control of reproductive function
	Follicle-stimulating hormone		
Posterior	Antidiuretic hormone	kidney	Conservation of body water

Pituitary ***(neurohypophysis)***	Oxytocin	Breast and uterus	Stimulates milk ejection and uterine contractions

+PITUITARY GLAND

-Anatomocally the gland is divided into two lobes, ***anterior (adenohypophysis)*** **and** ***posterior (neurohypophysis).***

ANTERIOR PITUITARY (ADENOHYPOPHYSIS); This pituitary lobe secretes (produces) at least 6 types of hormones, all of which are proteins or peptides. These hormones are: growth hormone (GH), prolactin(PRL), thyroid stimulating hormone (TSH), adenocorticotropic hormone (ACTH), follicle stimulating hormone (FSH) and luteinizing hormone (LH).

- The activity of the anterior pituitary is under the control of the **hypothalamus**, which synthesizes small peptides(neuropeptides) known as **releasing** or **inhibiting hormones or factors**.These hypothalamic factors are:- **corticotrophin-releasing hormone(CRH), thyrotropin-releasing hormone(TRH), growth hormone-releasing hormone (GRH), somatostatin(also called growth hormone- inhibiting hormone,GHIH), gonadotropin-releasing hormone(GnRH), GnRHis sometimes reffered to as LHRH (luteinizing hormone releasing hormone.** Therapeutic use of releasing hormones may be indicated in conditions where insufficient secretion of anterior pituitary hormones is due to hypothalamic dysfunction.

(1) GROWTH HORMONE AND SOMATOMEDINS

PHYSIOLOGICAL ACTION:- The overall action of GH is to promote growth of cartilage, bone and many soft tissues by stimulating protein synthesisMost GH effects are delayed rather than immediate.

LAB INVESTIGATION

BY analysing GH in blood specimen using either immunoassays or enzyme linked fluorescence assay (ELFA).

.NB/---LH,FSH,TSH,PRL,ACTH-----reffer to appropriate section

POSTERIOR PITUITARY (NEUROHYPOPHYSIS)- The two hormones secreted by this part of the pituitary are the ***ADH(VASOPRESSIN or ANTIDIURETIC HORMONE), and OXYTOCIN***

(1)ADH (ANTIDIURETIC HORMONE)

- **The prinicipal physiological action of ADH is stimulation of water reabsorption from the renal tubular fluid**.

CLINICAL SIGNIFICANCE OF ADH

-**POLYURIC STATES**---deficient production or action of ADH results in polyuria due to failure of the renal tubule to reabsorbed solute –free-water. POLYURIC STATES as seen in diabetes mellitus and diabetes insipindus.

(2) OXYTOCIN

- oxytocin is present in both male and female but its physiolocal effects are only in females.
- -The primary stimulus for oxytocin release is suckling. Oxytocin also stimulates lactation by the mammary gland.
- Oxytocin stimulates contraction of the estrogen – primed uterus during delivery. It is useful therapeutic agent in inducing labor.

REPRODUCTION AND HORMONES (GONADAL HORMONES)

-PUBERTY

-During childhood, circulatory levels of the sex steroids and gonadotropins are low and similar in both sexes.

- In girls, precocious puberty is defined as the onset of pubertal development before the age 8.5 years and puberty is said to be delayed if there has been no development by the the age 12-14 years and if menarche (first menstrual cycle) has not occurred by age 16.5 years.

-In boys, puberty is considered precocious if it occurs before the age of 10 years and delayed when the onset is later than age 16 years. Nb precocious puberty is more common in girls.

MALE REPRODUCTIVE SYSTEM AND HORMONES

- Sperm cell development starts at about the age of 11 years.
- This is influenced by the hormone testosterone. By the time the boy is 15 years, sufficient levels of testosterone exist so that the testis become capable of full spermatogenesis. Before the age of 15 years most of the sperm cells produced in the testis are incapable of fertilization.
- The production of fully mature sperm (spermatozoa) is triggered by FSH. LH influences the secretion of testosterone by the leyding cells of the testis.

MALE HORMONAL REPRODUCTIVE DISORDERS=(INFERTILITY)

(1) IMPOTENCE -This is the persistent inability to develop or maintain a penile erection sufficient for intercourse and ejaculation.

- Causes of impotence are-:

-low levels of testosterone.

- hyperprolactinemia.

-a number of systemic diseases that affect the pituitary/ gonadal axis eg CRF, Chronic liver cirrhosis and diabetes mellitus.

-severe malnutrition, stress,

- alcohol may have a direct effect on the testis impairing spermatogenesis.

- Low and elevated levels of the following hormones can cause impotence eg cortisol, Thyroid hormones, and low levels of LH and FSH.

(2) GYNECOMASTIA -This is the growth of glandular breast tissue in male.

- gynecomastia may develop due to excessive use of alcohol, marijuana or certain medication.

- gynecomastia may be associated with hyper- and hypo thyroidism.

-Tumors that produce chorionic gonadotropin, estradiol producing tumors of the adrenals and testis and prolactin-producing pituitary adenomas all may cause gynecomastia.

- The presence of glandular breast tissue in pubertal males is quite common and almost always resolves sportenously,

FEMALE REPRODUCTIVE SYSTEM AND HORMONES

THE MENSTRUAL CYCLE(MC)

Each month or so the inner wall of the uterus prepares for a possible pregnancy. Incase a pregnancy does not occur (as in the case throughout most months of a woman's fertile years),the linning of the uterus must be released and a new one prepared. The breakdown of the endometrial wall and the resultant discharge of blood and endometrial tissue are known as **menstruation (or menses)**

-The Cyclic timing of menstruation is governed by the hormones released from the pituitary and the ovaries.

- Girls generally have their first menstrual cycle, the onset of which is called menarche sometimes between 12-14 years of age. Body weight, nutrition, heredity, and overall health are factors related to menarche.

- This cycle activity continuous until about age 45-55 years.

- The normal MC lasts for 28 days but very few women perfects 28- day cycle. Most women fluctuates by a few days to a week around this 28 day pattern and some are extremely from this cycle.

- Day 1 of the cycle starts with the first day of bleeding or menstrual flow.

- The normal cycle occurs in three phases i.e. Follicular, ovulation and luteul

FOLLICULAR PHASE----This is the phase after the menstrual flow and takes around 2 weeks up to the period of ovulation. During this phase the hormonal status are that Estradiol and progesterone are low, FSH and LH are elevated .

OVULATORY PHASE----This is the phase that comes at the mid cycle and last for 48 hours. Ovulation takes place in this phase and the released ovum travels in the fallopian tube for 48 hours awaiting fertilization. The hormonal status is that there is a rise in estradiol, LH and FSH. Progesterone is low.

-LUTEUL PHASE----This is the last half of the MC. It comes between the ovulation periods up to the time of bleeding. The hormonal status is that there is increasing production of progesterone and estradiol and low production of LH and FSH.

HORMONAL STATUS DURING MC

(a) Putuitary hormone levels (LH and FSH) AND (b) Ovarian hormones (progesterone and estradiol)

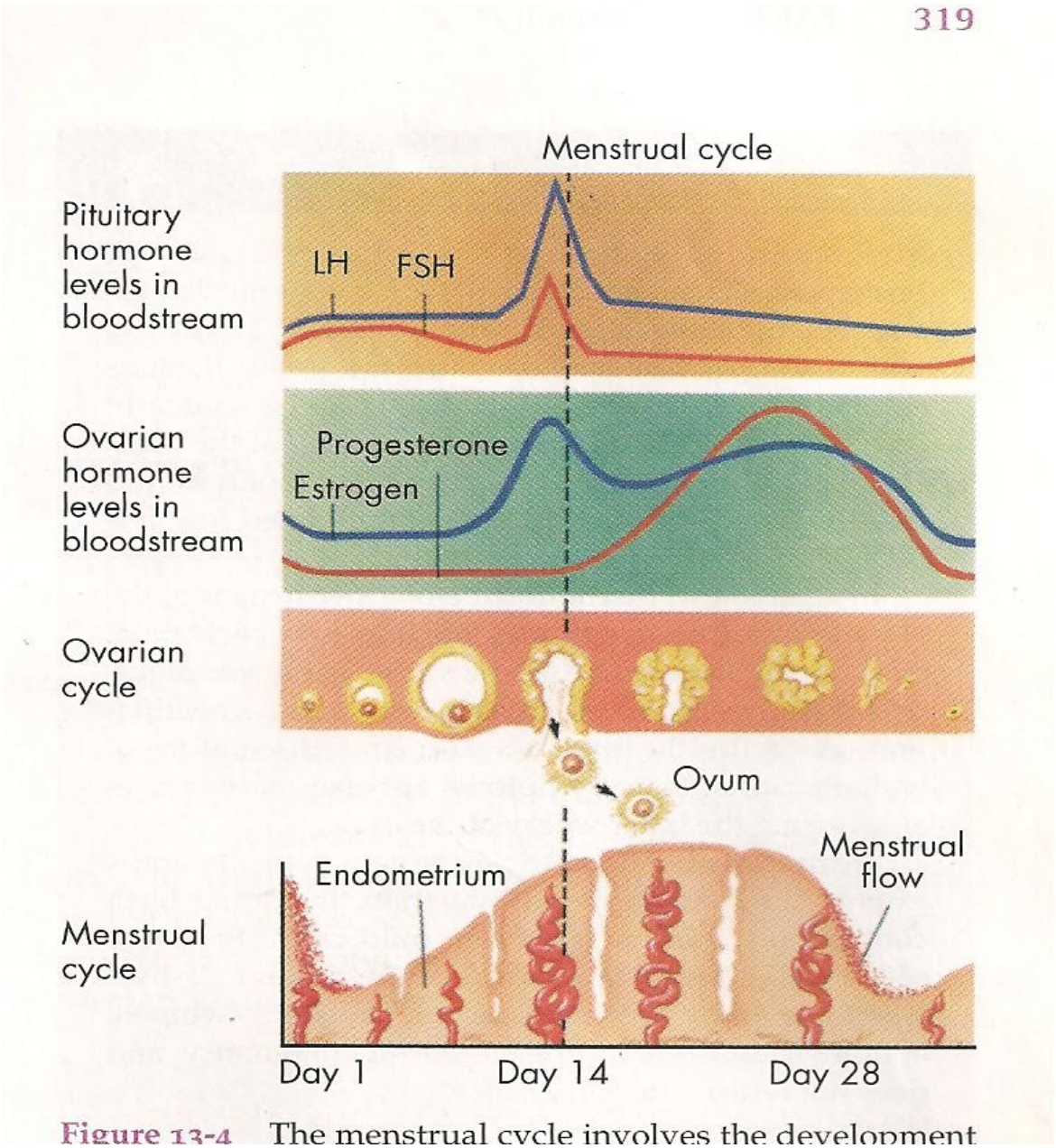


Figure 13-4 The menstrual cycle involves the development

FEMALE HORMONAL REPRODUCTIVE DISORDERS=(INFERTILITY)

(1)-IRREGULAR MENSES

-In a normal ovulatory menstrual cycle, bleeding usually occurs 14 to 15 days after the mid cycle. In infertile women, luteal phase may be shortened and menses occurs 5-10 days after mid cycle peak. The cycle may be normal but no ovulation occurs ie anovulatory.

(2)AMENORRHEA

- This is of two types ie primary and secondary.
- **Primary amenorrhea** is a disorder whereby no menses has begun by the age of 16.5 years or the patient has never menstruated and this is encountered in delayed puberty.
- The cause of this are endocrine disturbances such as hirsutism, virilism (testicular ferminization)
- **Secondary amenorrhea** is a disorder which occurs when previously established MC has stopped. This could be due to physiological factors eg pregnancy and menopause which are not abnormal, or stress and some contraceptive medication.
- Other causes are severe illness, excessive and rapid weight loss and obesity.

-Hyperprolactinemia is an important cause of secondary amenorrhea. High levels of prolactin inhibit normal release of GnRH and this affects the fertility hormone s release directly.

(2)MENOPAUSE

-This is a condition whereby the ovulation and menstrual cycle stops.

- This occurs in women at the age of 45-55 years and above. If it occurs at the age less than 40 years it is regarded as immature.

- Menopause is a normal physiological process and not a disease condition.

- Other signs associated with menopause are:- hot flashes (temporary feeling of warmth caused by blood vessel dilation), vaginal wall dryness and breast changes.

- Ovarian function and hormonal production diminishes.

(3) HIRSUTISM

- This is excess of facial and body hair in a woman.

- Could be caused by:- excess testosterone production which could be due to some medication or from a neoplasm in the ovaries or the adrenal glands.

(4) VIRILISM (TESTICULAR FEMINIZATION)

-This is the enlargement of the clitoris that resembles a penis (clitomegaly).

- Caused by excessive testosterone production.

- It is characterized by hair growth of male distribution, deepening of the voice and breast atrophy (no female breasts)

- Other causes are ovarian tumor, congenital adrenal hyperplasia.

5) PRE-MENSTRUAL SYNDROME(PMS)

- The cause of PMS appears to be hormonal .A woman's body is insensitive to a normal level of progesterone or her ovaries fail to produce a normal amount of progesterone. These reasons seems plausible because PMS types of symptoms do not occur during pregnancy , during which natural progesterone levels are very high , and because women with PMS seem to feel better after receiving high doses of natural progesterone in suppository form.

(6)FIBROCYSTIC BREAST CONDITION

This mostly affects those women who have never been pregnant . Stimulation of the breast tissues by estradiol and progesterone during mentraul cycle results in an unusually high degree of secretory activity by the cells linning the the ducts. This fluid finds its way into the fibrous connective tissue in the lower half of the breast , where in pocketlike cysts the fluid presses against neighbouring tissues. Excessive secretory activity produces in many women a fibrocystic breast condition characterized by swollen, firm or hardened, tender breast tissue before menstruation.

FERTILITY HORMONAL PROFILE

Fertility hormonal profile analysis plays an important role in the diagnosis and management of various pathological disorders that contribute to infertility in an individual. Infertility is defined as the absence of pregnancy following 12 months of unprotected intercourse.

To investigate primary and secondary infertility that affect both male and female the following diagnostic parameters are carried out: testosterone (TESTO), prolactin (PRL), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2 (EII) and progesterone (PRG)

TESTOSTERONE (TESTO)

In males, testosterone levels rise briefly in the 12th week of gestation and again shortly after birth. A sharp increase occurs at puberty, subsequently the levels remain relatively constant except for slight diurnal variation. The highest level occurs in the morning and gradually deceases to 25 % of the peak by evening. A

slow and progressive decline begins after the 5th decade. On average, an adult human **male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone. In females testosterone increases the libido which is expressed by clitoral engorgement. The levels are increased at puberty and mid-cycle where it plays a great role during ovulation (Davis and Tran, 2001).**

PROLACTIN (PRL),

Produced in the anterior pituitary by the pituitary mammotropic cells.***. It stimulates the mammary glands to produce milk (lactation). Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands of the breasts and prepare the production of milk***. ***Unusually high amounts are suspected to be responsible for impotence and loss of libido* .Hypersecretion of prolactin is more common than hyposecretion**. **Hyperprolactinemia is the most frequent abnormality of the anterior pituitary tumors. Clinical signs include inappropriate lactation, lack of menses, infertility in females, and impotence in males**.

FOLLICLE STIMULATING HORMONE (FSH)

Follicle-stimulating hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and Luteinizing hormone (LH) act synergistically in reproduction. FSH is a glycoprotein. Each monomeric unit is a protein molecule with a sugar attached to it; two of these make the full, functional protein. Its structure is similar to those of LH, TSH, and HCG (human chorionic gonadotropin) **(**Radu *et al*., 2010).

In males, FSH stimulates maturation of seminiferous tubules and spermatogenesis.

LUTEINIZING HORMONE (LH)

Luteinizing hormone is a hormone produced by the anterior pituitary gland. In both males and females, LH is essential for reproduction (Smellie, 2007). In females, an acute rise of LH called the LH surge triggers ovulation and development of the corpus luteum. In males, where LH had also been called interstitial cell-stimulating hormone (**ICSH**), it stimulates Leydig cell production of testosterone.

ESTRADIOL 2 (EII)

Estradiol is the predominant sex hormone present in females. It is also present in males, being produced as an active metabolic product of testosterone. It represents the major estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs including the bones. In the female, estradiol acts as a growth hormone for tissue of the reproductive organs, supporting the lining of the vagina, the cervical glands, the endometrium, and the lining of the fallopian tubes. The development of secondary sex characteristics in women is driven by estrogens, to be specific, estradiol. These changes are initiated at the time of puberty most enhanced during the reproductive years, and become less pronounced with declining estradiol support after the menopause. Thus, estradiol enhances breast development, and is responsible for changes in the body shape, affecting bones, joints, fat deposition.

PROGESTERONE (PRG)

Progesterone is sometimes called the "hormone of pregnancy", and it has many roles relating to the development of the fetus: Progesterone converts the endometrium to its secretory stage to prepare the uterus for implantation.

THYROID GLAND, HORMONES DISORDERS AND TESTS PROFILE

THYROID GLAND

The thyroid gland is a butterfly-shaped endocrine gland that is normally located in the lower front of the neck. It is one of the largest endocrine glands in the body. The gland makes thyroid hormone, which is secreted into the blood and then carried to every tissue in the body. Thyroid hormone is essential to help each cell in each tissue and organ to work right. For example, **thyroid hormone helps the body use energy, stay warm, and keep the brain, heart, muscles, and other organs working as they should**.

THYROID GLAND HORMONES

The major thyroid hormone secreted by the thyroid gland is thyroxine, also called T4 because it contains four iodine atoms. *To exert its effects, T4 is converted to triiodothyronine (T3) by the removal of an iodine atom. This occurs mainly in the liver and in certain tissues where T3 acts, such as in the brain.* The amount of T4 produced by the thyroid gland is controlled by another hormone, which is made in the pituitary gland located at the base of the brain, called thyroid stimulating hormone (abbreviated TSH) .

Thyroid glands disorders

Disorders of the thyroid gland fall into the following categories: hyperthyroidism, hypothyroidism, initial hyperthyroidism followed by hypothyroidism, cancers and non-cancerous nodules.

Hyperthyroidism

Hyperthyroidism, or overactive thyroid, is the overproduction of the thyroid hormones T_3 and T_4, and is most commonly caused by the development of Graves' disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones.

Hypothyroidism

Hypothyroidism is the underproduction of the thyroid hormones T_3 and T_4. Hypothyroid disorders may occur as a result of congenital thyroid abnormalities (see congenital hypothyroidism), autoimmune disorders such as Hashimoto's thyroiditis, iodine deficiency, especially in poorer countries, or the removal of the thyroid following surgery to treat severe hyperthyroidism.

Thyroid cancer

Cancers do occur in the thyroid gland and are more common in female occuring in the third, fourth and fifth decade of life. In most cases, the thyroid cancer presents as a painless mass in the neck. It is very unusual for the thyroid cancers to present with symptoms, unless it has been neglected.

Non-cancerous nodules

Many individuals may find the presence of small masses (nodules) in the neck. The majority of these thyroid nodules are benign (non cancerous). The presence of a thyroid nodule does not mean that one has thyroid disease. Most thyroid nodules do not cause any symptoms, and most are discovered on an incidental examination. Doctors usually perform a needle aspiration biopsy of the thyroid to determine the status of the nodules. If the nodule is found to be non-cancerous, no other treatment is required. If the nodule is suspicious then surgery is recommended

ADRENAL HORMONES AND FUNCTION TESTS

ADRENAL GLANDS- ***adrenal glands are situated in the kidney . It is divided into two parts ie adrenal cortex and adrenal medulla. Adrenal cortex is the site of synthesis and secretion of (A)adrenocortical steroids hormones and (B) adrenal medulla is the site of production of catecholamines (epinephrine and norepinephrine).***

(A)ADRENOCORTICAL STEROIDS

- The **corticosteroids** are physiologically and quatitatively the most important group of adrenal steroids. The major **corticosteroids are cortisol(most potent type of glucocorticoids) and aldosterone(most potent type of mineralocorticoid)**. Glucocorticoids tend to elevate blood glucose through promotion of gluconeogenesis and decrease carbohydrate utilization. The term mineralocorticoids refers to those steroids that regulate salt metabolism

REGULATION AND CORTISOL SECRETION

-There is diurnal variation in the secretion of cortisol and ACTH .In the early morning hours, blood levels of ACTH and cortisol start to rise, reaching a maximum between 6am and 8 am (6-8am), after which there is a gradual decline to the lowest level between early evening and mid-night (3pm-12 mid night). The levels at 8pm are normally 2/3 the levels at 8am.

The regularity of this rhythm is a function of sleep-wake habits and can be altered by consistent revision of the time and duration of the sleep-wake schedule. The normal regulatory system can be superseded by stimulatory factors such as trauma and stress, regardless the time of the day and independent of the plasma levels of cortisol. The response of normal individuals to stressful situation is a sharp rise in ACTH secretion and a consequent increase in cortisol secretion. In certain adrenal diseases (eg Cushings syndrome), the diurnal rhythm of cortisol secretion may be absent.

-Pathological conditions that leads to the measurement of cortisol levels are : ***GLUCOCORTICOID DEFICIENCY (ADDISON"S DISEASE)---***

CLINICAL SIGNIFICANCE OF RENIN AND ALDOSTERONE MEASUREMENTS

-The interaction of rennin, angiotensin and aldosterone is important in the regulation of extracellular fluid volume , blood pressure and the balance of sodium and potassium. Achange in one of these variables leads to changes in the others.

(1) ***HYPER ALDOSTERONISM***----this is overproduction of aldosterone which may be due to an adenoma of adrenal gland, or adrenal carcinoma

(2) ***HYPOALDOSTERONISM***—low production of aldosterone which cause hyponatramia and hypokalemia

LABORATORY INVESTIGATION

- BY analysing cortisol either in urine or in blood specimen using either immunoassays or enzyme linked fluorescence assay (ELFA).

(B) CATECHOLAMINES

-Catecholamines are benzene rings bearing two hydroxyl groups (catechol) and an alkylamine chain..The most important endogenously produced compounds of this group are:-

(1)epinephrine (adrenalin),

(2)norepinephrine (noradrenalin)

(3)dopamine (3,4-dihydroxyphenylethylamine).

- The main site of production of catecholamines are the brain, chromaffin cells of the adrenal medulla, and sympathetic neurons. Epinephrine is quantitatively the most important substance produced by the adrenal medulla. Norepinephrine is the major substance liberated by the postganglionic sympatheyic nerves..

- Each of these catecholamine hormones have a characteristic physiolocal functionand pharmacological actions:-

(1)Dopamine and norepinephrine are importantneurotransmitters in the central nervous system (the brain and the spinal cord). Both have a lot opf influence on the vascular system

(2) Epinephrine influences metabolic processes, especially carbohydrate metabolism

LABORATORY INVESTIGATION

Urine is used for the screening test of VMA.

ANALYTICAL METHODS FOR HORMONES

The current analytical technique employed in the analysis of all the hormones use the principle of immunochemical reaction based on an antiboby/antigen reaction. The set up of the reagent could either have the the antigen to the hormone being determined and this will react with the specific antibody (being the hormone in the specimen) in the specimen.