DETOXIFICATION OF XENOBIOTICS

A variety of toxic substances or potentially toxic substances may enter human body. They are food additives, poisons, toxins, certain drugs, chemicals, environmental pollutants, pesticides and other foreign substances. They are called as Xenobiotics (Xenos (Greek) - Strange). When they are ingested either accidently or some other way they may be absorbed from the gastrointestinal tract and gain access to the organs and tissues of the body. In the body xenobiotics undergo changes. These changes reduce the toxicity of xenobiotics. The conversion of highly toxic xenobiotics to less toxic substances is called detoxification or detoxication or biotransformation.

MEDICAL IMPORTANCE

1. Detoxification protects body and its organs from deleterious effects of toxins.
2. Detoxification removes most of drugs consumed from the body. Because of this drugs must be taken frequently during recovery from illness or disease.
3. Occasionally detoxification may generate toxic substance from relatively non-toxic substance.
4. Many anticancer agents work by inducing enzymes of detoxification.
5. Polymorphisms of enzymes of detoxification is associated susceptibility to diseases like myocardial infarction, cancer, inflammatory disease, alcoholic cirrhosis etc.

Generally detoxification converts less soluble toxic substance to more polar water soluble and hence the compound is easily excreted in urine. Some detoxified compounds may be excreted in feces through the bile. Liver is the organ involved in detoxification reactions. Detoxification of xenobiotics occur mainly in two stages (phases). In the first phase (stage) xenobiotics undergo three types of chemical reactions. They are oxidation, reduction (hydroxylation) and hydrolysis. The second phase involves conjugation of xenobiotics with variety of substances. Occasionally the detoxified products are sometimes more toxic than the original substance. Biotoxification is the word used to indicate such process.

I(*a*) Oxidation. Indole and Skatole are produced from tryptophan by the action of microbes. They are responsible for the disagreeble odour of the feces. They undergo oxidation.

Skatole → Skatoxyl, indole → Indoxyl

Benzene → Phenol, Benzaldehyde → Benzoic acid

Chloral → Trichloro acetic acid, Toluene → Benzoic acid

Ethylalcohol may be oxidized completely to CO2 and water. Similarly methanol may be oxidized to formaldehyde and formate.

Methanol → Formaldehyde → Formate

(*b*) **Reduction.** It is less common and less important than oxidation.

Picric acid → Picramic acid

Chloral hydrate (Sedative) → Trichloro ethyl alcohol

(*c*) **Hydroxylation.** Detoxification of number of drugs and steroids occur by hydroxylation. These reactions are catalyzed by cytochrome P450 dependent monooxygenases.

Phenobarbitol → Hydroxy phenobarbitol

Meprobamate (Tranquilizer) → Hydroxy meprobamate
Felbamate is structurally related to meprobamate. It is used in the treatment of epilepsy. It is eliminated by hydroxylation.
Felbamate → Hydroxyfelbamate

Cytochrome P450 (CYP) Enzymes

They are most important phase-I enzymes. They are involved in the detoxification and bio activation of xenobiotics present in food, organic solvents, tobacco smoke, drugs, pesticides, environmental pollutants and alcoholic drinks. They are products of CYP super family of genes. Over 100 mammalian CYP genes and their products are studied extensively. Some members of CYP super family with their function are given below :

CYP Form Function

CYP1A1 Inducible member of CYP super family helps in detoxification of carcinogens, toxins.
CYP1A2 Catalyzes activation of carcinogenic aryl amines and aflatoxin B.
CYP3A4 Involved in biotransformation of many drugs.
CYP2E1 Involved in oxidation of volatile environmental chemicals and anesthetics.

Medical Importance

1. CYP enzymes are involved in biotransformation of several endogenous compounds and activation of certain carcinogens. Certain compounds of dietary origin inhibit activities of these enzymes thus acting as selective inhibitors of carcinogens or toxicity of chemicals.
2. Polymorphisms in the genes coding for CYP enzymes is associated with susceptibility to different diseases including alcohol related diseases like alcoholic cirrhosis and alcoholic pancreatitis.

(*d*) **Hydrolysis.** Many drugs are detoxified by hydrolysis.
Aspirin (Acetyl salicylic acid) → Salicylic acid + Acetic acid
Atropine (Psychoactive) → Tropic acid + Tropine

II. Conjugation. Conjugation means the chemical combination of one compound with another compound. Many toxic substances are detoxified after combining with compounds like glucuronic acid, glutathione, sulfate, cysteine, acetate, glycine and glutamine.

(*a*) **Conjugation reaction using glucuronic acid.** Glucuronic acid participates in detoxification reactions as its UDP derivative.
Phenol is detoxified by conjugation with glucuronic acid as UDP-Glucuronic acid. The enzyme is UDP Glucuronyl transferase
Phenol→ Phenyl glucuronide
Paraacetamol→ Conjugated product. The conjugating agent is UDP-Glucuronic acid
Benzoic acid→ Glucuronide monobenzoate
Antibiotic chloramphenicol undergo conjugation with glucuronate.
Chloramphenicol→Complex with glucuronate
Lamotrigine an antiepileptic drug is conjugated with glucuronic acid and excreted in

urine.
Lamotrigine→ Conjugated product
Diclofenac sodium an analgesic and antipyretic is eliminated from the body by conjugation with glucuronic acid.
Diclofenac sodium→Conjugated product
Morphine, menthol, camphor, chloralhydrate, salicylic acid, PABA are excreted in conjugation with glucuronic acid.
(*b*) **Conjugation with glutathione.** Aliphatic or aromatic halogen substituted hydrocarbons are conjugated with glutathione. The conjugation is catalyzed by an inducible enzyme glutathione-S-transferase.
Dichloronitrobenzene is a halogen substituted aromatic hydrocarbon undergo conjugation with glutathione. The conjugated product is further acted upon by other enzymes to produce mercapturic acids which are excreted in urine.
Dichloronitrobenzene→Conjugated product→Mercapturic acid→Urine
Glutathione transferases (GST)
Glutathione-S-transferases are major enzymes of detoxification. They are involved in bioactivation
and detoxification of xenobiotics present in food, tobacco smoke, alcoholic drinks, pesticides, drugs, environmental pollutants, antitumor agents etc. They catalyze binding of large variety of electrophiles to sulfhydryl group of glutathione. Three types of mammalian glutathione-Stransferases
are identified. They are cytosolic, mitochondrial and microsomal GST.
Medical importance
1. Glutathione-S-transferases are involved in removal of chemical carcinogens. Since reactive ultimate carcinogenic form of chemical carcinogens are electrophiles GST is considered as important detoxification mechanism of carcinogen.
2. GST are involved in activation of unsaturated aldehydes, quinones, epoxides and hydroperoxides formed during oxidative stress.
3. Mammalian cytosolic GST exhibits polymorphism which increases susceptibility to carcinogenisis and inflammatory diseases.
4. Polymorphism of human microsomal GST is associated with increased risk of myocardial infarction and stroke.

(*c*) **Conjugation reactions using sulfate.** Paraacetamol, phenol, cresol, indoxyl and skatoxyl are compounds conjugated with sulfate. PAPS or active sulfate donates sulfate group.
Paraacetamol→Ethereal sulphate. The enzyme for the reaction is PAPS Transferase
PAPS is 3'-phosphoadenosine-5'-phosphosulphate
Skatoxyl/ Indoxyl→Ethereal sulphate. The enzyme is PAPS Transferase
Pain killer diclofenac sodium is conjugated with sulfate and excreted as ethereal sulphate.

(*d*) **Conjugation reactions using cyteine.** Naphthalene, anthracene, bromobenzene, chlorobenzene, iodobenzene and benzyl chloride are converted to mercapturic acids by conjugation with cysteine and acetylation.
(*e*) **Conjugation reactions using acetate.** Sulfa drugs are detoxified by acetylation.
Zonisamide an epilepsy drug is acetylated and excreted in urine.
Isonicotinic acid hydrazide used in treatment of tuberculosis undergo acetylation.
(*f*) **Conjugation reactions using glycine.** An example of conjugation with glycine is the detoxification of benzoic acid.
(*g*) **Conjugation with glutamine.** Phenyl acetate is conjugated with glutamine.
Detoxification of cyanide : Cyanide is converted to thiocyanate. The reaction is catalyzed by Rhodanase.
Methylation. Some compounds are detoxified by methylation. S-adenosyl methionine serve as methyl donor.
BAL (British anti Lewisite) is methylated and excreted. BAL removes toxic metals such as arsenic, mercury and cadmium from body.
BAL is used as antidote for arsenic poisoning.

Biomethylation
Arsenic ingested is detoxified by methylation and excreted in urine. Biomethylation reduces toxicity of arsenic and facilitates its elimination from the body. Initially inorganic arsenic is methylated to monomethylarsenic acid and finally to dimenthyl arsenic acid.
Anti carcinogens and enzymes of detoxification
1. Several anticarcinogens exert their effect by inducing phase-I and phase-II enzymes. Most important phase-I enzymes are CYP enzymes.
2. Phase-II enzyme induction is common feature of many chemoprotectants of cancer. Induction of phase-II enzymes before or during exposure to carcinogen decreases or inhibits carcinogensis.
3. Glucuronyl transferases and GST of phase-II enzymes are induced by some anti-carcinogens.
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VBB 301

BIOCHEMISTRY OF AGING AND DIEASE.

AGING

Aging is the accumulation of irreversible processes of deterioration which follows the development of an organism. It is generally characterized by declining ability to respond to external or environmental stresses as a result of impaired adaptive and homoeostatic mechanisms. Aging is also known as **senescence.**

Physical/physiological changes in aging

- Vision and hearing decline
- Reduction in muscle strength and size
- Decreased flexibility of soft tissue, blood vessels, skin, joint cartilages that can result into arthritis etc
- Overall decline in body tone including intestinal motility, movement and decreased effectiveness of body organ functions
- Diminished sensitivity to triggers/stimulations
- Loss of number of functional cells in tissues and organs e.g. the brains loses some amount of neurons with age.
- Lowered metabolic activity, immune functions, heart, kidney, lungs, liver functions etc
- Graying of hair (occurs in animals too!)
- Some animals develop dull hair coat, brittle nails and had foot/hand pads
- Dental/gum diseases leading to teeth loss
- Bone marrow progressively gets replaced by fat

THEORIES OF AGING

Many of these theories are interlinked, in the same complex way the biological processes of the body and the many factors affecting it are linked.

DNA and Genetic theory: closely related to this theory is also the programmed theory of aging this theory implies that aging is regulated by biological clocks operating throughout the life span; it focuses upon the encoded programming within the DNA. DNA is the blue-print of individual life obtained from our parents. It depends on changes in genes relating to the body repair, defense and maintenance mechanisms

Evolution theory; here it is suggested that that longevity is a product of evolutionary forces e.g. body weight brain weight and flight etc different species of animals have different life span, this provides evidence that longevity is genetically influenced. All adaptations that afford protection from predators and other hazards e.g. spines in porcupines justify greater developmental resources to build more durable animal and a longer maximum lifespan.

Neuroendocrine or hormonal Theory ; this theory Suggests the role of specific hormones in the aging process, notably cortisol, a hormone known to increase as organisms age and plays crucial role in stress; estrogen and so on. Generally it is known that the hypothalamus loses it precision regulatory ability and the receptors which uptake individual hormones become less sensitive to them. Accordingly, with increasing age the secretion of many hormones declines and their effectiveness (compared unit to unit) is also reduced due to the receptors down-grading.

Telomere or Telomerase Theory of Aging. Telomeres (the sequences of *nucleic acids* extending from the ends of *chromosomes*), shorten every time a cell divides. This shortening of telomeres is believed to lead to cellular damage due to the inability of the cell to duplicate itself correctly. Each time a cell divides it duplicates itself a little worse than the time before, thus this eventually leads to cellular dysfunction, aging and indeed death. Telomerase is an enzyme that appears to repair and replace telomeres helping to re-regulate the clock that controls the life-span of dividing cells, it is found only in germ and cancer cells.

Mitochondria theory; this theory is closely related to the free radical theory but emphasis of damage by FRs is placed on the mitochondria which is the power house of the cell where most endogenous generation of FRs occur. Mitochondria are the only cellular organelles possessing their own DNA, these DNA unlike nuclear DNA do not have protective heat shock and histone proteins, also lack DNA repair mechanisms, hence are liable to quick damage by FRs. The

mitochondrial theory of AGING postulates that damage to mitDNA occur at a rate 10-20 times more than damage to nuclear DNA due to deficiencies in the oxidative phosphorylative pathway leading to loss of mitochondria functions (mitDNA code for the protein complexes of the electron transport chain) and imminently cellular function (due to insufficient production of energy). About 1-2% of oxygen leak from the respiratory chain to for reactive oxygen species.

Free radical theory; this theory of aging was developed by Denham Harman, free radicals (FR) are molecules that have one or more free electrons (unpaired electron) and is capable of existing independently, and this property makes it react with healthy molecules in a destructive way. Reactive species is a term used to describe FRs and other molecules that are easily converted to FRs and are powerful oxidizing agents. These compounds are found both intra and extracellularly and maybe produced endo and exogenously.

It is known that diet, lifestyle, drugs (e.g. tobacco and alcohol) and radiation etc., are all accelerators of free radical production within the body. However, there is also natural production of free-radicals within the body. This is the result of the production of energy, particularly from the mitochondria as a byproduct of oxidative metabolism. Other endogenous sources include phagocytic processes, prostaglandins, detoxification processes etc

Free radicals are known to attack long lived biopolymers in the body such as structural proteins, DNA, lipids (membranes of cells), prostaglandins etc. for instance attack on lipids in cell membrane can damage the membrane by disrupting fluidity and permeability, while lipid peroxidation (oxidative change caused by free radical on lipids) of mitochondrial membranes reduces electrical potential and the mitochondria's ability to generate energy through the electron transport chain. Also FR damage cause fragmentation of DNA, loss of function and structural integrity of proteins, disrupt protein synthesis etc

Oxidative stress is caused by FRs.

REDUCTION OF OXYGEN TO REACTIVE SPECIES

$O_2 + e + H^+$---------HO_2^* (hydroperoxyl radical)

HO_2^*---------$H^+ + O_2^-$ (Superoxide radical)

$O_2^- + 2H^+ + e$----------H_2O_2 (hydrogen peroxide)

$H_2O_2 + e$----------$OH^- + {}^{\cdot}OH$ (hydroxyl radical)

${}^{\cdot}OH + e + H^+$---------- H_2O

Lipid peroxidation.

$^{\cdot}OH + LH$--------$^{\cdot}L + H_2O$; hydroxyl radical reacts molecules (LH) in the membranes of cells to produce lipid molecule radical (alkyl= $^{\cdot}L$)

$^{\cdot}L + O_2$ ------$LOO^{\cdot}$; The lipid radical then reacts with oxygen to form lipid peroxides (lipid peroxyl radicals, lipid molecules containing paired oxygen groups)

$LOO^{\cdot}$ + LH----------LOOH + $^{\cdot}L$.

The lipid hydroperoxides can promote a Fenton reaction;

Fe^{++} + LOOH----------Fe^{+++} + $^{\cdot}OL + H_2O$

The lipid alkoyl radical ($^{\cdot}OL$) is more reactive and damages more than the lipid peroxide radical ($LOO^{\cdot}$). However if two alkoyl, alky or peroxide radicals collide they nullify each other after creating a cross link between two lipids.

FACTORS INFLUENCING THE OCCURRENCE OF OXIDATIVE STRESS

Antioxidants; these group of compounds delay or inhibit the occurrence of oxidative damage to target molecules by acting as replacement to such target cells, keeping formation of reactive species to a minimum, replacing and repairing damaged molecules, scavenging FRs, and binding metal ions required for the formation of highly reactive species e.g. Fe^{2+} , Cu^{+} etc. Antioxidants could be enzymes, minerals or compounds.

- Antioxidant enzymes found endogenously which play a crucial role in scavenging FRs these include superoxide dismutase (SOD), glutathione peroxidase and catalase. These enzymes are found in all cells
- SOD – catalyzes the reaction betw 2 superoxide ions to prd H_2O_2 and triplet oxygen. Catalase catalyzes the formation of water and free oxygen from H_2O_2,it is present in membrane limited organelles called peroxisomes which contains other enzymes involved in degrading amino acids and fatty acids with the production of H_2O_2 as a by prdt. Glutathione peroxidase (GP) catalyses the reduction of H_2O_2 to water by using the antioxidant compound glutathione.
- Glutathione is a tripepetide and a major antioxidant in the non-lipid portion of cells. It exists as reduced glutathione GSH and oxidized GSSG. GP takes hydrogen molecules from glutathione and transfers to H_2O_2 to yield water.
- Vitamin E is the main Fr trap in the lipid bilayer of membranes.

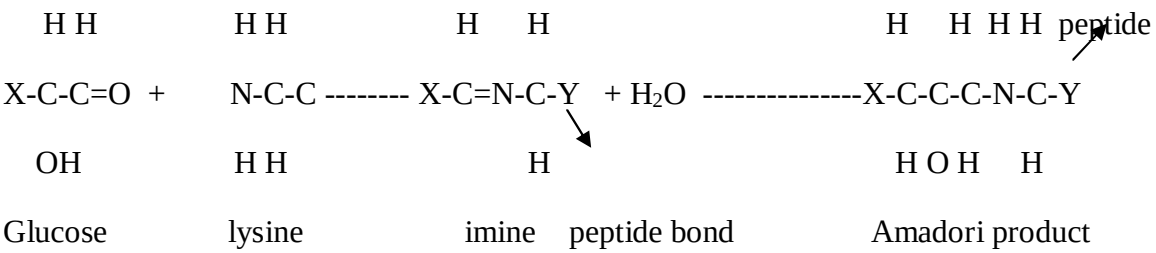
- Vitamin C acts as an antioxidant in the non lipid portion of cells and blood stream. Melatonin is a hormone produced by the pineal gland in decreasing quantities with age and it has been shown to be effective in protecting molecules against ·OH.
- Uric acid (produced from purine degradation) can also act as an antioxidant by binding to ion metal like Fe.
- A number of other compounds and chemicals notably found in plants e.g. lycopene, resveratrol, kolavirion etc has also been shown to have Fr radical scavenging capabilities.

Increase in FRs or reactive species; this can be influenced by

- excessive activation of phagocytes which produce FRs that may impose oxidative stress on tissues
- toxins form the environment e.g. cigarette smoke known to stimulate FRs production
- products of detoxification of toxins include FRs
- increased oxygen concentration or tension
- caloric restriction has been shown to increase life span of yeast cells , drosophila, worms and rodents, it is hypothesized that caloric restriction slows and reduces the overall metabolism (energy production, electron transport chain) hence also reduced production of reactive oxygen species.

Glycation theory; glycation is the formation of double bond between the glucose aldehyde and the lysine groups of amino acids with the elimination of water. An end product AGES- advanced glycation end products - is formed. AGES in tissues increases the rate of FR production to 50 times the rate of prdn in unglycated proteins. AGES attached to LDL-cholesterol accelerates oxidation and subsequent atherosclerosis. It can also aggravate protein cross linking; AGES may also be ingested in food. These compounds are known universal symptoms of aging and can adversely affect skin, lings, muscles, blood vessels and organ function in general.

The damage of proteins by FRs and glycation is also called Maillards reaction.



(Schiff base)

Oxidation catalysed by transition metals

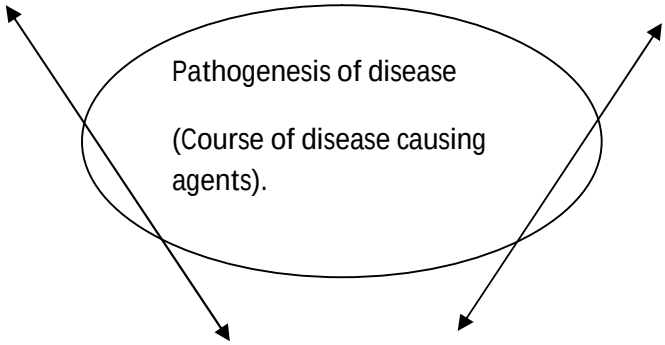
Advanced glycation end products (AGES)

BIOCHEMISTRY OF DISEASE

Biochemical changes occur as the basis for occurrence of disease. An understanding of the physiological biochemistry of the organism forms a baseline in understanding biochemistry of diseases.

Summarily;

CELL INJURY ⟷ FREE RADICALS



TISSUE DAMAGE

Changes therefore in the body of organisms with disease occurs as a result of the basic mechanism above and due to the body's effort to contain these changes for examples cell death to remove non-functional cells (apoptosis) such situation occurs to red cells as diseased ones are

rapidly removed from the circulation by spleen leading to anemia a common feature of many parasitic blood diseases e.g. trypanosomosis.

SOME CHANGES IN DISEASE AND BIOCHEMICAL BASES

1. Anemia- rapid breakdown of infected erythrocytes by the RES.
2. Hypoglycemia- excessive utilization of energy body cells to fight on going infections, and by the invading organisms to the detriment of host.
3. Hypergammaglobulinemia-increased synthesis of globulins to fight on-going invasion
4. Elevation of plasma enzymes- due to rapid tissue/cellular breakdown and release of contents into blood. The cell death is self induced as protective mechanism; apoptosis
5. Damage to more cells as a result of rapid release of FRs from invading organisms, phagocytes etc

Functions of the Liver

- Detoxification of endogenous and exogenous toxins
- Metabolism of CHOS, fats, and proteins
- Bile production
- Blood filtration
- Blood glucose regulation

Liver Function tests

The various liver function tests can be clarified broadly used on the major functions of the liver, including:-

1) Excretory functions

The liver is responsible for conjugating bilirubin, a production formed from the catabolism of hence to diglucuronide which is readily excreted in bile Bilirubin and other dyes like urobilinogens and sterobilinogen on be measured is blood (serum), and urine as important that for liver function. Bilirubin is estimated by Van Der Bergh reaction where diazotized suphanilic acid reacts with bilirubin to form a people colored complex-azobilirubin. For conjugated bilirubin the color change is produced immediately (direct), while for unconjugated color is produced only after addition of alcohol (indirect)

Only conjugated bilirubin is soluble in water and excretable wine, hence when there is obstructive jaundice, urine contains bilirubin as a means of excreting it from the body.

- Bromsulphthalein (BSP) test or sulphobromophthalein (organic anion)

When this dye is injected the hepatic cells conjugation it with glutathione although a significant faction is excreted unconjugated, when a single bolus dose to 50g/l is given, the retention of the dye after 45minutes in normal individuals (is less than 5% impairment of the liver cell function causes an increase in BSP retention.

Indocyacine green (ICG) is another dye also used.

2) Metabolic functions

The liver function tests are based on substances that are selectively metabolized by the liver e.g. galactose, ½ life of galactose in blood is about 10-15 minutes, but in defective liver is prolonged, antipyrine is rapidly and completely absorbed from the intestine and mostly metabolized by hepatic monoxygenase system, normal subject excrete 5-8% of this compound in their breathe on 2hrs while patients with cirrhosis excrete 2-3% and hepatitis 2-4%.

3) Synthetic functions liver

The liver functions in synthesis of almost all plasma proteins excepts Igs, so levels of plasma proteins may be arrested to determine the condition of the liver serum Albumin is appreciably reduced in all chronic liver disease but is not a good indicator of acute liver disease b/c of its long half life. Haptoglobulin and transferrin are better indicators of acute liver changes. Prolong prothrombin time is used an indicator of poor prognosis in chronic liver disease. Others are alpha feto-protein which is a tumor marker, whole level is markedly increased is blood during hepatocellular damage.

4) Serum Enzymes

Amino transferases levels in serum are used to indicate liver disease as they are elevated usually in almost all liver disease. Alkaline phosphatase (ALP) whose synthesis is induced by bile duct obstruction, have elevated levels in serum cholestasis and hepatic carcinomas as compared to parenchymal liver disease. Gamma glutamyl transferase levels are also used and are sensitive to biliary tract disease (usually obstructions).

Others include 5-nucleotidase and leucine amino peptidase and in special circumstances glutathione-S-transferase. Others are Arginase, Sorbitol dehydrogenase (esply used in large

animals as against ALT in small animals). All in small animals) Glutamate dehydrogenase, gamma glutamyl transpeptidase etc.

Jaundice

Definition: Yellowish coloration of tissues as a result of higher than normal concentrations of bilirubin in plasma.

Types

In Hemolytic jaundice unconjugated bilirubin is increased hence the Van der Bergh test is indirect position, while in obstructive jaundice, conjugated bilirubin is elevated and the test direct. In hepatocellular jaundice and biphasic reaction is observed because both conjugated and unconjugated bilirubin is seen.

Claim of Jaundice	**Type of Bilirubin**	**Causes**
Prehepatic/hemolytic	Unconjugated	Abnormal red cells, Abs drugs and toxins, thalassemias, Hemoglobinopathies, Gilbert's syndrome etc
Hepatic/hepatocellular	Conjugated/unconjugated	Viiral hepatitis, toxic hepatitis, intrahepatic cholestasis
Post-hepatic/obstructive	Conjugated	Extra hepatic cholestasis, gall stories, tumors of bile duct, carcinoma of pancreas, Lymph Node enlargement etc