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PG AND RESEARCH DEPARTMENT OF BIOCHEMISTRY

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UNIT-III

DISORDERS OF PROTEIN METABOLISM AND CLINICAL ENZYMOLOGY

Disorders of protein metabolism - non-protein nitrogenous constituents in blood - urea, uric acid and creatinine. Plasma protein abnormalities - deficiency, agammaglobulinemia, multiple myeloma, proteinuria, glomerulonephritis, nephrotic syndrome. Haemoglobinopathies - sickle cell anaemia and thalassimia. Phenylketonuria, tyrosinosis, alkaptonuria, maple syrup urine disease, Hartnup disease, homocystinuria, albinism.

Serum enzyme activities in diseases - Principle and assay of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, acid phosphatase, streptokinase, asparaginase, isocitrate dehydrogenase, ceruloplasmin, y -glutamyl transpeptidase, creatine kinase and lactate dehydrogenase.

Disorders of protein metabolism

The non–protein nitrogen fraction of serum (and blood) is composed of all nitrogenous compounds other than protein. The kidney plays an important role in the elimination of this compound.

There are more than 15 NPN compounds in plasma. Urea nitrogen comprises approximately of 45% of the total. Other compounds and there percentages of the total NPN in plasma include amino acids (20%), uric acid (20%), creatinine (5%), creatine (1–2 %) and ammonia (0.2%). NPN in blood is approximately 75% greater than the plasma value owing to the glutathione content of erythrocytes. NPN determination is most useful in connection with kidney disease.

The test for NPN has been reduced by the more useful and convenient test for urea nitrogen. The NPN does not offer any information in addition to that provided by the urea nitrogen determination. An exception to this may be the simultaneous determination of NPN and urea nitrogen in patient with hepatic failure in the presence of renal disease. Under such conditions, the ration of NPN to urea nitrogen may be higher than that normally found. This is due to the decreased ability of the liver to synthesize urea and deaminate amino acid.

Increase in NPN fraction are mainly a reflection of an increased in urea nitrogen. As the total NPN rises, the proportion of the urea also increases.

Requirement common to all NPN compound

1. **Deproteinization**

All proteins must be removed from the samples before actual measurement. Somogyi type filtrates should not be used since some NPN compounds are adsorbed on the precipitate.

2. **Digestion**

This consists of breaking down the nitrogenous compound after separating proteins and the conversion of nitrogen into a common measurable form. This is done by using acid digestion mixture and heat. There are many digestion mixtures which achieve the same results. These are digestion mixtures consists of an oxidizing agent, a catalyst and a chemical which increases the boiling point of the solution.

An example of an acid digestion mixture contains the following:

a. Sulfuric acid – oxidizing agent
b. Copper sulfate or perchloric acid – catalyst
c. Phosphoric acid or potassium sulfate – used to raise the boiling point

During the digestion process, the various nitrogenous substances are broken down and nitrogen is converted to ammonia held in the form of ammonium sulfate by sulfuric acid.

3. Measurement of ammonia by either

a. Distillation
b. Nesslerization
c. Gasometrically
d. Color reaction

Three common procedures in the determination of NPN

1. Folin Wu method

In this method, a protein free filtrate is made. To the filtrate, an acid digestion mixture is added to help liberate ammonia. This is heated to decompose nitrogenous substances in the form of ammonium salts. Nessler's solution is added to convert ammonium salts to yellow simercuric ammonia iodide which is measured colorimetrically.

Reagents:

a. Acid digestion mixture – a mixture of concentrate sulfuric acid and 85% phosphoric acid. Concentrated sulfuric acid is used to convert N_2 to NH_3 and to oxidize other organic compounds while H_3PO_4 is used to raise the boiling point.

b. Nessler's reagent – alkaline potassium mercuric iodide – used as color developer

c. Gum ghatti – used to prevent cloudiness

d. Glass beads – used to prevent bumping of the solution

e. Yellow – end color

2. Koch–McMackin method

This is the same as the Folin–Wu method except for the digestion mixture. Hydrogen peroxide is used instead of phosphoric acid as catalyst. Sulfuric acid is used for stronger concentration.

3. Berthelot's color reaction

PFF is prepared. Sulfuric acid is added to liberate ammonia. It is then heated to decompose nitrogen substance. Phenol color reagent and alkali hypochlorite reagent is added and in the presence of sodium nitroprusside as catalyst, blue color is produced which is measured colorimetrically.

UREA

It constitutes about 40–50% of the total NPN in the blood. It is the chief end product of protein catabolism.

Urea is synthesized in the liver from NH3 produced as the result of deamination of amino acids and CO_2 and the process is known as the ornithine cycle. From the liver, urea enters the blood to be distributed to all intracellular and extracellular fluids, since urea is freely diffusible across most cell membranes. Most of the urea is ultimately excreted by the kidney by glomerular filtration but minimal amounts are also excreted in the sweat and degraded by bacteria in the intestine.

It is customary in most laboratories to express urea as urea nitrogen. The same is through the desire to compare the quantity of nitrogen in urea with that of other components included in the non–protein category. Since its molecular mass is 60 daltons, and it contains 2 nitrogen atoms with a combined weight of 28, a urea nitrogen value can be converted to urea by 60 ÷ 28 or 2.14

Urea = BUN x 2.14

Example:

BUN = 15mg%
Urea = 15 mg% x 2.14
= 32.10 mg%

Methods of BUN determination

1. Colorimetric determination by its reaction with diacetyl monoxime

Fearon reaction and Diacetyl monoxime (DAM)

In 1939, Fearon found that reaction of ammonia with diacetyl monoxime followed by oxidation gives a color.

In 1942, Ormsby applied this reaction to the determination of urea. The sample is heated with diacetyl monoxime in acid solution and the resultant color is intensified by oxidation with potassium persulfate of the hydroxylamine formed in the reaction.

Disadvantages of this method:

a. Color develops rapidly and fades rapidly
b. Color is photosensitive
c. Color does not follow Beer's law.
d. Unpleasant odor and irritant fumes of the reagent.
e. The time of heating for maximal color development is dependent on the urea concentration.
f. The reaction is not completely specific.

2. Enzymatic method by the action of urease on urea

a. Karr method

To protein free filtrate, a buffer solution is added to control the pH. Enzyme urease is then added and the mixture is incubated to decompose urea and form ammonium carbonate. Gum ghatti is added as protective colloid. Lastly, Nessler's is added to yellow dimercuric ammonium iodide. This is then measured photometrically.

b. Van Slyke Cullen method

A buffer solution is added to oxalated blood to control the pH. Enzyme urease is added to decompose urea and form ammonium carbonate. Potassium carbonate is added to liberate ammonia which is collected in boric acid solution. This is then titrated with a standard acid solution and urea nitrogen is determined by calculation.

c. Gentzkow Massen method

Oxalated blood is diluted and enzyme urease is added to decompose urea and form ammonium carbonate. Then, the proteins are precipitated. Nessler's reagent is then added to the filtrate to convert ammonium carbons to yellow dimercuric ammonium iodide which is measured photometrically.

d. Folin–Svedberg method

A PFF is made and a buffer solution is added to control the pH. An ezyme is added to decompose urea and form ammonium carbonate. Sodium borate is added to liberate ammonia which is then collected in dilute hydrochloride acid. Nessler's solution is added to convert ammonium salts to yellow dimercuric ammonium iodide which is then measured colorimetrically.

e. Berthelot color reaction

Buffered urease is added to serum or plasma to decompose urea and form ammonia. The mixture is incubated to enhance the reaction. Phenol color reagent and alkali hypocholorite reagent are added which is measured colorimetrically.

f. Leiboff method

The principle is similar to the Folin method except that a special Leiboff pressure tube is used and the sample and sulfuric acid are placed in Leiboff and immersed in an oil both heated at 150oC for about 10 minutes. After cooling, it is nesslerized and the yellow color formed is measured colorimetrically.

g. Urograph (Urastrat) method

This consists of chromatography paper bonded by controlled amount of reagent (buffered urease, potassium carbonate and bromcresol green acidified with quantitative titrated tartaric

acid). The method will measure directly in 30 minutes the BUN concentration from 10–75 mg%. The urograph chemical reaction parallels closely those of the Conway microdiffusion method:

(1) Digestion with urease
(2) Release of ammonia by K_2CO_3
(3) Titration with acid

When the serum or plasma in the bottom of the test tube travels up through the urograph, it passes through a series of chemical adventures. The sample first encounters a highly potent urease which produces ammonia from the urea present in the sample.

In the next band containing potassium carbonate, the ammonia is liberated as free gas, the quantity of which is proportional to the original urea nitrogen concentration of the sample. The upward migration of the serum or plasm is eventually stopped by the plastic barrier. The indicator band of the bromcresol green tartaric acid located above the plastic layer quantitatively traps and reads the ammonia released. The urea nitrogen is quantitatively indicated by the height of the portion of the indicator band which has changed color at the end of 30 minutes incubation period.

The height of the indicator band in which color change is noted is measured in terms of millimeters which is then multiplied by 5 and 10 is added. This will be mg%.

3. Xanthydral reaction (direct determination of urea)

Urea is precipitated with xanthydrol and then the resulting dixanthydryl urea is estimated. The method requires a special apparatus, time consuming and expensive to manipulate.

Reference range for BUN is 8 – 26 mg / dl:

A value within this range, however, does not imply that renal function is unimpaired. A patient with baseline of 12 mg/dl whose level increases to 24 mg/dl in a steady state of hydration and protein intake may well have significant reduction of renal function regardless of the fact that the level is within the reference range.

Precautions in BUN determination:

1. BUN determination is affected by high protein diet.
2. Whole blood should be deproteinized to eliminate interferences of hemoglobin.
3. Ammonium–containing anticoagulants are contraindicated in enzymatic method.
4. Sodium fluoride inhibits the action of urease.
5. Upon prolonged standing, ammonium concentration in the sample raises 2 – 3 times the original value due to enzymatic deamination of labile amides like glutathione.

Clinical significance

The determination of serum urea nitrogen is presently the most popular screening test for the evaluation of kidney function. The test is frequently requested along the serum creatinine test since simultaneous determination of these two compounds appears to aid in different diagnosis of pre–renal, renal and post–renal hyperuremia.

1. Pre–renal causes – conditions in which circulation through the kidney is less efficient than usual.

    a. Cardiac decompensation
    b. Water depletion due to decreased intake or excessive loss.

2. Renal causes – with lesions of the renal parenchyma

    a. Glomerulonephritis
    b. Chronic nephritis
    c. Polycystic kidney
    d. Nephrosclerosis
    e. Tubular necrosis

3. Post–renal – due to obstruction of the urinary tract

    a. Stone
    b. Enlarged prostate gland
    c. Tumors

Azotemia – biochemical abnormality that refers to an increase in BUN and creatinine levels which is largely related to decreased GFR.

Uremia – increase in urea and creatinine values with accompanying clinical signs and symptoms of renal failure like:

a. Metabolic acidosis due to failure of the kidneys to eliminate acidic products of metabolism
b. Hyperkalemia due to failure of potassium excretion.
c. Generalized edema due to water retention.

CREATINE

Creatine is synthesized in the liver and pancreas from three amino acids: arginine, glycine and methionine. After synthesis, creatine diffuses into the vascular system and is supplied to many kinds of cells, particularly the muscle, where it is phosphorylated. Creatine phosphate serves as a reservoir of high energy and is readily convertible to adenosine triphosphate in muscles and other tissues.

Creatine and creatine phosphate total approximately 400 mg per 100 gram of fresh muscles. Both creatine and creatine phosphate are spontaneously converted into creatine at a rate of approximately 2% per day.

Method of determination:

It is determined as the difference between the preform creatinine and the total creatinine that results after the creatine present has been converted to creatinine by heating at an acid pH.

Total creatinine – Preformed creatinine = Creatine

CREATININE

Creatinine is a wasted product derived from creatine and creatine phosphate. It is an anhydride formed when creatine loses a water molecule and creatine phosphate loses a phosphoric acid molecule. The reaction occurs spontaneously. Approximately 2% of creatine is transformed into creatine every 24 hours. The body content of creatinine is proportional to muscle mass; therefore, the levels of creatinine in the body are also proportional to muscle mass. The conversion of creatine to creatinine occurs at an accelerated rate in acid or alkaline solutions. Creatinine is removed from the plasma almost entirely by glomerular filtration with a small contribution from tubular secretion. No reabsorption of creatinine occurs in the renal tubules. Urine contains significantly more creatinine than creatine because of the differences in renal handling.

Method of determination

1. **Jaffe Reaction**

PFF is treated with alkaline sodium picrate solution to produce a red – orange tautomer of creatine picrate (Jaffe reaction) and this is measured phtometrically.

The Jaffe reaction is not specific for creatine since there are many substances in the red cells which give the same reaction as creatinine. This is the reason why plasma and serum are preferred to whole blood since considerable amount of non – creatinine chromogens are present in the red cells. Lloyd's reagent is used for isolation of creatinine from interfering substances. After deproteinization of the specimen, creatinine is absorbed from an acid medium on Lloyd's reagent, an aluminum silicate, and subsequently described in an alkaline solution. This method is highly specific and gives true creatinine level.
Notes on creatinine determination:

a. Alkaline sodium picrate solution is unstable and should therefore be prepared and stored for 30 minutes before use.

b. Solution should be thoroughly mixed after the addition of alkaline picrate.

c. The end color in the Jaffe reaction slowly fades and should be read within ½ hour.

Chief source of difficulty of the Jaffe reaction

a. Lack of specificity
b. Sensitivity to certain variable like ascorbic acid, pyruvate, acetone, glucocyanidine, amino–hippurate, diacetic acid, glucose and protein.

Variables involved in Jaffe reaction:

a. Picric acid – commercial preparations must be purified.
b. Temperature – color development between 15 – 25°C is not much important but the temperature of solution while it is being read is important.
c. Protein precipitation – 85 – 90% recovery at pH 3 – 4.5; complete recovery at pH below 2.
d. pH – color intensity of alkaline picrate decreases with decreasing pH, conversely, color intensity of alkaline picrate increases as pH increases.

2. Enzymatic method

3. Development of a purple rose color formed between creatinine and 3,5– dinitrobenzoic acid in alkaline solution.

4. Turbidimetric method employing a modified Nessler's reagent.

5. Reaction of creatinine with potassium mercury thiocyanate

6. Degradation to methyl guanidine followed by Sakaguchi color reaction.

7. Isolation of creatinine by absorption of an ion exchange resin and quantitation by measurement of absorbance at its peak.

Clinical significance

Creatinine is considered as the best index for prognosis of kidney impairment. As renal function diminishes, serum creatinine rises but the rise is less than the change in BUN. An elevated serum creatinine level indicates severe, long standing renal impairment.

By virtue of its relative independence from such factors as diet (protein intake), degree of hydration and protein metabolism, the plasma creatinine is a significantly more reliable screening test or index of renal function than BUN.

Most experts advocate the establishment of a baseline GFR with creatinine clearance test, and then the serum creatinine can be used to establish changes in GFR.

Creatinine in serum represents a small part of the non – protein nitrogen function. Its determination has little clinical value in kidney diseases. It is used principally in evaluating muscle disorders. It is a product of endogenous muscle breakdown and when this breakdown is accelerated, as in muscular dystrophy, large amount of creatine maybe excreted in urine.

URIC ACID

Uric acid is a waste product derived from purine of the diet and those synthesized in the body. It has been shown that the healthy adult human body contains about 1.1 grams of uric acid and that about one sixth of this is present in the blood, the remainder being in other tissues. Normally, about one half of the total uric acid is eliminated and replaced each day, partly by way of urinary excretion and partly through destruction in the intestinal tract by microorganisms. Uric acid is one of the components of the NPN fraction of plasma.

Plasma uric acid is filtered by the glomeruli and is subsequently reabsorbed to about 90% by the tubules. It is the end product of purine metabolism in man. Other mammals are able to metabolize the uric acid molecule to a more soluble end product, allantoin. It is greatly affected by extra–renal as well as renal factors.

Methods of determination

1. Colorimetric method

Most methods of uric acid determination are based on the reducing property of this substance. The end result of most of the procedure is the production of a blue color by the action of uric acid on phosphotungstic acid.

Phosphotungstic acid serves both as protein precipitating agent and color agent.

A glycerine–silicate reagent increases the sensitivity and also provides alkalinity for the reduction of phosphotungstic acid to tungsten blue.

Sodium polyanethol sulfonate is added to prevent turbidity

a. Benedict's method

The disadvantage of this method is that 5% NaCN, which is added to intensify the color is poisonous. Uric acid is reacted with phosphotungstic acid solution in an alkaline media. The phosphotungstate complex is reduced to phosphotungstite (blue) in which intensity of color is directly related to the concentration of uric acid in the sample.

b. Folin method

A PFF is treated with urea cyanide and phosphotungstic acid to form a phosphotungstate complex. This is reduced by the uric acid present to form blue phosphotungstite complex. This then measured colorimetrically with a standard.

c. Brown method

A PFF is treated with sodium cyanide, urea and phosphotungstic acid to form a phosphotungstate complex. The uric acid present reduces the phosphotungstate to the blue phosphotungstite complex. The depth of the color is measured in a colorimeter and compared with a standard.

d. Newton method

A special PFF is made and treated with urea–cyanide solution and lithium arsenotungstate to form arsenotungstate complex. The depth of the color is measured in a colorimeter and compared with a standard.

e. Archibald method

A special PFF is made and glycerol silicate polyanethol sodium sulfonate and phosphotungstic acid are added to form phosphotungstic complex which reduced to blue phosphotungstite complex. This is measured in a colorimeter and compared with a standard.

The specificity of the method is enhanced by pre–treatment of the serum with sodium hydroxide which causes an oxidative destruction of ascorbic acid and sulfhydryl compound which would lead to false high values.

f. Henry method

Uric acid in serum, plasma and urine reduce an alkaline phosphotungstate solution to a "tungsten blue." The alkali used is sodium carbonate. The depth of the color is measured photometrically and compared with a standard.

g. Caraway method

Similar and identical with Henry method. The recent methods of Henry and Caraway returned to the older methods of providing alkalinity due to the disadvantages and hazards when one is using cyanide.

h. Kern–Stransky method

There are several disadvantages with the cyanide technique:

a. Reagent blanks with cyanide usually have appreciable absorbance which varies with age of the reagent and brand.
b. Standard curves with cyanide are not continuously reproducible.
c. Cyanide is undesirable because of its highly poisonous character.
d. Extra care in the storing of the reagent is needed as it requires refrigeration.

One of the problems involved in the colorimetric method of uric acid determination is the appearance of turbidity in the final colored solution.

Methods or reagents used to avoid the formation of turbidities are:

a. Purification of the cyanide reagent because of their carbonate contents.

b. Others add lithium salts to the phosphotungstic acid reagent. This reagent helps to avoid turbidity by inhibiting the formation of sodium or potassium phosphotungstate.

c. Addition of urea to the cyanide technique.

d. Kern and Stransky introduced a method employing sodium silicate as the source of the alkali.

e. Archibald, unable to avoid turbidities by the Kern – Stransky method introduced a technique retaining glycerine silicate but added sodium polyanethol sulfonate to prevent turbidity formation.

f. Henry, et.al. included lithium sulfate in the phosphotungstic acid reagent. He found that the same color intensity could be obtained with sodium carbonate, sodium hydroxide and sodium metasilicate.

With regards to the problem of specificity of the reaction, the following techniques have been employed:

a. Isolation of uric acid on ion exchange resin.

b. Bulger and Johns (1941) used the specific enzyme uricase. The end products resulting from the oxidation of uric acid by uricase are dependent on pH and specific buffer employed.

(1) Tris buffer or phosphate buffer at pH 7.2 – 8.5; uric acid is oxidized mainly to allantoin

(2) Presence of borate buffer and at pH 7.2, the main products are urea and allaxonic acid

(3) Borate buffer at pH 9, the products of uric acid oxidation are urea, allaxonic acid and allantoin.

c. Kalokar introduced the technique of differential spectrophotometry for the determination of uric acid. Uric acid has an absorption peak in the region of 290 –293 mu, whereas, the end product after destruction by uricase has no absorption at this wavelength. Thus, the decrease

in absorbance in this wavelength resulting from uricase action is proportional to the uric acid originally present.

2. Spectrophotometric method

Uricase is an enzyme found in most mammals except man, which catalyzes the complicated transformation of uric acid into allantoin. Uric acid has a maximum absorption peak at about 203 – 290 mu. When uricase destroys uric acid, the resultant products have no absorption at this wavelength. The decrease in optical density of the specimen, after incubating it with uricase, is proportional to the amount of the uric acid present. This method has great specificity because uricase acts on uric acid alone. It is more time consuming and requires the use of an instrument capable of reading in the ultraviolet region; therefore, it is seldom used routinely.

Normal values: Female = 2 – 6 mg%
Male = 3 – 7 mg%

Clinical significance:

Determination of serum uric acid levels is most helpful in the diagnosis of gout. It is also increased whenever there is increased metabolism of nucleoproteins, such as leukemia and polycythemia or after the intake of food rich in nucleoproteins, e.g. liver, kidney or sweetbread. It is also a constant finding in familial idiopathic hyperuricemia, of which there are two types. In one type, there is an overproduction of uric acid in the presence of normal excretion and in the other; there is a decreased rate of excretion in the presence of normal uric acid production. Uric acid levels are elevated in decreased renal function and are valuable for early diagnosis of kidney impairment. It is excreted least daily.

Gout – a chronic disturbance of metabolism in which there is a noted accumulation of uric acid in the blood as a result of the disturbance of the exogenous and endogenous uric acid formation. In gout, the blood uric acid level is elevated and there is often deposition of crystalline uric acid around various joints called “tophi.”

Plasma protein abnormalities:
Agammaglobulinemia:

X-linked agammaglobulinemia (a-gam-uh-glob-u-lih-NEE-me-uh), also called XLA, is an immune system disorder that's passed through families, called inherited. XLA makes it hard to fight infections. People with XLA might get infections of the inner ear, sinuses, respiratory tract, bloodstream and internal organs.

XLA almost always affects males. But females can carry the genes linked to the condition. Most people with XLA are diagnosed in infancy or early childhood, after they've had repeated infections. Some people aren't diagnosed until they're adults.

Symptoms

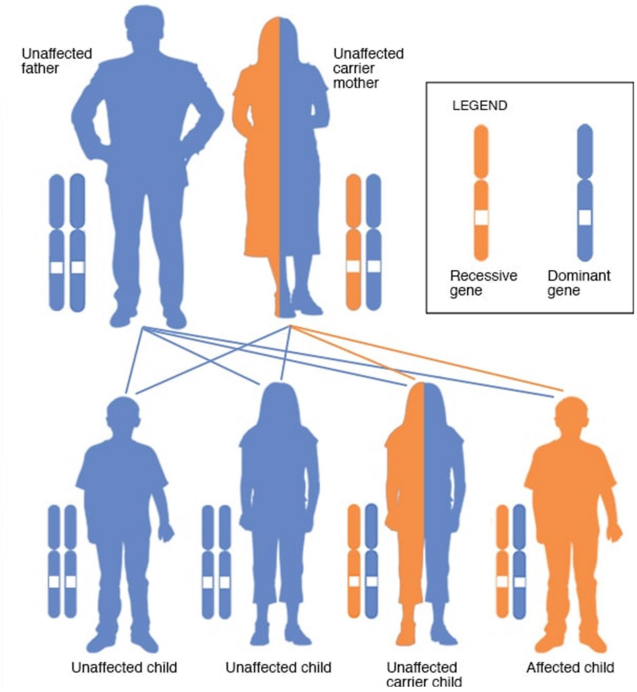
Most babies with XLA appear healthy for the first few months. They're protected by the proteins called antibodies they got from their mothers before birth.

When these antibodies leave their systems, the babies begin to get repeat bacterial infections. The infections can be life-threatening. Infections might involve the ears, lungs, sinuses and skin.

Male infants born with XLA have:

- Very small tonsils.
- Small or no lymph nodes.

Causes



X-linked inheritance pattern with carrier mother

X-linked agammaglobulinemia is caused by a change in a gene. People with the condition can't produce proteins called antibodies that fight infection. About 40% of people with the condition have a family member who has it.

Complications

People with XLA can live mostly typical lives. They should try to take part in regular activities for their ages. But repeat infections linked to XLA will likely need careful watching and treatment. They can damage organs and be life-threatening.

Possible complications include:

- Long-lasting, called chronic, lung disease.

- Increased risk of certain cancers.
- Infectious arthritis.
- Increased risk of central nervous system infections from live vaccines.

Diagnosis

Diagnosis involves a medical history of repeat infections and a physical exam. Blood tests and maybe genetic testing can confirm the diagnosis.

Treatment

There's no cure for XLA. Treatment aims at boosting the immune system to prevent infections. There also is quick treatment for infections as they happen.

Medications

Medicines to treat XLA include:

- **Gammaglobulin.** This is a type of protein found in blood that contains antibodies against infections. It's put into a vein, called infusion, every 2 to 4 weeks or given with weekly shots.

  Reactions to gammaglobulin can include headache, chills, backache and nausea. Reactions are more likely to happen during a viral infection, such as a cold.

- **Antibiotics.** Some people with XLA take antibiotics all the time to prevent infections. Others take antibiotics for bacterial infections longer than do people without XLA.

Multiple myeloma

Multiple myeloma refers to a malignant disorder of plasma cells (mature B lymphocytes).

Multiple myeloma (MM) is the second most common haematological malignancy. It is characterised by excess secretion of a monoclonal antibody. We term it a monoclonal antibody, because it is derived from a single clone of plasma cells that have undergone abnormal proliferation.

MM accounts for 60-70 cases per 1,000,000 people each year, although the overall prevalence of the condition is increasing due to the improved survival with newer treatments. Unfortunately, it still accounts for 2% of cancer-related deaths and it is associated with a number of severe complications including spinal cord compression, renal impairment and hypercalcaemia,

Immune response

Our immune system is broadly divided into the innate and adaptive immune response.

All blood cells are derived from haematopoietic stem cells (HSCs), which differentiate into three main cell lineages: erythroid, myeloid, lymphoid. See our notes on haematopoiesis.

We have a variety of different cell types that are important in both innate (e.g. neutrophils) and adaptive (e.g. lymphocytes) immunity. The chemical messengers of the immune system are small molecules, such as cytokines, which are released by a variety of cell types including immune cells and endothelial cells.

Innate immunity

This is our first line of defence against microorganisms, which involves both cells (e.g. phagocytes, dendritic cells) and molecules (e.g. complement, cytokines). It involves recognition of foreign material by identification of conserved constitutes of microorganisms. We call these pathogen-associated molecular patterns (PAMPs). This stimulates a pro-inflammatory response and activates the adaptive immune response.

Adaptive immunity

This immune response is divided into cellular immunity and antibody-mediated immunity. There are three hallmarks of adaptive immunity:

- Antigen specificity: it can detect almost an unlimited number of antigens with high accuracy.
- Delayed onset: takes 2-4 days to mount an adaptive immune response (on first encounter).
- Immune memory: rapidly protective on re-exposure to a foreign antigen.

Lymphocytes are the primary immune cell in adaptive immunity. T lymphocytes are important in both cellular immunity and mediating the antibody response (through activation of B lymphocytes). B lymphocytes are important in antibody-mediated immunity, which mature into plasma cells.

Antibody-mediated immunity

The secretion of antibodies forms a key part of our adaptive immune response.

B lymphocytes are the antibody secreting cells in the body. Immature cells mature within the bone marrow. On exposure to an antigen, naive B cells mature into plasma cells.

Antibodies

Antibodies are composed of heavy and light chains. There are five types of heavy chain (A, G, M, D, E) and two types of light chain (Kappa, lambda). Two heavy chains form with two

light chains to create the complete antibody.

Maturation and proliferation

The body contains thousands of antigen-specific B cells. Once activated they multiply into a clone of plasma cells that secrete a specific antibody to a foreign antigen. Collectively, the pool of antibodies from all the different clones of B cells are termed polyclonal antibodies (i.e. each antibody has a different antigen-specificity).

If one clone acquires an abnormal mutation and proliferates uncontrollably, it will secrete its own antigen-specific antibody in excess of the others. These are termed monoclonal

antibodies (i.e. a group of the same antibodies from a clone plasma cells). These monoclonal antibodies are associated with a single light chain (kappa/lambda).

Aetiology and pathophysiology

The pathophysiology of MM is still poorly understood but there appears to be a two-step model.

The pathophysiology of MM is centred around the development of a malignant clone of plasma cells, which can secrete excess amounts of a monoclonal antibody. This is due to the development of cytogenetic abnormalities, which refers to structural chromosomal changes, mutations or cellular dysregulation (e.g. cell cycle or apoptosis dysregulation).

There appears to be two key steps in the pathophysiology of MM: development of monoclonal gammopathy of undetermined significance (MGUS) and progression from MGUS to MM.

- Development of MGUS: almost all cases of MM arise from this premalignant plasma cell disorder. Affects 3% of patients > 50 years old. Initial cytogenetic abnormality occurs (inciting event). Thought to be an abnormal plasma cell response to antigen stimulus. Leads to creation of plasma cell clone that secretes a monoclonal antibody. Most will not develop MM.
- Progression from MGUS to MM: rate of progression estimated at 1% per year. Further cytogenetic abnormalities and changes to the bone marrow microenvironment occur, which promotes proliferation. Associated with systemic problems due to plasma cell infiltration of bone marrow and excess light chain secretion.

There is a further intermediate stage between MGUS and MM. This known as smouldering myeloma or asymptomatic myeloma. It is a more advanced premalignant stage due to a higher burden of clonal plasma cells in the bone marrow.

Clinical presentations

The clinical presentation of MM is generally related to the infiltration of plasma cells and secretion of monoclonal antibodies.

Patients with MM may have constitutional features of malignancy including weight loss, fatigue, loss of appetite and/or generalised weakness. There are a number of typical clinical presentations that relate to the excess proliferation and infiltration of plasma cells (usually in bone marrow) and the excess secretion of monoclonal antibodies.

Presenting clinical features

- Bone disease: widespread due to clonal proliferation in bone marrow. Seen as lytic lesions on imaging. Can lead to fractures.
- Impaired renal function: >50% have raised creatinine at diagnosis. Kidneys affected in multiple ways. Commonly due to light chain nephropathy (tubules blocked by light chain casts).

- Anaemia: seen in >90% at some point during disease course. Normal bone marrow destroyed by proliferation of malignant plasma cells. Renal disease may contribute (EPO deficiency).
- Hypercalcaemia: MM-induced bone demineralisation. More common in active disease. At high levels (≥ 2.9 mmol/L) should be treated as a medical emergency. See our notes on hypercalcaemia.
- Recurrent or persistent bacterial infection: immune dysfunction and hypogammaglobulinaemia due to suppression of normal plasma cell function.

Mnemonic

These key clinical features of MM can be remembered using the mnemonic 'CRAB'.

- C - calcium levels high
- R - renal impairment
- A - anaemia
- B - bone disease

Other syndromes

MM can present with a myriad of other clinical features, some presenting as medical emergencies. These may include paraesthesia, fever (<1%), splenomegaly (1%), hepatomegaly (4%) or lymphadenopathy (1%). Neurological involvement can result from hyperviscosity syndromes, spinal cord compression, peripheral neuropathy or radiculopathy.

- Hyperviscosity syndrome: may develop with high paraprotein levels (i.e. high IgA or IgG). Typical symptoms include blurred vision, headaches, mucosal bleeding and dyspnoea due to heart failure. Requires urgent plasma exchange.
- Spinal cord compression: can occur in 5% of patients during course of disease. Highly variable depending on lesion causing compression, location, and rate of development. See our notes on cord compression.

There should be a low threshold for myeloma screening in any patient with suggestive clinical features.

Myeloma should be suspected in any patient with typical features, but particularly those over 60 years old with:

- Unexplained bone pain (and pathological fractures)
- Fatigue
- Symptoms of hypercalcaemia: bone pain, abdo pain, constipation, confusion, polyuria
- Weight loss
- Symptoms of cord compression: back pain, new leg weakness, bladder/bowel dysfunction

- Symptoms of hyperviscosity: headache, blurred vision, shortness of breath, mucosal bleeding
- Recurrent infections

Screening for myeloma

'Screening' for myeloma involves looking for monoclonal antibodies, which are the secretion product of the malignant clones.

When we 'screen' for myeloma we are looking for the secretion product of the malignant clone of plasma cells - the monoclonal antibodies. We can do this using protein electrophoresis and immunofixation. Electrophoresis tells us whether there is an increased number of antibodies. This is followed by immunofixation, which tells us what type of antibody has increased (i.e. is it a monoclonal antibody). MM is usually the result of IgG, IgA or the accompanying light chain. It rarely occurs with IgM.

The presence of an IgM monoclonal antibody suggests another haematological malignancy termed Waldenstrom macroglobulinemia.

Protein electrophoresis

This is a quantitative test that separates proteins into different bands using an electric current. The distance individual proteins travel is dependent on their shape, size and electrical charge. Electrophoresis gives us characteristic band patterns including normal, polyclonal and monoclonal.

Immunofixation

Immunofixation is a qualitative test that 'fixes' proteins in place by using antibodies. It is important for the identification of proteins after separation by electrophoresis.

Urine electrophoresis and serum free light chains

Protein electrophoresis assumes that all myelomas secrete an intact antibody. In fact, around 20% of myelomas only secrete light chains. To help detect these myelomas we can send off serum free light chains (SFLCs) or urine for electrophoresis.

SFLCs is a newer test that looks at the amount of light chain unbound to heavy chains within the blood. Light chains are secreted in healthy individuals as plasma cells produce more light chains than heavy chains. Therefore, it is the ratio between the light chains kappa and lambda, which is the most important factor. An elevated ratio is suggestive of myeloma and needs further work-up.

Alternatively, a urine electrophoresis can be completed. Light chains within the serum may be filtered by the kidneys into the urine. Monoclonal light chains detected in the urine are known as Bence-Jones proteins. NICE recommend the use of serum protein electrophoresis and SFLCs in the work-up of suspected myeloma. Depending on the centre, urine electrophoresis may still be used in combination with the above two tests or as an alternative to SFLCs.

A small percentage of patients with MM do not have detectable paraprotein levels (i.e. protein electrophoresis and SFLCs are negative). These patients have non-secretory myeloma.

Diagnostic criteria

Diagnosis of MM involves identifying a monoclonal antibody, bone marrow analysis and assessing organ damage.

The work-up and diagnosis of MM is dependent on identification of a monoclonal antibody (sometimes referred to as M protein or paraprotein), analysis of the bone marrow to look for a population of malignant clonal plasma cells and further laboratory tests and imaging to assess for myeloma-related organ damage.

- Monoclonal antibody detection: protein electrophoresis & immunoglobulins, SFLCs +/- urine electrophoresis for Bence-Jones protein
- Bone marrow infiltration: bone marrow aspirate and trephine with cytogenetics
- Myeloma-related organ damage: FBC, U&Es, bone profile, imaging (whole body MRI or low-dose whole body CT if MRI not suitable). Skeletal survey (x-rays) only used if CT/MRI not possible
- Staging if confirmed myeloma: beta-2 microglobulin, albumin

Collectively, these investigations are important as part of the diagnostic criteria of myeloma. They help differentiate between MGUS, smouldering/asymptomatic myeloma and multiple myeloma.

Treatment principles of myeloma

MM is an incurable condition, treatments aim to increase periods of disease remission.

There are many options for the treatment of myeloma. Management depends on a patients fitness and co-morbidities, disease severity, initial response to treatment, relapse(s) and previous therapy. All patients should be discussed in an MDT specialising in myeloma and have access to psychological services, palliative care and support, specialist nurses and clinical research.

Unfortunately, there is no cure for myeloma. The aim is to induce disease remission and then maintain disease free survival for as long as possible with ongoing monitoring for disease relapse. The four key areas of management include: induction therapy, autologous stem cell transplantation (ASCT), maintenance therapy and managing relapse or refractory disease.

- Induction therapy: initial treatment option. Aim to induce remission. Usually combination of three drugs. Choice depends on high-risk features, co-morbidities and plan for ASCT. Example is VRd (Velcade - bortezomib / Revlimid - lenalidomide / dexamethasone - steroid)
- ASCT: if suitable for transplant, provides best option for long period of remission. Stem cells mobilised, harvested and stored following induction. Subsequently given high-dose chemotherapy (e.g. melphalan). Stem cells then re-infused.

- Maintenance: used to maintain disease remission as long as possible. Given post-induction or post-transplant. Choices include bortezomib or lenalidomide. Typically given until progression.
- Relapse or refractory disease: almost all patients will relapse, even if they respond to treatment. Therapy indicated if a clinical relapse or rapid rise in paraproteins. Choices include ASCT, rechallenge with previous regimen, or new therapy.

Treatment of myeloma complications

Myeloma is associated with a number of complications, which require further management.

Myeloma can lead to devastating complications due to its proliferation in bones, affect on the kidneys and neurological involvement. The management of individual complications is beyond the scope of these notes, but use the linked below for further information on these topics (albeit not specifically linked to myeloma).

- Myeloma bone disease: Bisphosphonates used for boney pain.
- Hypercalcaemia
- Cord compression
- Renal impairment
- Anaemia

Prognosis

MM is an incurable disease with a variable natural history.

Patients with myeloma will invariably relapse following treatment. Subsequent relapses are associated with reducing response to treatment. The median survival is highly variable between patients depending on response to treatment, age of onset and cytogenetic abnormalities.

Beta-2 microglobulin is often used as a prognostic tool in the International Staging System (ISS) for myeloma. This divides patients into three groups (I, II, III) based on serum beta-2 microglobulin and albumin levels, which predicts the median survival.

- Stage I: median survival 62 months
- Stage II: median survival 44 months
- Stage III: median survival of 29 months

Other factors associated with a worse prognosis include high plasma cell counts, high levels of monoclonal antibody in blood/urine or development of complications (e.g. diffuse multiple bone lesions, marked anaemia, hypercalcaemia and renal impairment).

Proteinuria

Proteinuria is high levels of protein in your urine. Causes may include relatively harmless conditions, including dehydration or intense exercise, or more serious, including kidney

disease or immune disorders. Testing can confirm proteinuria, and a treatment plan can help you manage it. Proteinuria (pro-tee-nyur-ee-uh) is a high level of protein in your urine (pee). This condition can be a sign of kidney damage.

Proteins have many important functions, including:

- Building muscles and bones.
- Regulating the amount of fluid in your blood.
- Fighting off infection.
- Repairing damaged tissues.

Proteins should remain in your blood. If proteins enter your pee, they ultimately leave your body, which can harm your overall health.

Proteinuria is an early sign of chronic kidney disease (CKD), although you can have CKD and have normal levels of protein in your urine. CKD is a gradual loss of your kidney functions, which may eventually require a kidney replacement therapy, dialysis or kidney transplant. Diabetes and high blood pressure (hypertension) pressure can damage your kidneys. They're the two most common causes of kidney disease.

Glomeruli (glo-mare-yoo-lye) are groups of tiny blood vessels in your kidneys. They perform the first stage of filtering waste products and excess water from your blood. The waste products and excess water leave your body through your pee. Glomeruli don't allow passage of larger proteins or blood cells into your pee. If smaller proteins sneak through your glomeruli, then long, thin tubes in your kidneys (tubules) recover the proteins and keep them in your body.

Proteins may flow into your pee if there's:

- Damage to your glomeruli or tubules.
- A problem with the reabsorption process of the proteins.

Symptoms and Causes

In advanced stages of proteinuria, symptoms may include:

- Swelling (edema) in your face, belly, feet or ankles.
- More frequent urination.
- Shortness of breath.
- Tiredness.
- Nausea and vomiting.
- Lack of appetite.
- Muscle cramping at night.
- Puffiness around your eyes, especially in the morning.

- Foamy or bubbly urine.

These symptoms are also symptoms of chronic kidney disease.

Causes

In many cases, relatively benign (noncancerous) or temporary medical conditions cause proteinuria. These conditions may include:

- Dehydration.
- Inflammation.
- Low blood pressure (hypotension).
- Kidney stones.

Intense exercise, stress, taking aspirin every day (aspirin therapy) and exposure to cold temperatures can also trigger proteinuria.

More serious medical conditions can also damage your kidneys and cause proteinuria. These conditions may include:

- Certain immune disorders, including lupus and Goodpasture's syndrome.
- Acute kidney inflammation (glomerulonephritis).
- Plasma cell cancer (multiple myeloma).
- The destruction of red blood cells, which causes hemoglobin to release into your bloodstream (intravascular hemolysis).
- Cardiovascular disease.
- The simultaneous development of proteinuria and hypertension in a pregnant person (preeclampsia).
- Poisoning.
- Trauma.
- Kidney cancer.
- Congestive heart failure.

Treatment

The underlying kidney disease may be treatable with drugs. The type of treatment depends on the cause. Information is contained in the sections on each individual disease (see above for the types of kidney diseases). Water retention can be treated by reducing the amount of salt and water taken in your diet each day. Some cases also require drugs to make the kidneys produce more urine. High blood pressure can be treated by reducing salt in your diet, and often with drugs to take each day. A high cholesterol level may be treated with dietary control (eating less fat) and, in some cases, with cholesterol lowering drugs.

Glomerulonephritis

Glomerulonephritis is a kind of kidney disease. It involves damage to your glomeruli, tiny filters inside your kidneys. Some people don't show any symptoms. Infections and immune system disorders are one of the many causes. Sometimes, glomerulonephritis is mild and goes away without treatment. Other times it leads to kidney failure and other complications.

Causes

Glomerulonephritis can be caused by various factors including:

- Toxins or medicines
- Viral infections, such as HIV, hepatitis B and C viruses
- IgA nephropathy
- Lupus-related kidney inflammation
- Bacterial infections that commonly cause throat and skin infections, such as strep or staph bacteria

Symptoms of glomerulonephritis

The kidneys can be badly damaged before any symptoms appear. These are the most common symptoms:

- Fatigue
- High blood pressure
- Swelling of the face, hands, feet, and belly
- Blood and protein in the urine (hematuria and proteinuria)
- Decreased urine output

The symptoms of glomerulonephritis may look like other medical conditions or problems. Always talk with your healthcare provider for a diagnosis.

Diagnoses

Tests may include:

- **Urinalysis.** This test checks urine for red and white blood cells, infection, or too much protein.
- **Blood tests.** Tests to measure the levels of waste products to find out how well the kidneys are filtering.
- **Ultrasound of the kidney.** This test uses high-frequency sound waves and a computer to make images of blood vessels, tissues, and organs. It's done to see whether the shape or size of the kidney is abnormal. Ultrasounds are used to view organs as they work, and to check blood flow through blood vessels.

- **Kidney biopsy.** In this test, tissue samples are removed from the kidney and checked under a microscope.

Treatment

Your healthcare provider will figure out the best treatment based on:

- How old you are
- Your overall health and medical history
- How sick you are
- How well you can handle specific medicines, procedures, or therapies
- How long the condition is expected to last
- Your opinion or preference

Unfortunately, kidney disease cannot be cured. Treatments focus on slowing the progression of the disease and preventing complications. Treatment may include:

- **Blood pressure medicines** such as ACE (angiotensin-converting enzymes) inhibitors that protect blood flow into the kidneys.
- **Corticosteroids** may be used to decrease inflammation that leads to scar tissue.
- **Diuretics** (water pills) may be used to remove excess fluid in the body through more urine production.
- **Diet changes** including eating less protein, sodium, and potassium.
- **Dialysis** to remove wastes and fluid from the blood after the kidneys has stopped working.
- **Kidney transplant** that replaces your diseased kidney with a healthy kidney from a donor.

Nephrotic syndrome

Nephrotic syndrome is a kidney disorder that causes your body to pass too much protein in your urine. Nephrotic syndrome is usually caused by damage to the clusters of small blood vessels in your kidneys that filter waste and excess water from your blood. The condition causes swelling, particularly in your feet and ankles, and increases the risk of other health problems.

Kidney cross section

The kidneys remove waste and excess fluid from your blood through filtering units called nephrons. Each nephron contains a filter (glomerulus) that has a network of tiny blood vessels called capillaries. When blood flows into a glomerulus, tiny molecules — water, essential minerals and nutrients, and wastes — pass through the capillary walls. Large molecules, such as proteins and red blood cells, do not. The filtered solution then passes into another part of the nephron called the tubule. The water, nutrients and minerals your body

needs are transferred back to the bloodstream. The excess water and waste become urine that flows to the bladder.

Treatment for nephrotic syndrome includes treating the condition that's causing it and taking medications. Nephrotic syndrome can increase your risk of infections and blood clots. Your doctor might recommend medications and dietary changes to prevent complications.

Symptoms

Signs and symptoms of nephrotic syndrome include:

- Severe swelling (edema), particularly around your eyes and in your ankles and feet
- Foamy urine, a result of excess protein in your urine
- Weight gain due to fluid retention
- Fatigue
- Loss of appetite

Causes

Nephrotic syndrome is usually caused by damage to the clusters of tiny blood vessels (glomeruli) of your kidneys.

The glomeruli filter your blood as it passes through your kidneys, separating things your body needs from those it doesn't. Healthy glomeruli keep blood protein (mainly albumin) — which is needed to maintain the right amount of fluid in your body — from seeping into your urine. When damaged, glomeruli allow too much blood protein to leave your body, leading to nephrotic syndrome.

Many possible causes

Many diseases and conditions can cause glomerular damage and lead to nephrotic syndrome, including:

- **Diabetic kidney disease.** Diabetes can lead to kidney damage (diabetic nephropathy) that affects the glomeruli.
- **Minimal change disease.** This is the most common cause of nephrotic syndrome in children. Minimal change disease results in abnormal kidney function, but when the kidney tissue is examined under a microscope, it appears normal or nearly normal. The cause of the abnormal function typically can't be determined.
- **Focal segmental glomerulosclerosis.** Characterized by scarring of some of the glomeruli, this condition can result from another disease, a genetic defect or certain medications or occur for no known reason.
- **Membranous nephropathy.** This kidney disorder is the result of thickening membranes within the glomeruli. The thickening is due to deposits made by the immune system. It can be associated with other medical conditions, such as lupus, hepatitis B, malaria and cancer, or it can occur for no known reason.

- **Systemic lupus erythematosus.** This chronic inflammatory disease can lead to serious kidney damage.
- **Amyloidosis.** This disorder occurs when amyloid proteins accumulate in your organs. Amyloid buildup often damages the kidneys' filtering system.

Risk factors

Factors that can increase your risk of nephrotic syndrome include:

- **Medical conditions that can damage your kidneys.** Certain diseases and conditions increase your risk of developing nephrotic syndrome, such as diabetes, lupus, amyloidosis, reflux nephropathy and other kidney diseases.
- **Certain medications.** Medications that might cause nephrotic syndrome include nonsteroidal anti-inflammatory drugs and drugs used to fight infections.
- **Certain infections.** Infections that increase the risk of nephrotic syndrome include HIV, hepatitis B, hepatitis C and malaria.

Complications

Possible complications of nephrotic syndrome include:

- **Blood clots.** The inability of the glomeruli to filter blood properly can lead to loss of blood proteins that help prevent clotting. This increases your risk of developing a blood clot in your veins.
- **High blood cholesterol and elevated blood triglycerides.** When the level of the protein albumin in your blood falls, your liver makes more albumin. At the same time, your liver releases more cholesterol and triglycerides.
- **Poor nutrition.** Loss of too much blood protein can result in malnutrition. This can lead to weight loss, which can be masked by edema. You may also have too few red blood cells (anemia), low blood protein levels and low levels of vitamin D.
- **High blood pressure.** Damage to your glomeruli and the resulting buildup of excess body fluid can raise your blood pressure.
- **Acute kidney injury.** If your kidneys lose their ability to filter blood due to damage to the glomeruli, waste products can build up quickly in your blood. If this happens, you might need emergency dialysis — an artificial means of removing extra fluids and waste from your blood — typically with an artificial kidney machine (dialyzer).
- **Chronic kidney disease.** Nephrotic syndrome can cause your kidneys to lose their function over time. If kidney function falls low enough, you might need dialysis or a kidney transplant.
- **Infections.** People with nephrotic syndrome have an increased risk of infections.

Diagnosis

Tests and procedures used to diagnose nephrotic syndrome include:

Urine tests. A urinalysis can reveal abnormalities in your urine, such as large amounts of protein. You might be asked to collect urine samples over 24 hours.

Blood tests. A blood test can show low levels of the protein albumin and often decreased levels of blood protein overall. Loss of albumin is often associated with an increase in blood cholesterol and blood triglycerides. The creatinine and urea nitrogen levels in your blood also might be measured to assess your overall kidney function.

Kidney biopsy. Your doctor might recommend removing a small sample of kidney tissue for testing. During a kidney biopsy, a needle is inserted through your skin and into your kidney. Kidney tissue is collected and sent to a lab for testing.

Treatment

Treatment for nephrotic syndrome involves treating any medical condition that might be causing your nephrotic syndrome. Your doctor might also recommend medications and changes in your diet to help control your signs and symptoms or treat complications of nephrotic syndrome.

Medications might include:

- **Blood pressure medications.** Drugs called angiotensin-converting enzyme (ACE) inhibitors reduce blood pressure and the amount of protein released in urine. Medications in this category include lisinopril (Prinivil, Qbrelis, Zestril), benazepril (Lotensin), captopril and enalapril (Vasotec).

Another group of drugs that works similarly is called angiotensin II receptor blockers (ARBs) and includes losartan (Cozaar) and valsartan (Diovan). Other medications, such as renin inhibitors, also might be used, though angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) are generally used first.

- **Water pills (diuretics).** These help control swelling by increasing your kidneys' fluid output. Diuretic medications typically include furosemide (Lasix). Others include spironolactone (Aldactone, Carospir) and thiazides, such as hydrochlorothiazide or metolazone (Zaroxolyn).

- **Cholesterol-reducing medications.** Statins can help lower cholesterol levels. However, it's not clear whether cholesterol-lowering medications can improve the outcomes for people with nephrotic syndrome, such as avoiding heart attacks or decreasing the risk of early death.

Statins include atorvastatin (Lipitor), fluvastatin (Lescol XL), lovastatin (Altoprev), pravastatin (Pravachol), rosuvastatin (Crestor, Ezallor) and simvastatin (Zocor).

- **Blood thinners (anticoagulants).** These might be prescribed to decrease your blood's ability to clot, especially if you've had a blood clot. Anticoagulants include heparin, warfarin (Coumadin, Jantoven), dabigatran (Pradaxa), apixaban (Eliquis) and rivaroxaban (Xarelto).

- **Immune system-suppressing medications.** Medications to control the immune system, such as corticosteroids, can decrease the inflammation that accompanies some

of the conditions that can cause nephrotic syndrome. Medications include rituximab (Rituxan), cyclosporine and cyclophosphamide.

Haemoglobinopathies - Sickle cell anaemia and Thalassaemia

Haemoglobinopathies are genetic disorders (usually autosomal recessive) that alter the structure of haemoglobin. This may result in deformed structures, or reduced production of particular globin chains – called thalassaemias. This results in reduced oxygen carrying capacity of the blood, and produces symptoms of anaemia. The most common types of haemoglobinopathies are thalassaemias and sickle cell disease.

Sickle cell anaemia

Sickle cell anemia, or sickle cell disease (SCD), is a genetic disease of the red blood cells (RBCs). Normally, RBCs are shaped like discs, which gives them the flexibility to travel through even the smallest blood vessels. However, with this disease, the RBCs have an abnormal crescent shape resembling a sickle. This makes them sticky and rigid and prone to getting trapped in small vessels, which blocks blood from reaching different parts of the body. This can cause pain and tissue damage.

Symptoms

Symptoms of sickle cell anemia usually show up at a young age. They may appear in babies as early as 4 months old, but generally occur around the 6-month mark.

While there are multiple types of SCD, they all have similar symptoms, which vary in severity. These include:

- excessive fatigue or irritability, from anemia
- fussiness, in babies
- bedwetting, from associated kidney problems
- jaundice, which is yellowing of the eyes and skin
- swelling and pain in hands and feet
- frequent infections
- pain in the chest, back, arms, or legs

Types of sickle cell disease

Hemoglobin is the protein in red blood cells that carries oxygen. It normally has two alpha chains and two beta chains. The four main types of sickle cell anemia are caused by different mutations in these genes.

Hemoglobin SS disease

Hemoglobin SS disease is the most common type of sickle cell disease. It occurs when you inherit copies of the hemoglobin S gene from both parents. This forms hemoglobin known as Hb SS. As the most severe form of SCD, individuals with this form also experience the worst symptoms at a higher rate.

Hemoglobin SC disease

Hemoglobin SC disease is the second most common type of sickle cell disease. It occurs when you inherit the Hb C gene from one parent and the Hb S gene from the other. Individuals with Hb SC have similar symptoms to individuals with Hb SS. However, the anemia is less severe.

Hemoglobin SB+ (beta) thalassemia

Hemoglobin SB+ (beta) thalassemia affects beta globin gene production. The size of the red blood cell is reduced because less beta protein is made. If inherited with the Hb S gene, you will have hemoglobin S beta thalassemia. Symptoms are not as severe.

Hemoglobin SB 0 (Beta-zero) thalassemia

Sickle beta-zero thalassemia is the fourth type of sickle cell disease. It also involves the beta globin gene. It has similar symptoms to Hb SS anemia. However, sometimes the symptoms of beta zero thalassemia are more severe. It is associated with a poorer prognosis.

Hemoglobin SD, hemoglobin SE, and hemoglobin SO

These types of sickle cell disease are more rare and usually don't have severe symptoms.

Sickle cell trait

People who only inherit a mutated gene (hemoglobin S) from one parent are said to have sickle cell trait. They may have no symptoms or reduced symptoms.

Risks

Children are only at risk for sickle cell disease if both parents carry sickle cell trait. A blood test called a hemoglobin electrophoresis can also determine which type you might carry. People from regions that have endemic malaria are more likely to be carriers. This includes people from:

- Africa
- India
- the Mediterranean
- Saudi Arabia

Complications

SCD can cause severe complications, which appear when the sickle cells block vessels in different areas of the body. Painful or damaging blockages are called sickle cell crises. They can be caused by a variety of circumstances, including:

- illness
- changes in temperature
- stress

- poor hydration
- altitude

The following are types of complications that can result from sickle cell anemia.

Severe anemia

Anemia is a shortage of RBCs. Sickle cells are easily broken. This breaking apart of RBCs is called chronic hemolysis. RBCs generally live for about 120 days. Sickle cells live for a maximum of 10 to 20 days.

Hand-foot syndrome

Hand-foot syndrome occurs when sickle-shaped RBCs block blood vessels in the hands or feet. This causes the hands and feet to swell. It can also cause leg ulcers. Swollen hands and feet are often the first sign of sickle cell anemia in babies.

Splenic sequestration

Splenic sequestration is a blockage of the splenic vessels by sickle cells. It causes a sudden, painful enlargement of the spleen. The spleen may have to be removed due to complications of sickle cell disease in an operation known as a splenectomy. Some sickle cell patients will sustain enough damage to their spleen that it becomes shrunken and ceases to function at all. This is called autosplenectomy. Patients without a spleen are at higher risk for infections from bacteria such as *Streptococcus*, *Haemophilus*, and *Salmonella* species.

Delayed growth

Delayed growth often occurs in people with SCD. Children are generally shorter but regain their height by adulthood. Sexual maturation may also be delayed. This happens because sickle cell RBCs can’t supply enough oxygen and nutrients.

Neurological complications

Seizures, strokes, or even coma can result from sickle cell disease. They are caused by brain blockages. Immediate treatment should be sought.

Eye problems

Blindness is caused by blockages in the vessels supplying the eyes. This can damage the retina.

Skin ulcers

Skin ulcers in the legs can occur if small vessels there are blocked.

Heart disease and chest syndrome

Since SCD interferes with blood oxygen supply, it can also cause heart problems which can lead to heart attacks, heart failure, and abnormal heart rhythms.

Lung disease

Damage to the lungs over time related to decreased blood flow can result in high blood pressure in the lungs (pulmonary hypertension) and scarring of the lungs (pulmonary fibrosis). These problems can occur sooner in patients who have sickle chest syndrome. Lung damage makes it more difficult for the lungs to transfer oxygen into the blood, which can result in more frequent sickle cell crises.

Priapism

Priapism is a lingering, painful erection that can be seen in some men with sickle cell disease. This happens when the blood vessels in the penis are blocked. It can lead to impotence if left untreated.

Gallstones

Gallstones are one complication not caused by a vessel blockage. Instead, they are caused by the breakdown of RBCs. A byproduct of this breakdown is bilirubin. High levels of bilirubin can lead to gallstones. These are also called pigment stones.

Sickle chest syndrome

Sickle chest syndrome is a severe type of sickle cell crisis. It causes severe chest pain and is associated with symptoms such as cough, fever, sputum production, shortness of breath, and low blood oxygen levels. Abnormalities observed on chest X-rays can represent either pneumonia or death of lung tissue (pulmonary infarction). The long-term prognosis for patients who have had sickle chest syndrome is worse than for those who have not had it.

Diagnoses

All newborns in the United States are screened for sickle cell disease. Prebirth testing looks for the sickle cell gene in your amniotic fluid. In children and adults, one or more of the following procedures may also be used to diagnose sickle cell disease.

Detailed patient history

This condition often first appears as acute pain in the hands and feet. Patients may also have:

- severe pain in the bones
- anemia
- painful enlargement of the spleen
- growth problems
- respiratory infections
- ulcers of the legs
- heart problems

Blood tests

Several blood tests can be used to look for SCD:

- Blood counts can reveal an abnormal Hb level in the range of 6 to 8 grams per deciliter.
- Blood films may show RBCs that appear as irregularly contracted cells.
- Sickle solubility tests look for the presence of Hb S.

Hb electrophoresis

Hb electrophoresis is always needed to confirm the diagnosis of sickle cell disease. It measures the different types of hemoglobin in the blood.

Treatment

A number of different treatments are available for SCD:

- Rehydration with intravenous fluids helps red blood cells return to a normal state. The red blood cells are more likely to deform and assume the sickle shape if you're dehydration.
- Treating underlying or associated infections is an important part of managing the crisis, as the stress of an infection can result in a sickle cell crisis. An infection may also result as a complication of a crisis.
- Blood transfusions improve transport of oxygen and nutrients as needed. Packed red cells are removed from donated blood and given to patients.
- Supplemental oxygen is given through a mask. It makes breathing easier and improves oxygen levels in the blood.
- Pain medication is used to relieve the pain during a sickle crisis. You may need over-the-counter drugs or strong prescription pain medication like morphine.
- (Droxia, Hydrea) helps to increase production of fetal hemoglobin. It may reduce the number of blood transfusions.
- Immunizations can help prevent infections. Patients tend to have lower immunity.

Bone marrow transplant has been used to treat sickle cell anemia. Children younger than 16 years of age who have severe complications and have a matching donor are the best candidates.

Thalassemia

Thalassemia is an inherited blood disorder wherein the body produces an inadequate amount of haemoglobin. Haemoglobin is a protein molecule that carries oxygen in the red blood cells. This disorder causes the destruction of the red blood cells which leads to anaemia. Anaemia is a condition in which the haemoglobin or red blood cells are less than the normal count.

It is an inherited disease which is mainly caused due to abnormal haemoglobin synthesis. It is transferred by one of the parents who is a carrier of this disease due to either deletion of particular key gene fragments or a genetic mutation.

Mild thalassemia requires no treatment, but acute thalassemia might require regular blood transfusions.

Types of Thalassemia

There are two types of thalassemia:

- **Alpha-thalassemia** – A disorder in which one of the genes of alpha-globin has a mutation or abnormality.
- **Beta-thalassemia** – The genes of beta-globin are abnormal.

Causes of Thalassemia

It develops when there is some abnormality in any one of the genes that are involved in the production of haemoglobin and this defect is inherited from the parents. If any of the parents have thalassemia, the baby is more likely to develop this disease so-called thalassemia minor. If both the parents suffer from this disease, you are more likely to get the disease.

There are no symptoms at an early stage but are likely to be a disease carrier. It is the most common disease in people of Asia, Africa, the Middle East, Turkey, and Greece.

Symptoms of Thalassemia

Beta-thalassemia

Beta thalassemia occurs in two different forms namely thalassemia intermedia and thalassemia major.

Thalassemia symptoms appear generally before a child's second year of age and severe anaemia concerned with this condition can be fatal. Some of the major signs of thalassemia major include:

- Paleness
- Jaundice
- Fussiness
- Poor appetite

This kind of thalassemia is so serious that it needs frequent blood transfusions. Thalassemia intermedia is a less serious kind of beta-thalassemia and do not require the patient to go through blood transfusions.

Beta-Thalassemia trait is found in individuals where there is only one *HBB* gene mutation in each cell possesses mild anaemia.

Alpha-thalassemia

It consists of two major forms namely, Hydrops fetalis or Haemoglobin H disease.

Haemoglobin H can be responsible for bone complexities. The forehead, cheeks, and jaw may overgrow. Moreover, haemoglobin H can cause:

- An intensely enlarged spleen
- Malnourishment

Treatment for Thalassemia

The treatment depends on the type and severity of the disease. The doctor provides a course of treatment that suits best for a particular case.

Some of the treatments, which are opted for maximum cases include:

- Bone marrow transplant (BMT)
- Supplements and Medications
- Blood transfusions

Few precautions are prescribed by the doctors that include not taking supplements or vitamins and minerals containing iron. This is true when there is a need for blood transfusions. Patients who go through blood transfusions obtain extra iron which a body cannot lose.

If you are receiving a blood transfusion, you may need chelation therapy. It includes taking a chemical injection that combines with other heavy metals and iron. This helps eliminate extra iron from the body.

Prevention of Thalassemia

Thalassemia cannot be prevented since it is a genetically inherited disorder. However, these disorders can be detected during prenatal tests before birth. Also, genetic counselling helps to detect whether people have altered or missing haemoglobin genes that cause thalassemia.

Phenylketonuria

Phenylketonuria also called PKU, is a rare inherited disorder that causes an amino acid called phenylalanine to build up in the body. PKU is caused by a change in the phenylalanine hydroxylase (PAH) gene. This gene helps create the enzyme needed to break down phenylalanine.

Without the enzyme necessary to break down phenylalanine, a dangerous buildup can develop when a person with PKU eats foods that contain protein or eats aspartame, an artificial sweetener. This can eventually lead to serious health problems.

Symptoms

Newborns with PKU initially don't have any symptoms. However, without treatment, babies usually develop signs of PKU within a few months.

Signs and symptoms of untreated PKU can be mild or severe and may include:

 A musty odor in the breath, skin or urine, caused by too much phenylalanine in the body

 Nervous system (neurological) problems that may include seizures

 Skin rashes, such as eczema

 Lighter skin, hair and eye color than family members, because phenylalanine can't transform into melanin — the pigment responsible for hair and skin tone

 Unusually small head size (microcephaly)

 Hyperactivity

 Intellectual disability

 Delayed development

 Behavioral, emotional and social problems

 Mental health disorders Severity varies

The severity of PKU depends on the type.

 Classic PKU. The most severe form of the disorder is called classic PKU. The enzyme needed to break down phenylalanine is missing or severely reduced. This results in high levels of phenylalanine that can cause severe brain damage.

 Less severe forms of PKU. In mild or moderate forms, the enzyme still has some function, so phenylalanine levels are not as high, resulting in a smaller risk of significant brain damage.

Pregnancy and PKU

Women who have PKU and become pregnant are at risk of another form of the condition called maternal PKU. If women don't follow the special PKU diet before and during pregnancy, blood phenylalanine levels can become high and harm the developing baby.

Even women with less severe forms of PKU may place their unborn children at risk by not following the PKU diet.

Babies born to women with high phenylalanine levels don't often inherit PKU. But a child can have serious problems if the level of phenylalanine is high in the mother's blood during pregnancy. At birth, the baby may have:

 Low birth weight

 Unusually small head

 Problems with the heart

In addition, maternal PKU can cause the child to have delayed development, intellectual disability and problems with behavior.

Causes

Autosomal recessive inheritance

A gene change (genetic mutation) causes PKU, which can be mild, moderate or severe. In a person with PKU, a change in the phenylalanine hydroxylase (PAH) gene causes a lack of or reduced amount of the enzyme that's needed to process phenylalanine, an amino acid.

A dangerous buildup of phenylalanine can develop when a person with PKU eats protein-rich foods, such as milk, cheese, nuts or meat, or grains such as bread and pasta, or aspartame, an artificial sweetener.

Inheritance

For a child to inherit PKU, both the mother and father must have and pass on the changed gene. This pattern of inheritance is called autosomal recessive.

It's possible for a parent to be a carrier — to have the changed gene that causes PKU, but not have the disease. If only one parent has the changed gene, there's no risk of passing PKU to a child, but it's possible for the child to be a carrier.

Most often, PKU is passed to children by two parents who are both carriers of the changed gene, but don't know it.

Risk factors

Risk factors for inheriting PKU include:

 Having both parents with a gene change that causes PKU. Two parents must pass along a copy of the changed gene for their child to develop the condition.

 Being of a certain racial or ethnic descent. PKU affects people from most ethnic backgrounds worldwide. But in the United States, it's most common in people of European ancestry and much less common in people of African ancestry.

Complications

Untreated PKU can lead to complications in infants, children and adults with the disorder. When women with PKU have high blood phenylalanine levels during pregnancy, it can harm their unborn baby.

Untreated PKU can lead to:

 Irreversible brain damage and marked intellectual disability beginning within the first few months of life

 Neurological problems such as seizures and tremors

 Behavioral, emotional and social problems in older children and adults

 Major health and developmental problems

Prevention

If you have PKU and are considering getting pregnant:

 Follow a low-phenylalanine diet. Women with PKU can prevent harm to their developing baby by sticking to or returning to a low-phenylalanine diet before becoming pregnant. Nutritional supplements designed for people with PKU can ensure enough protein and nutrition during pregnancy. If you have PKU, talk to your health care provider before you start trying to conceive.

 Consider genetic counseling. If you have PKU, a close relative with PKU or a child with PKU, you may benefit from genetic counseling before becoming pregnant. A specialist in medical genetics (geneticist) can help you better understand how PKU is passed through your family. The specialist can also help determine your risk of having a child with PKU and assist with family planning.

Tyrosinosis

Tyrosinemia is hereditary; in order to have the disease, a child must get a mutation in the gene for tyrosinemia from each parent. In families where both parents carry a mutation, there is a one in four risk that a child will have tyrosinemia. There is now a genetic test available, so that couples at high risk of being carriers can determine their risk of having a child with tyrosinemia. This is a very rare disease; only about one person in 100,000 has it.

In tyrosinemia, the body doesn't have an enzyme it needs [called fumarylacetoacetate hydrolase (FAH)] to metabolize tyrosine. Metabolism is a process in which our bodies break down substances as we use them for energy; in this case tyrosine. Tyrosine is an amino acid that is found in most proteins. When people with tyrosinemia break down protein, abnormal toxic break down products of tyrosine build up in their bodies. This causes progressive damage to the liver and kidneys, but mainly the liver. This is because the liver is normally the primary place tyrosine is metabolized.

Symptoms

Tyrosinemia symptoms tend to fall into two categories, acute and chronic. In the acute form of tyrosinemia, babies experience symptoms within months of birth. They may not gain weight properly, have an enlarged liver and spleen and a swollen abdomen, which are symptoms of other liver diseases. Jaundice is unusual. Babies with tyrosinemia also have swelling of the legs, and an increased tendency to bleed, particularly nosebleeds. These babies may need liver transplants right away.

The chronic form of tyrosinemia presents after 6 months with a more gradual onset and less severe symptoms. Enlargement of the liver and spleen are the main symptoms, the abdomen is distended with fluid, and these children may have trouble gaining weight. They may vomit or have diarrhea. Liver disease develops more slowly, eventually leading to cirrhosis.

Tyrosinemia Diagnosis

Tyrosinemia is diagnosed based on blood tests and urine tests. In both the acute and chronic forms of the disease, liver function tests are often abnormal. Low serum albumin and clotting factors are also frequently found. Because of the biochemical defect, the abnormal product Succinylacetone may be measured in the urine, which confirms the diagnosis.

In the United States tyrosinemia is included in the newborn screening programme, so nowadays children are usually detected before they become unwell. It is possible to test for tyrosinemia while the baby is still developing in the womb. Doctors can detect mutations or measure succinylacetone in the amniotic fluid.

Tyrosinemia Treatment

The treatment for tyrosinemia is a combination of a low-protein diet and a drug called Nitisinone. Nitisinone prevents the buildup of toxic breakdown products. Meats, dairy products, and other protein rich foods such as nuts and beans should be avoided. Good nutrition and adequate vitamin and mineral intake allow children to grow normally. Children with tyrosinemia do require careful monitoring to ensure normal growth and because there is a risk of developing liver cancer. Children who are treated following newborn screening do not seem to develop liver disease in childhood. For unknown reasons some children with tyrosinemia have learning difficulties.

Liver transplantation is still the only way to correct the metabolism of tyrosine, but this is rarely necessary nowadays. More than 90% of children respond very well to Nitisinone and diet. At present, liver transplantation is only needed where children with the acute form do not respond to Nitisinone rapidly or where liver cancer is suspected. After receiving a transplant, children can eat a normal diet and lead healthy, active lives.

Alkaptonuria

Alkaptonuria is a rare inherited disorder. It occurs when your body can't produce enough of an enzyme called homogentisic dioxygenase (HGD). This enzyme is used to break down a toxic substance called homogentisic acid. When you don't produce enough HGD, homogentisic acid builds up in your body. The buildup of homogentisic acid causes your bones and cartilage to become discolored and brittle. This typically leads to osteoarthritis, especially in your spine and large joints. People with alkaptonuria also have urine that turns dark brown or black when it's exposed to air.

Symptoms of Alkaptonuria

Dark stains on a baby's diaper are one of the earliest signs of alkaptonuria. There are few other symptoms during childhood. Symptoms become more obvious as you age.

Your urine may turn dark brown or black when it's exposed to air. By the time you reach your 20s or 30s, you may notice signs of early-onset osteoarthritis.

For example, you may notice chronic stiffness or pain in your lower back or large joints. Other symptoms of alkaptonuria include:

 dark spots in the sclera (white) of your eyes

 thickened and darkened cartilage in your ears

 blue speckled discoloration of your skin, particularly around sweat glands

 dark-colored sweat or sweat stains

 black earwax

 kidney stones and prostate stones

 arthritis (especially hip and knee joints)

Alkaptonuria can also lead to heart problems. The buildup of homogentisic acid causes your heart valves to harden. This can keep them from closing properly, resulting in aortic and mitral valve disorders. In severe cases, heart valve replacement may be necessary. The buildup also causes your blood vessels to harden. This raises your risk of high blood pressure.

Causes

Alkaptonuria is caused by a mutation on your homogentisate 1,2-dioxygenase (HGD) gene. It's an autosomally recessive condition. This means that both of your parents must have the gene in order to pass the condition on to you.

Alkaptonuria is a rare disease. According to the National Institutes of Health, the condition affects about 1 in 250,000 to 1 million people worldwide, but is more common in Slovakia and the Dominican Republic, affecting about 1 in 19,000 people.

Treatment

There's no specific treatment for alkaptonuria. Instead, treatment is focused largely on managing symptoms. There are many therapies that have been tried, but unfortunately they haven't been proven to be effective, and may be harmful or unhelpful in the long term. However, The National Institutes of Health warns that long-term use of vitamin C can sometimes increase the production of kidney stones and has generally proven ineffective for long-term treatment of this condition.

Other treatments for alkaptonuria are focused on preventing and relieving possible complications, such as:

 Arthritis

 Heart disease

 Kidney stones

Maple syrup urine disease (MSUD)

Maple syrup urine disease (MSUD) is a rare, inherited metabolic disorder. The disease prevents your body from breaking down certain amino acids. Amino acids are what remain after your body digests protein from the food you eat. Special enzymes process amino acids so they can be used to maintain all of your body functions. If some of the necessary enzymes are missing or defective, the amino acids and their byproducts, called keto acids, collect in your body. As the levels of these substances increase, it can result in:

- neurological damage
- coma
- life-threatening conditions

In MSUD, the body lacks an enzyme called BCKDC (branched-chain alpha-keto acid dehydrogenase complex). The BCKDC enzyme processes three important amino acids: leucine, isoleucine, and valine, also called BCAAs (branched-chain amino acids). BCAAs are found in foods rich in protein, such as meat, eggs, and milk. When untreated, MSUD can cause significant physical and neurological problems. MSUD can be controlled with dietary restrictions. The success of this method can be monitored with blood tests. Early diagnosis and intervention improve the chance of long-term success.

Types of MSUD

MSUD is also known as:

- BCKDC deficiency
- branched-chain alpha-keto acid dehydrogenase deficiency
- branched-chain ketoaciduria
- branched-chain ketonuria I

There are four subtypes of MSUD. All are inherited genetic disorders. They differ by their degree of enzyme activity, severity, and the age when the disease appears.

Classic MSUD

This is the most common and severe form of the condition. A person with this form has little, if any, enzyme activity — about 2 percent or less of normal activity. Symptoms are present in newborns within a few days of birth. Onset is usually triggered when the infant's body begins to process protein from feedings.

Intermediate MSUD

This is a rare version of MSUD. Symptoms and age of onset vary greatly. People with this type of MSUD have a higher level of enzyme activity than classic MSUD — about 3 to 8 percent of normal activity.

Intermittent MSUD

This form doesn't interfere with normal physical and intellectual growth and development. Symptoms usually don't appear until a child is between 1 and 2 years of age. It's a milder form of classic MSUD. Individuals have significant enzyme activity — about 8 to 15 percent of normal activity. The initial reaction of the disease often occurs when the child experiences stress, illness, or an unusual increase in protein.

Thiamine-responsive MSUD

This rare form of the condition often improves with large doses of thiamine, or vitamin B-1. Symptoms usually occur after infancy. Even though thiamine can be beneficial, dietary restrictions also are necessary.

Symptoms of MSUD

Some initial symptoms characteristic of classic MSUD are:

- lethargy
- poor appetite
- weight loss
- weak sucking ability
- irritability
- a distinctive maple sugar odor in earwax, sweat, and urine
- irregular sleep patterns
- alternating episodes of hypertonia (muscle rigidity) and hypotonia (muscle limpness)
- high-pitched cry

Signs of intermediate and thiamine-response MSUD include:

- seizures
- neurological deficiencies
- developmental delays
- feeding problems
- poor growth
- a distinctive maple sugar odor in earwax, sweat, and urine

Causes of MSUD

MSUD is a recessive genetic disorder. All forms of the disease inherited from your parents. The four varieties of MSUD are caused by mutations, or changes, in the genes that are related to the BCKDC enzymes. When those genes are defective, the BCKDC enzymes aren't produced or don't work properly. These gene mutations are inherited on the chromosomes you receive from your parents.

Typically, parents of children with MSUD don't have the disease and they possess one mutated gene and one normal gene for MSUD. Though they carry the defective recessive gene, they aren't affected by it. Having MSUD means that you inherited one flawed gene for BCKDC from each parent.

Diagnosis of MSUD

Data from the National Newborn Screening and Genetics Resource Center (NNSGRC) indicates that every state in the United States tests infants for MSUD as part of their newborn screening program, which is a blood test that also screens for more than 30 different disorders.

Identifying the presence of MSUD at birth is critical to preventing long-term damage. In cases when both parents are carriers and their child's test is negative for MSUD, additional tests may be advised to confirm the findings and prevent the onset of symptoms.

When symptoms show up after the newborn period, diagnosis of MSUD can be made by a urine analysis or blood test. A urine analysis can detect a high concentration of keto acids, and a blood test can detect a high level of amino acids. The diagnosis of MSUD also can be confirmed with an enzyme analysis of white blood cells or skin cells.

If you are concerned that you might be a carrier of MSUD, genetic testing can confirm if you possess one of the malformed genes that cause the disease. During pregnancy, your physician can use samples obtained by chorionic villus sampling (CVS) or amniocentesis to diagnose your baby.

Complications of MSUD

Complications from undiagnosed and untreated MSUD can be severe and even fatal. Even babies in a treatment plan can experience incidents of extreme sickness, called metabolic crises.

Metabolic crises occur when there is a sudden and intense increase of BCAAs in the system. If untreated, the situation can lead to serious physical and neurological damage. A metabolic crisis usually is indicated by:

- extreme fatigue or lethargy
- loss of alertness
- irritability
- vomiting

When MSUD is undiagnosed, or metabolic crises are untreated, the following severe complications can occur:

- seizures
- swelling of the brain
- lack of blood flow to the brain
- metabolic acidosis — a situation in which the blood contains high levels of acidic substances
- coma

When these conditions occur, they can result in:

- severe neurological damage
- intellectual disability
- blindness
- spasticity, or uncontrolled muscle tightness

Eventually, life-threatening complications can develop and lead to death, especially if they go untreated.

Treatment of MSUD

If your infant is diagnosed with MSUD, prompt medical treatment can avoid serious medical problems and intellectual disability. Initial treatment involves reducing the levels of BCAAs in your baby's blood.

Typically, this involves intravenous (IV) administration of amino acids that don't contain BCAAs, combined with glucose for extra calories. The treatment will promote the utilization of existing leucine, isoleucine, and valine in the body. At the same time it will reduce the BCAA level and provide necessary protein. The goal of the treatment plan is to provide your child with all the protein and nutrients needed for healthy growth and development. The plan will also avoid allowing too many BCAAS to collect in their blood.

Hartnup disease

Hartnup disease is also referred to as Hartnup disorder. It's a hereditary metabolic disorder. It makes it difficult for your body to absorb certain amino acids from your intestine and reabsorb them from your kidneys. Amino acids are essential building blocks for creating protein in your body. Hartnup disease was named for the Hartnup family of England, who were featured in a 1956 study of the condition. Four out of eight family members were found to have excessive amounts of amino acids in their urine. They also had skin rash and a lack of coordination of their voluntary muscle movements, known as ataxia. These are the signs and symptoms characteristic of Hartnup disease, which typically affects the skin and brain.

Symptoms

Your brain and skin remain healthy and function properly if you get the required amount of vitamin B complex. If you have Hartnup disease, you can't absorb certain amino acids properly. This impedes your body's ability to produce protein and to make vitamin B complex. It can trigger specific mental and physical symptoms, including:

- skin rash
- anxiety
- rapid mood swings
- delusions
- hallucinations
- intention tremor
- speech difficulties
- unsteady wide-based gait, in which you walk with your legs farther apart than normal
- abnormalities in muscle tone, in which your muscles become tighter or lose tone
- short stature
- sensitivity to light

A skin rash called “pellagra” is a common symptom. It usually results from exposure to sunlight. It’s an intermittent red and scaly rash that typically appears over your face, neck, hands, and legs. It’s initially red, but over time it can progress to an eczematous-like rash. With prolonged sun exposure, the changes in your skin pigmentation can become permanent.

Sunlight, poor nutrition, sulfonamide drugs, or emotional or physical stress may trigger symptoms.

Causes

Hartnup disease is caused by a mutation of the gene that controls your body’s amino acid absorption and reabsorption. It’s an autosomal recessive trait. That means that people who are born with the condition have inherited a mutated gene from both parents. Scientists aren’t sure why the mutation occurs.

In most people, your body absorbs specific amino acids into your intestines and then reabsorbs them in your kidneys. If you have Hartnup disease, you can’t properly absorb certain amino acids from your small intestine. You also can’t reabsorb them from your kidneys. As a result, an excessive amount of amino acids exits your body through urination. This leaves your body with an insufficient amount of these amino acids.

Among other amino acids, Hartnup disease affects your ability to absorb tryptophan. This is an important building block for proteins and vitamins. Without enough tryptophan, your body can’t produce enough niacin. A niacin deficiency can cause you to develop a sun-sensitive rash. It can also lead to dementia.

Diagnoses

If your doctor suspects you have Hartnup disease, they may order a urinalysis test. They will collect a sample of your urine to send to a laboratory to measure the amount of amino acids excreted through your urine. If there are high levels of “neutral” amino acids in your urine, it may be a sign of Hartnup disease.

This test alone isn’t enough to diagnose Hartnup disease. Your doctor will also review your personal and family medical history. They will ask you about your symptoms, how frequently you have them, and when they first began. They may also order a blood test to check your levels of vitamin B complex, including niacin.

Treatment

Dietary changes

Since those with Hartnup disease can’t produce enough niacin, consuming foods that contain niacin can significantly reduce your symptoms. Good sources of niacin include:

- red meat
- poultry
- fish
- peanut butter
- fortified grains
- whole grains
- potatoes

Red meat, poultry, fish, and peanuts are also excellent sources of protein. Choose lean cuts of red meat and skinless poultry. The fat and skin of meat and poultry are rich sources of saturated fat. Eating too much saturated fat can raise your risk of high cholesterol.

Homocystinuria

Homocystinuria is a genetic disorder that causes a buildup of homocysteine in your blood and urine. Homocysteine is an amino acid. With this disorder, your body lacks an enzyme that it needs to break down homocysteine properly. The condition can cause symptoms involving your eyes, bones, brain and heart. Treatment includes vitamin B6 supplements. Homocystinuria (HCU) is a rare genetic disorder that affects your body's ability to process the amino acid homocysteine. With this disorder, a harmful buildup of homocysteine in your blood and pee (urine) can occur. This buildup can cause severe complications involving your eyes, skeletal system, central nervous system and vascular system. Amino acids are the building blocks of protein. Your body produces some homocysteine from another amino acid called methionine. Your body gets more methionine from the food you eat, particularly high-protein foods. With homocystinuria, your body lacks an enzyme that it needs to metabolize homocysteine properly and keep it within a normal range. Enzymes are proteins that help speed up the chemical reactions in your body.

Types

Researchers classify homocystinuria into different types based on their underlying genetic causes. The two main types of homocystinuria include:

Cystathionine beta-synthase (CBS) deficiency (classical homocystinuria)

Classical homocystinuria is the most common type of the disorder. Cystathionine beta-synthase (CBS) is an enzyme that helps convert homocysteine into cysteine, another amino acid your body needs. This type of the disease occurs when the *CBS* gene makes little to no CBS enzyme or when it makes CBS enzyme that doesn't work properly. CBS enzyme requires pyridoxine (vitamin B6) to function properly. This type of homocystinuria is further classified by how well you respond to vitamin B6 supplements.

Cobalamin (cbl) cofactor metabolism defect

Your body needs to convert some homocysteine back into methionine. This process involves cobalamin (vitamin B12). Your body goes through a series of steps to change vitamin B12 into the form your body needs to convert homocysteine back into methionine. Homocystinuria caused by cbl defect occurs when your body can't complete these steps, doesn't make the correct enzymes or produces defective enzymes.

Symptoms

The symptoms of homocystinuria vary based on which type you have. They typically develop during the first few years of life. But some people don't develop any symptoms until adulthood.

The symptoms of the most common type of homocystinuria usually involve your:

- Eyes.
- Skeletal system.
- Central nervous system.
- Vascular system.

Homocystinuria symptoms may include:

Eyes

- Dislocation of the lenses of your eyes (ectopia lentis).
- Severe nearsightedness (myopia).

Skeletal system

- Excessive growth.
- Long arms, legs, fingers and toes.
- Knees bent inward that touch when legs are straight (knock knees).
- Sunken or protruding chest.
- Curvature of your spine (scoliosis).

People with homocystinuria are also at risk of developing early osteoporosis.

Central nervous system

- Developmental delays.
- Learning problems.

Vascular system

- Increased risk of blood clots, which can lead to stroke or pulmonary embolism.

Causes

Genetic changes (mutations) in many different genes cause most types of homocystinuria. A mutation in the *CBS* gene causes the most common type of homocystinuria. The *CBS* gene

tells your body how to make an enzyme called cystathionine beta-synthase. This enzyme is responsible for creating a chemical pathway for homocysteine to convert into methionine.

Mutations in the *MTHFR*, *MTR*, *MTRR* and *MMADHC* genes can also cause homocystinuria. All of these genes are responsible for converting homocysteine into methionine.

Mutations in any of these genes prevent their corresponding enzymes from working properly, which can lead to a buildup of homocysteine. Researchers don't know why excess homocysteine causes the symptoms associated with homocystinuria.

You inherit homocystinuria in an autosomal recessive pattern. That means both of your biological parents, who usually have no symptoms, would have to pass on a copy of the affected gene for you to inherit it.

Homocystinuria can also occur due to non-genetic reasons. A severe lack of vitamin B6, vitamin B9 (folate) or vitamin B12 may cause the condition.

Diagnosis

In the United States, the newborn screening test checks for metabolic conditions, including homocystinuria. The homocysteine test measures the levels of homocysteine and methionine in your baby's blood. If the test result is positive, your baby's healthcare provider will request additional tests to confirm the result.

Newborn screening tests aren't always 100% accurate. Sometimes, they don't detect certain conditions. Therefore, some people aren't diagnosed with homocystinuria until after symptoms appear. Most symptoms develop in infancy or toddlerhood, but they can develop in adulthood as well.

If you develop symptoms of homocystinuria, your healthcare provider will order a homocysteine test to confirm the condition. If the results show you have classical homocystinuria, your provider will request another test to determine which subtype you have. This test is called the vitamin B6 challenge. The test determines how you'll respond to vitamin B6 supplementation so your provider can develop the right treatment plan for you.

Classical homocystinuria can be:

- **Vitamin B6-responsive**: Your body is making enough CBS enzyme, so vitamin B6 may help the enzyme do its job.
- **Partially vitamin B6-responsive**: Your body is making some CBS enzyme, so vitamin B6 may partially help the enzyme do its job.
- **Vitamin B6-non-responsive**: Your body isn't making enough CBS enzyme, so vitamin B6 probably won't help the enzyme do its job.

Genetic testing can look for mutations in the genes that cause homocystinuria. But healthcare providers don't typically use them because the condition can usually be diagnosed based on the homocysteine test alone.

Treatment

Homocystinuria treatment involves managing your symptoms by controlling the homocysteine levels in your blood. Treatment usually includes taking a vitamin B6 supplement. If you have vitamin B6-responsive classical homocystinuria, vitamin B6 supplementation may be enough to reduce and control your homocysteine levels.

If you have vitamin B6-non-responsive or partially vitamin B6-responsive classical homocystinuria, then vitamin B6 supplements won't be enough. You'll need additional treatment options, including:

- A medication called betaine (cystadane): Betaine can help lower the levels of homocysteine in your blood.
- A special diet for homocystinuria: You may have to stay on a diet that restricts your protein and methionine intake.
- Additional supplements: If you have another type of homocystinuria, you may need to take folate (vitamin B9) or cobalamin (vitamin B12) supplements.

Albinism

Albinism is a rare genetic condition that causes the lack of pigment in skin, hair, and eyes, sometimes accompanied by visual impairment. Different types of albinism are caused by various gene mutations.

Causes

Albinism is an inherited disorder that's present at birth. Children have a chance of being born with albinism if both of their parents have albinism or both of their parents carry the gene for albinism.

The cause of albinism is a defect in one of several genes that produce or distribute melanin, the pigment that gives skin, eyes, and hair their coloring. The defect may result in the absence of melanin production or a reduced amount of melanin production.

For most types of albinism, both parents must carry the gene in order for their child to develop the condition. Most people with albinism have parents who are only carriers of the gene and don't have symptoms of the condition.

Other types of albinism, including one that only affects the eyes, mostly occur when a birthing parent passes the gene for albinism on to a child assigned male at birth.

Types of albinism

Different gene defects characterize the numerous types of albinism. Types of albinism include:

- oculocutaneous albinism (OCA)
- ocular albinism
- Hermansky-Pudlak syndrome

- Chediak-Higashi syndrome
- Griscelli syndrome

Oculocutaneous albinism (OCA)

OCA affects the skin, hair, and eyes. Around 1 in 70 people have a mutation in an OCA gene.

There are several subtypes of OCA.

OCA1

OCA1 is caused by a defect in the tyrosinase enzyme. There are two subtypes of OCA1:

- OCA1a. People with OCA1a have a complete absence of melanin. People with this subtype have white hair, very pale skin, and light eyes.
- OCA1b. People with OCA1b produce some melanin. They have light-colored skin, hair, and eyes. Their coloring may increase as they age.

OCA2

OCA2 is less severe than OCA1. It's caused by a defect in the OCA2 gene that results in reduced melanin production. People with OCA2 are born with light coloring and skin. Their hair may be yellow, blond, or light brown. OCA2 is most common in people of African descent and Native Americans. OCA1 and OCA2 are the most common subtypes globally. Around 1 in 40,000 people have OCA1, and 1 in 39,000 people have OCA2.

OCA3

OCA3 is the result of a defect in the TYRP1 gene. It usually affects people with dark skin, particularly Black people in southern Africa. People with OCA3 have reddish-brown skin, reddish hair, and hazel or brown eyes.

OCA4

OCA4 is caused by a defect in the SLC45A2 protein. It results in the minimal production of melanin and commonly appears in people of East Asian descent. People with OCA4 have symptoms similar to those in people with OCA2.

Other subtypes

OCA5, OCA6, and OCA7 are very rare subtypes of OCA.

OCA5 and OCA7 have both been reported in only one family each. OCA6 has been reported in one family and one separate individual.

Ocular albinism

Ocular albinism is the result of a gene mutation on the X chromosome and occurs almost exclusively in males.

People with ocular albinism have reduced coloring in the retina and iris. The condition doesn't affect the skin or hair.

Hermansky-Pudlak syndrome

Hermansky-Pudlak syndrome is a rare form of albinism that's caused by a defect in one of 10 genes. It produces symptoms similar to OCA. The syndrome occurs with lung, bowel, and bleeding disorders.

Chediak-Higashi syndrome

Chediak-Higashi syndrome is another rare form of albinism that's the result of a defect in the LYST gene. It produces symptoms similar to OCA, but it may not affect all areas of the skin. There have been fewer than 500 cases reported globally.

The skin is usually creamy white to grayish. Hair is usually brown or blond with a silvery sheen. People with this syndrome have a defect in the white blood cells, increasing their risk of infections.

Griscelli syndrome

Griscelli syndrome is an extremely rare genetic disorder. It's caused by a defect in one of three genes. There were only around 150 known cases of this syndrome worldwide between 1978 and 2018.

It occurs with albinism (but may not affect the entire body), immune issues, and neurological issues. Griscelli syndrome usually results in death within the first decade of life.

Symptoms

People with albinism will have the following symptoms:

- an absence of color in the skin, hair, or eyes
- lighter than normal coloring of the skin, hair, or eyes
- patches of skin that have an absence of color

Albinism occurs with vision problems, which may include:

- strabismus, or crossed eyes
- photophobia, or sensitivity to light
- nystagmus, or involuntary rapid eye movements
- impaired vision or blindness
- astigmatism

Diagnoses

The most accurate way to diagnose albinism is through genetic testing to detect defective genes related to albinism.

Less accurate ways of detecting albinism include an evaluation of symptoms by a doctor or an electroretinogram test. This test measures the response of the light-sensitive cells in the eyes to reveal eye problems associated with albinism.

Treatment for albinism

There's no cure for albinism. But treatment can help relieve symptoms and prevent sun damage.

Treatment may include:

- sunglasses to protect the eyes from the sun's ultraviolet (UV) rays
- protective clothing and sunscreen to protect the skin from UV rays
- prescription eyeglasses to correct vision problems
- surgery on the muscles of the eyes to correct abnormal eye movements

Enzymes are catalysts that increase the rate or velocity of physiologic reactions. Each and every reaction in our body takes place with the help of an enzyme. In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood.

Enzymes present in plasma can be classified into 2 types, they are

- Functional Plasma enzymes and
- Non-functional plasma enzymes

Functional plasma enzymes:

- Present in plasma at higher concentration than tissues
- They function in plasma
- Mostly synthesized by the liver
- Usually decreased in disease conditions
- Eg. Clotting enzymes, lipoprotein lipase

Non-functional plasma enzymes:

- Present in plasma at lower concentration than tissues
- Do not have any function in plasma
- Mostly synthesized by liver, skeletal muscle, heart, brain etc z Usually increased in disease conditions
- Eg. Creatine kinase, Alanine transaminase etc
- Measurement of these enzymes in plasma can be used to assess cell damage and proliferation i.e. diagnosis of disease.

AST (aspartate aminotransferase) is an enzyme that is found mostly in the liver, but it's also in muscles and other organs in your body. When cells that contain AST are damaged, they

release the AST into your blood. An AST blood test measures the amount of AST in your blood. The test is commonly used to help diagnose liver damage or disease.

Purpose of the test

A blood test measuring AST is used to detect damage to cells. Most often, it helps assess the condition of the liver, but it can provide insight into other health concerns as well.

Depending on the situation, AST testing, usually combined with other measurements in a panel test, can be used as a form of medical screening, diagnosis, or monitoring:

- **Screening** is the medical term for testing when you don't have any symptoms of a condition. A screening that includes AST may be prescribed if you have risk factors for liver disease such as obesity, diabetes, significant alcohol use, or a family history of liver problems. During routine health checkups, AST may also be tested on a CMP.
- **Diagnosis** takes place after symptoms have occurred and is the process of finding the cause. For example, AST may be measured if you have had jaundice, fatigue, swelling, unexplained weight loss, itching, nausea and vomiting, or other symptoms associated with liver problems.

Clinical significance

AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells.[citation needed] As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.

AST was defined as a biochemical marker for the diagnosis of acute myocardial infarction. However, the use of AST for such a diagnosis is now redundant and has been superseded by the cardiac troponins.

Alanine Aminotransferase (ALT)

Alanine Aminotransferase (ALT), also known as serum glutamic-pyruvic transaminase (SGPT), is an enzyme that catalyzes the transfer of an amino group from alanine to α-ketoglutarate, thereby producing glutamate and pyruvate (Figure 1). This pyridoxal phosphate dependent transaminase is found primarily in serum and the liver, but it can also be found in various other body tissues. ALT is usually measured as a clinical marker of liver function to ascertain liver health. Hepatocellular injury often leads to an increase in ALT levels, which are usually measured in units per liter (U/L).

Assay Principle

Alanine Aminotransferase (ALT) Assay Kit measures ALT activity through a series of enzyme driven reactions. ALT in samples reacts with alanine to transfer an amino group to another substrate, producing glutamate and pyruvate. Pyruvate is then detected with the

colorimetric probe. Samples and standards are incubated for 30-60 minutes and then read with a standard 96-well specrophotometric plate reader (540-570 nm). The ALT activity is directly proportional to the amount of pyruvate generated in the reaction. Sample ALT levels are determined by comparison with the known pyruvate standards.

Alkaline Phosphatase

The Alkaline Phosphatase assay is used for the quantitation of alkaline phosphatase in human serum or plasma. Human alkaline phosphatase (AlkP, EC.3.1.3.1) consists of a group of at least five tissue-specific isoenzymes which catalyzes the hydrolysis of phosphate mono-esters at alkaline pH. A variety of disease processes can result in the release of increased quantities of alkaline phosphatase into the blood.

Principles of Procedure

Several substrates have been used to measure alkaline phosphatase activity such as glycerophosphate,1 phenyl phosphate,1 and p-nitrophenyl phosphate.2 Bowers and McComb3 improved the method of Bessey et al. to include a kinetic measurement. Tietz et al.4 optimized this method to include a chelated metal-ion buffer of zinc, magnesium, and HEDTA. This Alkaline Phosphatase procedure is a modification of this method. Alkaline phosphatase in the sample catalyzes the hydrolysis of colorless p-nitrophenyl phosphate (p-NPP) to give p-nitrophenol and inorganic phosphate. At the pH of the assay (alkaline), the p-nitrophenol is in the yellow phenoxide form. The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

Acid Phosphatase

The Acid Phosphatase assay is used for the quantitation of acid phosphatase in human serum. This method is for the measurement of total acid phosphatase, and is not specific for prostatic acid phosphatase enzyme. The greatest concentration of acid phosphatase (ACP) activity occurs in liver, spleen, milk, erythrocytes, platelets, bone marrow, and the prostate gland. The last is the richest source, and it contributes a small proportion of the enzyme present in sera from healthy males.1 Increasing levels of ACP are consistent with prostatic cancer. The optimal pH for the individual ACPs varies depending on the tissues from which they are obtained. The observed pH optimum also varies with the substrate on which the enzyme acts; the more acidic the substrate, the lower the pH at which maximum activity is obtained. The ACPs are unstable, especially at temperatures above 37°C and at pH levels above 7.0. Some of the enzyme forms in serum (especially the prostatic enzyme) are particularly labile and more than 50% of the ACP activity may be lost in 1 hour at room temperature. Acidification of the serum specimen to a pH below 6.5 aids in stabilizing the enzyme.

Principles of Procedure

Acid Phosphatase catalyzes the hydrolysis of alpha-naphthylphosphate, liberating the alpha-naphthol and phosphate. The alpha-naphthol is then coupled with diazotized 2-amino-5-chlorotoluene (Fast Red TR) to form diazo dye which has a strong absorbance at 405 nm. The increase in absorbance is directly proportional to the level of ACP in the sample.2 The diazo dye is measured bichromatically at 412/660 nm.

Creatine Kinase

Creatine Kinase (EC 2.7.3.2; adenosine triphosphate: creatine Nphosphotransferase CK) CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle. The concentration gradients between some human tissues and serum for creatine kinase. The concentration gradient is logarithmic

Clinical significance
Normal range for total CK:
Male : 46-171 U/L= 0.78-2.90 μkat/L
Female: 34-145 U/L= 0.58-2.47 μkat/L

Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%- 80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity. Quite high values of Ck are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as: (a) Myasthenia gravis (b) Multiple sclerosis (c) Polimyeltis (d) Parkinsonism Serum enzyme activity is normal.

Isoenzymes of CK

CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.

CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than five per cent in skeletal muscle: its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than six per cent of the total. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal. Approximate concentrations of tissue CK activity (expressed as multiple activity concentrations in serum and cytoplasmic isoenzyme composition 23.3.3.2

Lactate Dehydrogenase

Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD+ oxidoreductase; LD) catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage.

LD has a molecular weight of 134 kDa and is composed of four peptide chains of two types:

- M (or A)
- H (or B)

Each under separate genetic control The subunit compositions of the five isoenzymes are listed below in order of their decreasing anodal mobility in an alkaline medium.

- LD-1 (HHHH; H4) = migrates fastest towards the anode
- LD-2 (HHHM; H3M)
- LD-3 (HHMM; H2M2)
- LD-4 (HMMM; HM3)
- LD-5 (MMMM; M4)

Clinical significance

Normal range of total LDH: 180-360 U/L= 3.1-6.1 μkat/L It is increased in plasma in Myocardial injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic disease and Myocardial. Injury.

Causes of Raised Plasma

Total LD Activity

- Artefactual: Due to in vitro haemolysis or delayed separation of plasma from whole blood.
- Marked increase (more than 5 times the upper reference limit in adults):
- Circulatory failure with ‘shock’ and hypoxia:
- Myocardial infarction
- Some haematological disorders. In blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas. very high levels (up to 20 times the upper reference limit in adults) may be found.
- Moderate increase. viral hepatitis: malignancy of any tissue: skeletal muscle disease: pulmonary embolism: infectious mononucleosis.

Isoenzymes of LD

- LD1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.
- LD5 is the most abundant form in the liver and in skeletal muscle. \Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value.
- Predominant elevation of LD1 and LD5. (LD1 greater than LD5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.
- Predominant elevation of LD2 and LD3 occurs in acute leukaemia: LD3 is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of LD5 occurs after damage to the liver or skeletal muscle. Other clinically important enzymes