AMINOACIDOPATHIES

PHENYLKETONURIA
- Methods of analysis
- Guthrie inhibition test
- Microfluorometric assay
- Genetics of the disease

TYROSINEMIA
- Type I and Type II

ALKAPTONURIA

MAPLE SYRUP URINE DISEASE
- Methods of analysis
- Modified Guthrie bacterial inhibition
- Microfluorometric assay
HOMOCYSTINEURIA
  – Methods of analysis
    • In plasma
    • In urine

CYSTINURIA

General guidelines for amino-acid analysis in plasma

General guidelines for amino-acid analysis in urine
AMINO ACIDOPATHIES

• Rare inherited disorders of amino acid metabolism
• Abnormality in either activity of specific enzyme, in metabolic pathway or in the membrane specific transport system for amino acid.
• These defects lead to accumulation of amino acid itself, its precursor or a by-product.
• Excessive accumulation in blood leads to physical symptoms of the disease.
PHENYL KETONURIA (PKU)

- Autosomal recessive trait

- Rate of incidence 1 in 14000 is considered relatively common compared to other aminoacidopathies. It's considerably increased in families with diseased cases.

- Deficiency of *phenylalanine hydroxylase* (PAH), that catalyses the conversion of phenyl-alanine into tyrosine.

- Phenylalanine is metabolized via alternative pathway leading to the accumulation of Phenyl pyruvic acid *(product of phenylalanine de-amination)*. It’s circulation is found in both blood and urine giving urine a characteristic *musty* odors.
• Phenyl pyruvic acid is neurotoxic and in undiagnosed newborn can cause severe mental retardation due to brain damage, which starts in 2nd and 3rd week post birth.

• Early detection allows diet control therapy low in phenylalanine up to the age of 5-6yrs until normal metabolism develops, however low IQ levels have been reported after termination of special diet.

• Women suffering from PKU if not diet controlled from the time of conception till birth can deliver micro-cephalic and mentally retarded child. Due to these reasons it’s now highly recommended to continue diet supplement low in phenylalanine for life.
Methods of analysis:

**Screening methods;**
Guthrie bacterial inhibition & Micro-fluorometric assay

**Reference or confirmatory method;**
High performance liquid chromatography (HPLC).

**Principle of Guthrie inhibition test:**
- Spores of organism *Bacillus subtilis* are incorporated into an agar plate containing β-2-thienylalanine (a metabolic antagonist to *B. Subtilis*).

- A filter paper disk, impregnated with dried blood sample is placed onto the agar
• If the blood phenylalanine level is higher than 2.5 mg/dl, the phenylalanine counteracts the antagonist and the bacteria grows.

• The sample *should be collected* prior to administration of antibiotics or transfusion of blood or blood products.
Principle of microfluorometric assay:

• Direct and quantitative measurement of phenylalanine in blood stained filter disks.

• More adaptable to automation and is not affected by the presence of antibiotics (advantage)

• The filter paper disk containing the sample is treated with trichloroacetic acid (TCA).

• The extract is then reacted with a mixture of ninhydrin, succinate and leucylalanine, in the presence of copper tartarate. The reaction takes place in a microtitre plate.

• Fluorescence of the complex is measured using excitation / emission wavelength of 360nm and 530nm respectively.
• Normal levels for serum phenylalanine in a full term, normal weight newborn ranges from 1.2-3 mg/dl

• Urine testing is useful for monitoring dietary therapy and this can be carried out by reagent strip test that involves the reaction of ferric chloride with phenyl pyruvic acid in urine to produce green colour.
Genetic pre-natal diagnosis of PKU:

• Prenatal diagnosis and detection of carrier status in families with PKU is now available using DNA analysis.

• However PKU is a complex polygenic disorder and results in multiple independent mutations at the PAH locus. Recombinant cloned human PAH cDNA probes (in-vitro "artificially" produced but 100% similar to parent gene fragment) are used to reveal the presence of various mutations in the patient's PAH gene. These mutation patterns and the disease traits have been proven to be tightly linked in PKU families. Therefore these mutation patterns can be used as indicators to study families at higher risk of developing PKU.
TYROSIINEMIA

• Excretion of tyrosine and tyrosine catabolytes in urine.

• Deficiency of *fumaryl- acetoacetate hydrolase* results in type I (more common) or defect in *amino transferase* results in type II tyrosinemia.

• Elevated levels of tyrosine lead to liver damage which may be fatal during infancy or to liver cirrohsis and liver carcinoma later on in life.

• Rate of incidence is 1 in 100,000
ALKAPTONURIA

- Lack of homogenisate oxidase in the tyrosine catabolic pathway

- Incidence rate is 1 in 250,000

- Very important physical symptom includes darkening of urine upon exposure to atmosphere which is due to homogensttic acid (HGA) oxidising to produce dark polymer.

- Initially patients have no significant symptoms but high level of HGA gradually starts depositing in connective tissues causing arthritis-like degeneration.
MAPLE SYRUP URINE DISEASE (MSUD):

• Hereditary disease with characteristic maple syrup or burnt sugar type odour of urine, breath and skin.

• Complete absence or reduction in activity of branched-chain keto acid decarboxylase enzyme hence blocking the normal metabolism of three essential amino acids i.e leucine, isoleucine and valine.

• Results in the accumulation of branched-chain amino acids and their corresponding keto-acids in blood, urine and CSF.
Physical symptoms of MSUD include:

- Mental retardation and convulsions due to presence in CSF. In blood however: keto-acidosis and hypoglycaemia takes place.

- Death occurs in 1st year after birth if untreated however the rate of incidence is very low i.e. 1 in 216,000

- Diet supplements low in protein should be strictly administered and therefore early diagnosis through routine screening is highly recommended. However due to rarity of disease many centers don't have the screening facility for MSUD
Methods of analysis for MSUD:

- **Modified Guthrie bacterial inhibition test**
  This time 4-azaleucine is added to the medium to inhibit growth of B. Subtilis however in a +ve MSUD test, increased leucine levels from the filter paper disc with blood sample dried on it, will overcome the activity of 4-azaleucine allowing bacterial growth.

- **Microfluorometric analysis for branched chain amino acids.**
  A filter paper treated with a solvent mixture of methanol and acetone will denature hemoglobin in the blood sample. Leucine dehydrogenase is added to the sample extract to produce NADH which is subsequently measured at an emission wavelength of 450 nm using an excitation wavelength of 360nm.
HOMOCYSTINURIA

• Rate of incidence 1 in 200,000

Methionine → Homocysteine → Cystathionine → Cysteine

Cystathionine β-synthase

• It's a hereditary disorder with impaired activity of Cystathionine-β Synthase

• There is accumulation of precursors i.e. homocysteine & methionine
Methods of analysis

**In plasma:** modified Guthrie test
- It is performed using L-methionine sulfoximine as a metabolic inhibitor of bacterial growth. Increased plasma levels of methionine will allow bacterial growth by suppressing the antagonistic effect of L-methionine sulfoximine.

**In urine:** Cyanide-nitroprusside spot test
- In this test cyanide-nitroprusside is added to urine sample. High levels of homocysteine will be detected by the development of *purple-red colour*. However there is another urinary metabolite called urinary cysteine that might *interfere* in this test to give *false +ve*.
- Therefore after the addition of cyanide-nitroprusside, a further *confirmatory step* is carried out, where by adding *silver*, homocysteine is reduced but *not* cysteine thus allowing homocysteine to react with nitro-prusside to produce *reddish colour*. 
CYSTINURIA

• Defect in *amino acid transport system* rather than metabolic pathway affecting renal system and other vital organs.

• 20 to 30 fold increase in urinary excretion of cysteine due to genetic defect in renal re-absorption mechanisms.

• Cysteine is insoluble therefore precipitates in kidney tubules.

• Laboratory analysis is the same as that of homocysteinuria where a *reddish-purple colour* is produced in cyanide-nitroprusside test.
General rules for amino acid analysis in plasma:

• Blood should be drawn after at least 6-8 hrs fasting to avoid the effect of aa being absorbed via dietary proteins.
• The sample should be collected in heparin and plasma must be promptly removed from the cells carefully without aspirating the platelets and leukocyte layers. These cells contain 100 fold increase in aspartic and glutamic acid as compared to plasma levels.
• Hemolysis should be avoided for the same reason.
• De-proteinisation must be carried out within 30 min of sample collection

General rules for amino acid analysis in urine:

• Random specimen is suitable for screening purposes
• For quantification purposes a 24hrs urine preserved with Thymol or organic solvents is required.