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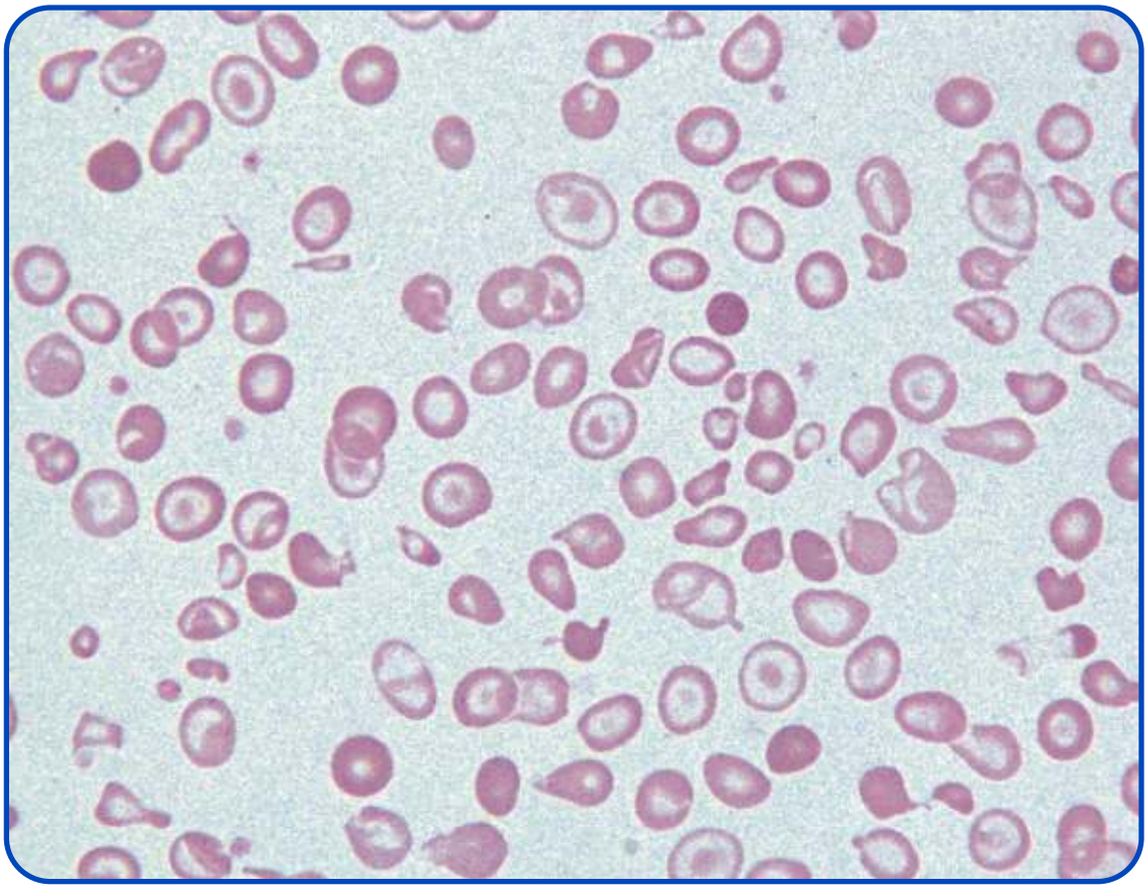

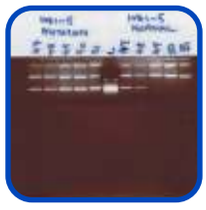

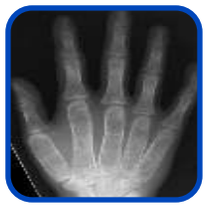

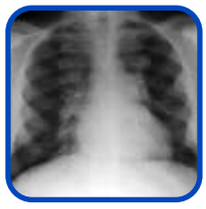

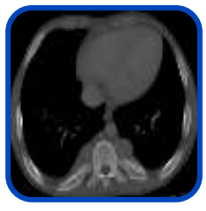

In this issue

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Detection of Amoebic Meningitis by Wet Film of Cerebrospinal Fluid (CSF)

Dr Sadia Shakoor,
Resident, Microbiology

Primary Amoebic Meningoencephalitis (PAM) is a fatal meningitis syndrome caused by the free-living amoeba *Naegleria fowleri*. This amoeba is found in freshwater ponds and lakes. At high temperatures it may contaminate urban water supplies resulting in sporadic cases of meningitis. The disease is rare resembling bacterial meningitis, except for the poor response to antibacterial therapy. These features lead to decreased recognition by clinicians and a high index of suspicion needs to be maintained to entertain this diagnosis.

Laboratory diagnosis depends on CSF analysis. This usually reveals hypoglycorrhachia, high protein content and high neutrophilic pleocytosis. Amoeba may easily be missed on routine cell count on hemacytometers owing to the usage of toxic diluting fluid. When a diagnosis is suspected, a simple wet film of the CSF without centrifugation (which destroys amoebae) usually reveals motile amoebic trophozoites. *Naegleria fowleri* moves sluggishly by means of rounded lobopodes/ psuedopods (Figure 1). Cysts are not visible on CSF films; however, brain biopsy which is usually not required can show cysts as well as its trophozoites.

The amoebae also exist in flagellar form. CSF samples can be directly suspended in distilled water and incubated for 30 minutes to demonstrate rapidly motile flagellar forms. This may further confirm the diagnosis. Isolation of amoebae is possible as it can be cultured on a non-nutrient agar covered with a lawn of *E.coli*. Growth can be seen within the next 48 hours.

Treatment regimens with antifungal agents such as Amphotericin have been suggested, but are largely ineffective. Prevention of the disease entails chlorination of water supplies and avoidance of unchlorinated freshwater activities.

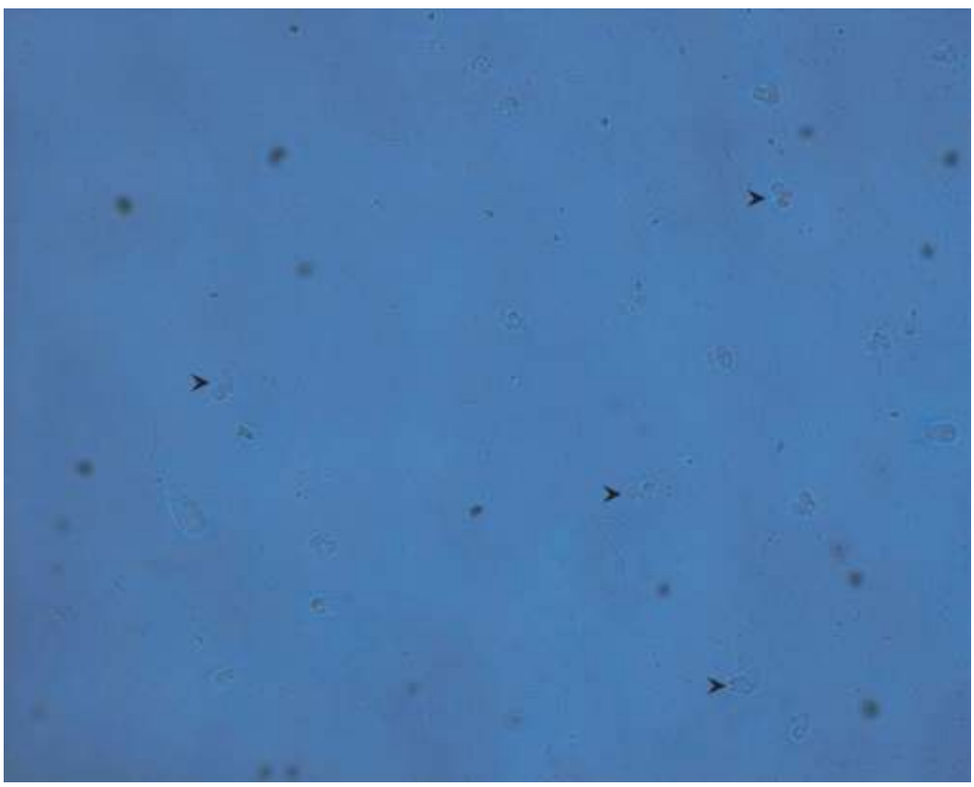

Figure 1: Amoebic trophozoites (marked by a black pointer) as visible on a wet preparation of CSF sample.

Biochemical Tests in the Diagnosis of Anaemia

Dr Sahar Iqbal,
Resident, Chemical Pathology

Anaemia may be defined as a disorder resulting from low concentration of haemoglobin or haematocrit in the blood in relation to age, sex or location. The combination of haematological and biochemical studies of blood, enable one to identify underlying aetiology of anaemia.

Anaemia can be classified morphologically as microcytic, normocytic and macrocytic depending on Mean Corpuscular Volume (MCV) of red cells. The reference ranges for these respectively are MCV<76, 76-96 and >96 fl for Pakistani population.

The most common differential diagnosis and biochemical findings of various types of anaemia are shown in the table1.

Table 1: Differential Diagnosis of Anaemia and Biochemical Findings

Microcytic anaemia	**Biochemical test findings**
Iron deficiency anaemia	low Serum iron, low ferritin, low SOT, increased TIBC, FEP increased
Thalassaemic disorders	Ferritin normal or high, SOT high (thalassaemia major) to normal (thalassaemia trait)
Anaemia of chronic disease (late, uncommon)	Ferritin normal to high, SOT low, iron low, TIBC low, FEP increased
Sideroblastic anaemia (for example congenital, lead, alcohol, drugs, uncommon)	Iron high, SOT high, ferritin high, TIBC normal, FEP normal or increased
Copper deficiency, zinc poisoning (rare)	Ferritin normal to high
Normocytic anaemia	
Acute blood loss	
Iron deficiency anaemia (early)	Described earlier
Anaemia of chronic disease (for example infection, inflammation, malignancy)	Described earlier
Bone marrow suppression (may also be macrocytic)	
Bone marrow infiltration (for example leukoerythroblastic blood picture)	
Acquired pure red blood cell aplasia	
Aplastic anaemia	
Chronic renal insufficiency	Erythropoietin levels decreased, abnormal renal function tests

Endocrine dysfunction	
Hypothyroidism	TSH increased
Hypopituitarism	
Macrocytic anaemia	
Folic acid deficiency	Folic acid levels decreased
Vitamin B12 deficiency	Vit B12 levels decreased
Myelodysplastic syndromes	
Acute myeloid leukemias (for example erythroleukemia)	
Reticulocytosis	
Hemolytic anaemia	Elevated total and indirect bilirubin, SGOT (liver enzyme) normal, LDH increased, haptoglobin reduced.
Response to blood loss	
Drug-induced anaemia (for example Hydroxyurea, AZT, chemotherapeutic agents)	
Liver disease	LFTs abnormal
Ethanol abuse	

SOT: saturation of transferrin, TIBC: total iron binding capacity, FEP: free erythrocyte protoporphyrin

Biochemical markers are very helpful to support the differential diagnoses made by hematological studies. When taken in conjunction with other clinical and laboratory features, simple biochemical tests can be of diagnostic value. Without hematological information these tests are nonspecific but they are useful for prompt and accessible orientation and avoidance of misdiagnosis of type of anaemia.

Iron deficiency is the most common nutritional disorder in humans and is also most frequent cause of anaemia globally. It is estimated that about 3% of adult men, 20% of women in their reproductive years, and 50% of pregnant women are deficient in iron. Iron deficiency develops in stages, the first of which is the depletion of storage iron in response to a prolonged negative iron balance. Once iron reserves are exhausted, biochemical tests of iron metabolism become abnormal. Next, a drop in hemoglobin concentration of blood is seen. The diagnostic usefulness of an isolated serum iron measurement has limitations as changes up to 20% in serum iron concentration may occur suddenly, even in the healthy people, because of momentary imbalances in iron inflow and outflow. Diurnal variations also occur as well, so serum iron values should always be interpreted in combination with TIBC, SOT and ferritin levels.

In AKUH clinical laboratory serum iron and TIBC are measured through timed-endpoint spectrophotometric and serum ferritin through electrochemical luminescence techniques however SOT is a calculated measurement.

Pathophysiology and Laboratory Investigation in Anaemia of Chronic Disease

Dr Raihan Sajid,
Assistant Professor, Haematology

Anaemia of Chronic Disease (ACD) is the second most common anaemia after Iron Deficiency Anaemia (IDA) and is the most frequently observed anaemia in the hospital setting. It is also known as anaemia of inflammation, anaemia of chronic inflammation and hypoferremia of inflammation. M. Wintrobe used the terminology of 'simple chronic anaemia' for the normocytic anaemia associated with infections and chronic systemic diseases in his famous text book of *Clinical Hematology* in 1961.

Causes of ACD include acute and chronic infections which may be viral (including HIV), bacterial, parasitic, fungal, various types of cancer (both hematologic and solid) and autoimmune disorders [like Systemic Lupus Erythromatosis (SLE), rheumatoid arthritis, vasculitis, sarcoidosis, inflammatory bowel disease], chronic kidney disease and chronic rejection subsequent to solid organ transplants.

Findings in ACD include mild to moderate anaemia (Hb 8-11 g/dl) while platelet and white cell counts are usually normal. Mostly the anaemia is normocytic and normochromic, however, in about 30% of cases it can be hypochromic and microcytic as well. There may be mild anisocytosis and poikilocytosis. Reticulocyte count is generally normal but is inappropriately low for degree of anaemia. Bilirubin levels are normal and erythropoietin levels are increased but the increase is blunt in comparison to the anaemia.

Mechanisms of development of ACD are many but the most important are erythropoiesis restriction as a result of iron unavailability, abnormal iron metabolism with trapping of iron in macrophages and inadequate erythropoietic secretion as well as resistance to erythropoietin. During inflammation, release of iron from macrophages and liver stores is inhibited. IL-6 which is released as a cytokine during inflammation induces hepcidin production form liver. The latter inhibits release of iron from macrophages leading to hypoferremia. This hypoferremia or functional iron deficiency (so called starvation in midst of plenty) is the defining feature of ACD.

It is very important to differentiate IDA from ACD. Sometimes the two may co-exist for example in rheumatoid arthritis. Table 1 summarizes the laboratory investigations that differentiate ACD from IDA. The laboratory diagnosis of ACD is made by low serum iron, low transferrin saturation and normal or increased ferritin level. This is in sharp contrast to iron deficiency where serum ferritin is low but transferrin receptors are increased. It is important to note that bone marrow examination is usually not required to discriminate iron deficiency anaemia from ACD.

	ACD	IDA	ACD + IDA
Iron	Reduced	Reduced	Reduced
Transferrin	Reduced to normal	Increased	Reduced
Transferrin saturation	Reduced	Reduced	Reduced
Ferritin	Normal to increased	Reduced	Reduced
Soluble transferrin receptor	Normal	Increased	Normal to increased
Cytokine levels	Increased	Normal	Increased

Table1: Laboratory investigations to differentiate anaemia of chronic disease from iron deficiency anaemia (IDA)

Haematogones: A Pitfall in the Diagnosis of Leukemias/Lymphoma in Children

Drs Raihan Sajid, Bushra Moiz,
Assistant Professors, Haematology

Haematogones are benign lymphoid precursors which appear morphologically and immunophenotypically similar to lymphoblasts in children. These cells have been observed after cessation of chemotherapy in acute lymphoblastic leukemia (ALL), following bone marrow transplantation, during infection, in children with non haemopoietic neoplasms and even in healthy children.

Morphologically, haematogones resemble lymphoid cells varying in size from 10 to 20 µ. They have round or oval nuclei having one or more indentations or shallow clefts and condensed homogeneous chromatin (Figure 1 and 2). Nucleoli are either absent or small and indistinct. There is generally scanty or no discernible cytoplasm which when present is moderate to deeply basophilic being devoid of granules, inclusions, or vacuoles. There can be various other cytologic features that make them blend with mature lymphocytes.

One of the clinical settings in which haematogones cause diagnostic confusion, is following treatment for ALL becaused hemtogones are frequently increased in regenerating marrow and can potentially be mistaken for residual disease. Definitive diagnosis requires evaluation of case by flow cytometry, as haematogones can be distinguished from ALL blasts because they tend to express TdT strongly and CD10 and CD19 weakly whereas the reverse pattern of reactivity is seen with ALL blast cells. One of the important features which are characteristic of haematogones is expression of markers ranging from maturity to immaturity - a fact that is recognized as smearing on flowcytometry. In sharp contrast, blasts usually express immature markers.

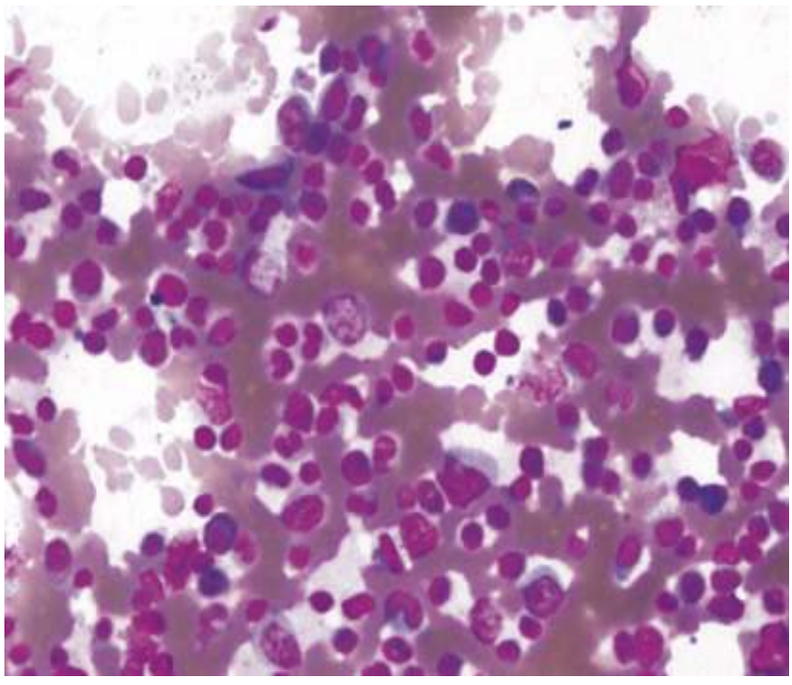

Figure 1: Haematogones as seen in bone marrow aspirate of an Acute Lymphoblastic Leukemia patient during remission phase.

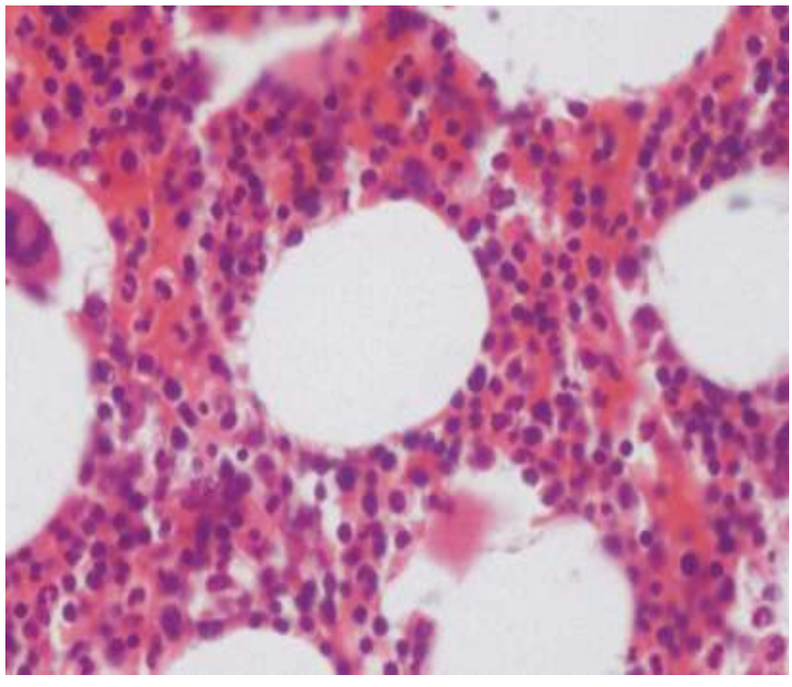

Figure 2: Bone trephine from same patient showing haematogones (40X).

Porphyria: The Mysterious Disease

Dr Lena Jafri,
Resident, Clinical Biochemistry

Porphyrins are a group of tetrapyrroles differing in the composition of their side chains. They are intermediate metabolites of haem synthetic pathway. In humans there are three main porphryins, namely uroporphyrin, coproporphrin and protoporphyrin.

Porphyrias are the disorders characterised by deficiency of specific enzymes catalyzing intermediary steps in haem synthetic pathway. As shown in figure 1, various enzymes may be affected giving rise to different forms of porphyrias. As a result of enzyme blockade, there is overproduction of haem intermediates, which are potentially toxic and causes the characteristic neurovisceral and photosensitizing symptoms seen in different types of porphyria (Table 1). Although the mechanism is unclear, the porphyrias associated with increased production of delta-aminolevulinic acid (ALA) and/or porphobilinogen (PBG) are associated with neurovisceral complaints. Porphyrins cause photosensitization and skin damage through exposure to ultraviolet light (Figure 2), with subsequent production of tissue-damaging free radicals. Water soluble precursors are excreted mainly in the urine (ALA, PBG, and uroporphyrin), protoporphyrin is excreted mainly in the faeces, and coproporphyrin is excreted in both.

The role of biochemical investigations in porphyrias is tremendous. Porphyria is diagnosed through spectroscopy and biochemical analysis of blood, urine, and stool.

In Acute Intermittent Porphyria (AIP), Hereditary Coproporphyria (HCP), or Variegate Porphyria (VP) but not in ALA Dehydratase Deficiency Porphyria (ALAD) increased excretion of PBG is seen, with typical values being ten or more times the upper limit of normal. Repeat testing during an attack and subsequent attacks may be necessary in order to detect a porphyria, as levels may be normal or near-normal between attacks. In severe cases of AIP, the urine may develop a port-wine color, due to a high concentration of porphobilin, an autooxidation product of PBG.

Urinary porphyrins are increased in all three acute porphyrias. Positive screening tests are followed by quantitative measurement of total porphyrins in a 24 hours urine sample. Elevated porphyrin is then identified.

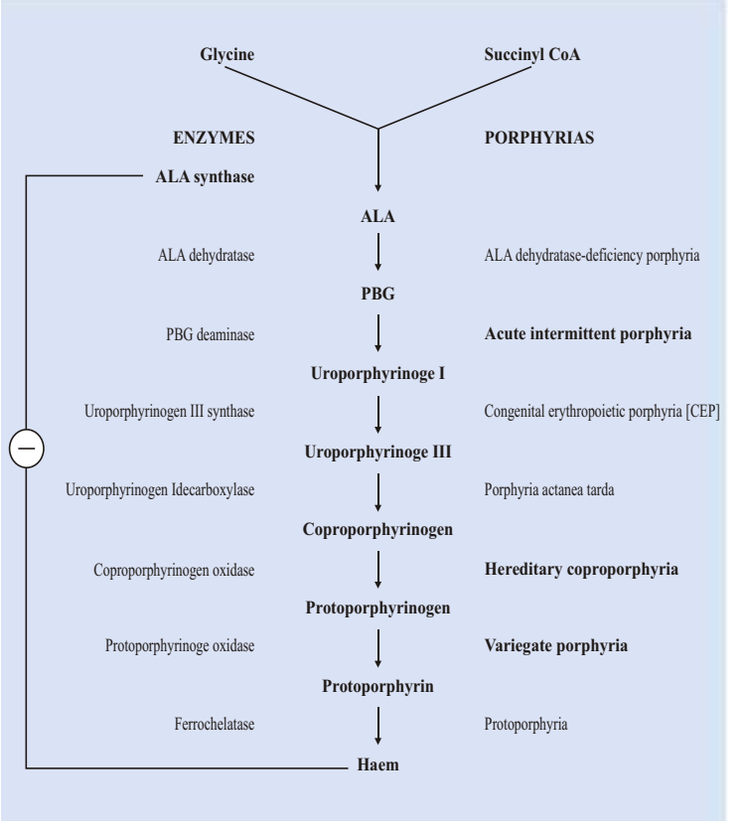

Figure 1: Haem synthesis pathway showing various types of Porphyrias

Increased excretion of faecal porphyrins is seen in VP and HCP. Fecal porphyrins consist of coproporphyrin and protoporphyrin. Both coproporphyrin and protoporphyrin are excreted in huge amounts in VP and only coproporphyrin is excreted in HCP.

Red blood cell porphyrins are measured as free erythrocyte protoporphyrin (FEP). Normally it reflects concentration of Zn protoporphyrin. These are greatly increased in Congenital Erythropoietic Porphyria (CEP) and protoporphyria but are normal in other porphyrias. It may also be increased in iron deficiency and lead poisoning.

At AKUH Clinical Lab the following four of the above mentioned tests for diagnosing porphyria are available: urinary ALA, urinary PBG, urinary uroporphyrin and urinary coproporphyrin.

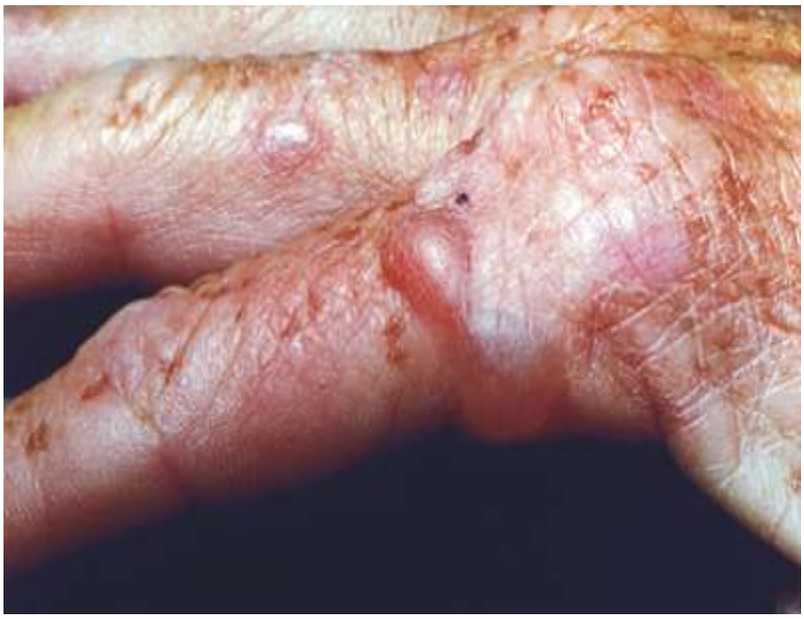

Figure 2: Skin of a patient suffering from Porphyria Cutanea Tarda showing typical skin lesions.

Type	Deficient Enzyme	Classification
Congenital Erythropoietic Porphyria (CEP)	Uroporphyrinogen III cosynthetase	Erythropoietic
Erythropoietic protoporphyria (EP)	Ferrochelatase	Erythropoietic
ALA dehydratase deficiency porphyria (ALAD)	ALA dehydratase	Hepatic
Acute Intermittent porphyria (AIP)	Porphobilinogen deaminase	Hepatic
Hereditary coproporphyria (HCP)	Coproporphyrinogen oxidase	Hepatic
Variegate porphyria (VP)	Protoporphyrinogen oxidase	Hepatic
Porphyria cutanea tarda (PCT)	Uroporphyrinogen decarboxylase	Hepatic
Lead Poisoning	Ferrochelatase	

Table 1: Various types of Porphyrias with their associated deficient enzymes

Imaging Features of Thalassaemia and Sickle Cell Anaemia

Drs Zishan Haider, Ishtiaq Chishti,
Farhan Ahmed, Dawar Khan,
Radiology

Thalassaemia and sickle cell anaemia are among the common diseases which can give distinct imaging features that point the diagnosis which are diagnostic. Although the imaging features of Thalassaemia and other chronic haemolytic anaemias are overlapping, it is important to recognize the distinguishing features for proper management in absence of adequate clinical clues. Sickle cell disease is also among those diseases which have a wide spectrum of manifestations including avascular necrosis and osteomyelitis.

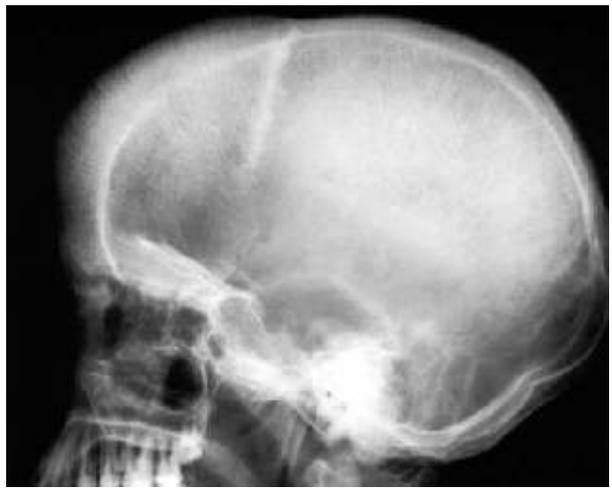

Figure 1: Skull X-ray showing hair-on-end appearance secondary to expansion of diploic space with relative sparing of occipital region.

Thalassaemia

The radiographic features of thalassaemia result from either a generalized marrow hypertrophy or hyperplasia, which may occur in other haemoglobinopathies, or a reticuloendothelial storage process including Gaucher disease. These may be diagnosed radiologically with a high degree of confidence, although additional clinical and laboratory information may be required for the differential diagnosis of the haemoglobinopathies.

Skeletal response to marrow proliferation consists of expansion of the medulla, thinning of cortical bone, and resorption of cancellous bone, which results in a generalised loss of bone density. Frequently, small areas of lucency resulting from focal proliferation of marrow may be present, often demarcated by coarsened but less numerous trabeculae. In addition, the hypertrophic and hyperplastic marrow may perforate the cortex, proliferate sub-periosteally, and stimulate a variety of periosteal responses.

In weight-bearing bones, the resorptive process preserves the primary trabeculae at the expense of secondary trabeculae. In the vertebral bodies, a striated appearance resulting from thickened vertical trabeculae that stands out against the paucity of horizontal trabeculae. In severely affected patients, biconcavity of the superior and inferior margins of the vertebral bodies or compression fractures may occur.

In the skull, widening of the diploic space (medulla) with a thinning of the tables (cortices) occurs, frequently with complete obliteration of the outer table. New bone forms in response to marrow proliferation beneath the periosteum. These bony spicules may be seen radiographically and result in a classic 'hair-on-end' appearance (Figure 1, 2).

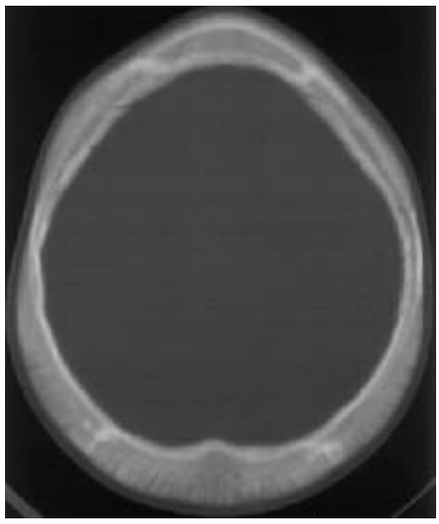

Figure 2: Axial CT scan of brain in bone window showing expansion of diploic space due to marrow expansion in skull.

Proliferation of marrow within the frontal and facial bones impedes pneumatisation of the paranasal sinuses. This results in hypertrophy of osseous structures and a consequent prominence of the lateral margins of the malar eminences, together with anterior and medial displacement of developing teeth.

In the hand, the phalanges reveal the changes of cortical thinning, expansion, osteopenia and coarsening of the trabeculae and may lose their normal tubulation, which frequently results in a squared or sausage-shaped configuration (Figure 3). A highly characteristic appearance consists of bulbous expansion of the posterior, and to a lesser extent, anterior segments of the ribs (Figure 4). Cardiomegaly may also be seen secondary to anaemia.

In severe cases, soft tissue densities may be present in the posterior mediastinum and, to a lesser degree, in the anterior mediastinum or pelvis, known as extramedullary hematopoiesis (Figure 5). Apart from these skeletal radiographic appearances, imaging can detect the presence of haepatosplenomegaly, gall stones and haemosiderosis using the help of ultrasound, CT scan or MRI.

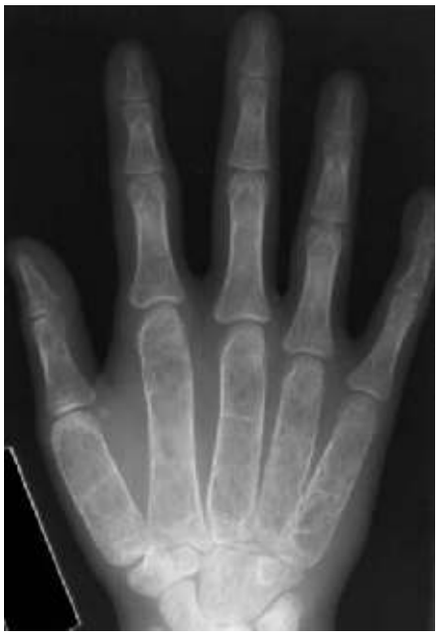

Figure 3: X-ray hand showing decreased bone density with expansion of metatarsals and phalanges resulting sausage shape, secondary to marrow expansion in thalassaemia.

Sickle Cell Anaemia (SCA)

Sickle Cell Anaemia is a haemolytic anaemia characterised by abnormally shaped (sickled) red blood cells (RBC), which are removed from the circulation and destroyed at increased rates in spleen, leading to anaemia. These sickled RBCs cause vascular occlusion, which in turn leads to tissue ischemia and infarction. For the vast majority of patients the vaso-occlusive complications of the disease are much more clinically troublesome than is the anaemia itself.

The skeletal changes of marrow hyperplasia are similar to other haemolytic anaemias such as widening of diploic space, biconcave vertebrae, coarsened ribs and trabeculae. Endosteal apposition of bone is seen as inward cortical thickening which is separated occasionally by a thin zone of translucency, resulting typical appearance in the long bones known as 'a bone within a bone'.

Skeletal complications include infarction and osteomyelitis. Bone infarction is estimated to be much more common than osteomyelitis, at least in children. Infarction typically occurs in the medullary cavity and the epiphysis, and it has been described in essentially every marrow-containing bone. Bone marrow infarction is thought to be the underlying cause of most pain crises.

Infarction manifests first with an ill-defined zone of radiolucency, which subsequently develops arc-like subchondral and intramedullary luceney, with patchy sclerotic areas. A peripheral rim of sclerosis is often seen,

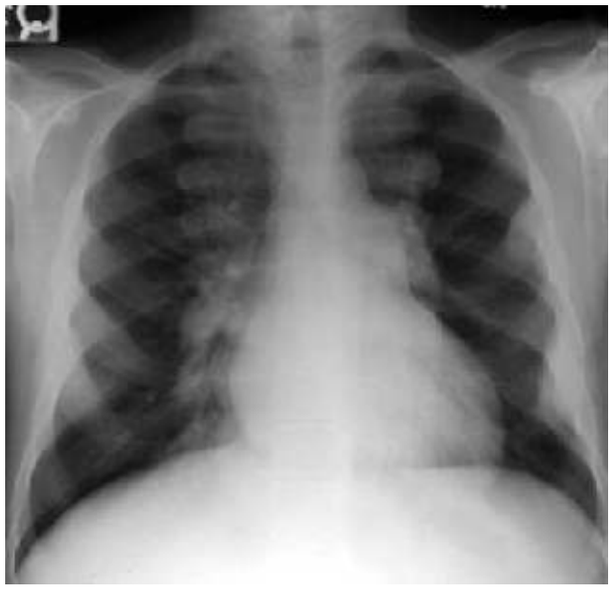

Figure 4: Chest X-ray showing expanded anterior and posterior rib expansion in thalassaemia.

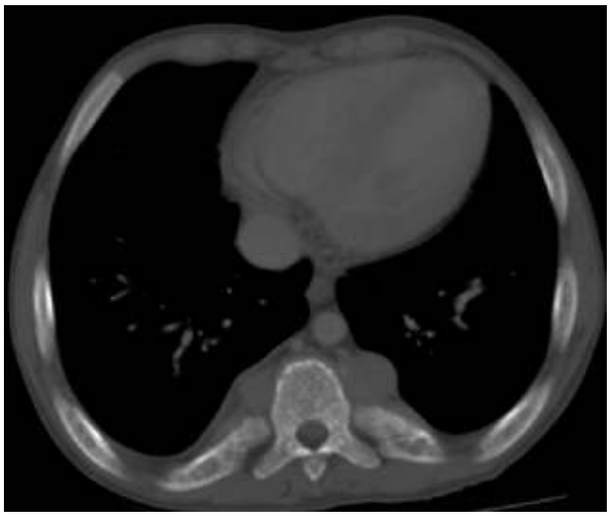

Figure 5: Post contrast CT scan of chest in bone window showing coarse trabeculated expanded ribs with para-vertebral soft tissues bilaterally along dorsal spine suggestive of extramedullary haematopoiesis.

especially with medullary bone infarcts. Over time, these areas will become sclerotic as fibrosis replaces the infarcted region. The common sites of bone infarct are femurs (Figure 6), humeral heads, vertebral bodies, hands and feet. The radiographic appearance of femoral head avascular necrosis is similar to Perthe's disease. In the proximal humerus, infarction gives rise to area of diffuse sclerosis known as 'snowcap sign'.

Central depressions in endplates of vertebral bodies on radiograph are common and give rise to typical H-shaped vertebra, may be seen in asymptomatic patients. Infarcts may be massive, causing bone destruction, sequestration, reactive sclerosis and even the formation of involucrum. When the metaphysis is involved significant deformity may occur due to growth arrest. Central metaphyseal defects and lucencies are typical. These in turn may produce fragmentation and deformity of the epiphyses. They may also be the sites of pathological fractures. In sickle cell anaemia superadded infection is very common and it is frequently challenging to differentiate it from infarcts even with use of CT scan, MRI and nuclear scintigraphy.

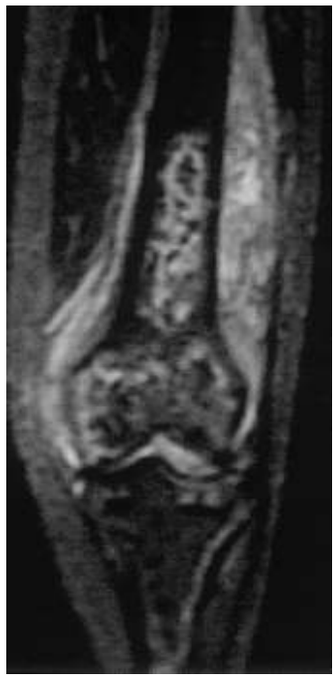

Figure 6: MRI showing Coronal STIR image of knee demonstrating heterogeneous increased signals in proximal femur suggestive of a bone infarct.

N-Terminal ProBNP for Rapid Diagnosis of Heart Failure

Ms Saba Azeem,
Technologist, Clinical Biochemistry

B-type natriuretic peptide (BNP) is a strong diagnostic predictor of left-ventricular dysfunction. Recently, the amino terminal portion of pro-BNP (NT-proBNP) has been introduced in our clinical lab, which is more sensitive because of its longer half-life.

BNP and NT-proBNP found in heart ventricles are the two brain natriuretic peptides derived from the prohormone, pro BNP (Figure 1). Unlike Atrial Natriuretic Peptide whose major storage sites include both atria and ventricles, the source of plasma BNP is cardiac ventricle, where it is synthesised as pre pro BNP and released as proBNP. It is then enzymatically cleaved to NT-proBNP and BNP upon stretching of ventricular myocyte. BNP and NT-proBNP are released when the ventricle is stretched due to hemodynamic pressure.

Both BNP and NT-proBNP are independent predictors of high left ventricular end diastolic

pressure and are useful for assessing mortality in patients with chronic congestive heart failure. Their levels increase progressively with increase in heart failure. Use of BNP and NT-proBNP as population screening tools for left ventricular systolic dysfunction has been shown to be of value in comparison with gold standard echocardiography. BNP and NT-pro BNP assays are useful for screening of heart disease, stratification of patients with heart failure, detection of left ventricular dysfunction, and differential diagnosis of dyspnea. They regulate blood pressure and body fluid volume by their natriuretic and diuretic actions, arterial dilation and inhibition of rennin-angiotensin system.

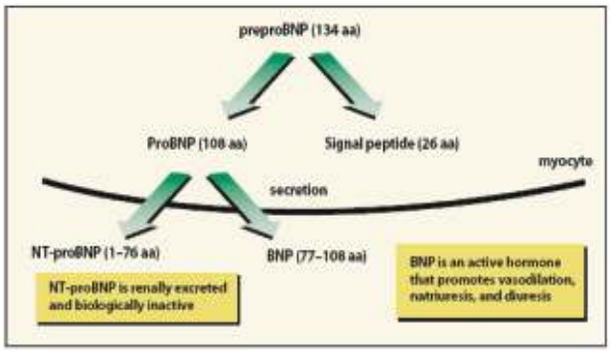

Figure 1. The cardiac natriuretic peptide family. BNP, B-type natriuretic peptide; NT, N-terminal; aa, amino acid.

BNP is cleared via specific natriuretic peptide receptors as well as neutral endopeptidase in the blood. In contrast NT-proBNP is assumed to be cleared solely by renal excretion. Due to differences in their molecular size and metabolism, there is a variation between the utility of BNP and NT-proBNP in diagnosis of congestive heart failure in patients with chronic kidney disease. BNP has been approved as a marker of CHF at a cut-off concentration of 100 pg/mL and NT-proBNP at a cut-off concentration of 125 pg/ml. McCullough et al. reported for the first time that the optimal cut-off for BNP to diagnose CHF should be increased for patients with an eGFR <60ml/min/1.73m2 (Table 1). Therefore NT-proBNP values should be interpreted with the clear understanding of renal function, body mass index and age of the patient.

The diagnosis of heart failure in emergency department is based on history and examination together with chest X-ray and echocardiography. The unreliability of history and examination findings makes both treatment and decision making challenging with potential for misdiagnosis and increased morbidity. BNP or NT-pro BNP are important new tests that can have a considerable impact on the way we manage patients presenting to the emergency with dyspnea and heart failure. It aids in differentiating between cardiac and pulmonary disease in patients with acute dyspnoea. It is a quick and easy test for assessment of heart failure.

The test is performed by electrochemiluminescence in AKUH Clinical Laboratory

Characteristic	BNP	NT-proBNP
Hormonally Active	Yes	No
Half life	20 minutes	1-2 hours
Clearance from body	Via specific natriuretic peptide receptors as well as neutral endopeptidase	Cleared solely by renal excretion
Effect of age on the levels	Increases with normal aging +	Increases with normal aging ++++
Approved cutoff for congestive heart failure diagnosis	100 pg/ml	Age <75 years 125 pg/ml Age >75 years 450 pg/ml
Effect of kidney function on the levels	Correlation with estimated GFR = 0.20	Correlation with estimated GFR = 0.60

Table 1: Comparison between BNP and NT-pro BNP.

Frozen Section: A Rapid Diagnostic Tool

Dr Sabeen Mujtaba,
Resident, Histopathology

Frozen section is an imperfect but rapid method of diagnosis while the patient is still in the operating room. This is achieved through solidifying small pieces of tissue in order to make thin sections for histologic purpose. Similar to emergency cases, the frozen section must solve the most urgent problems for which the entire procedure needs to be immensely fast.

It is performed to provide rapid, gross or microscopic diagnoses that can guide intra or perioperative management of patient, to confirm the presence and adequacy of tissue in the lesion for a subsequent diagnosis on permanent sections and to process tissue for certain special studies for example enzyme histochemistry, immuno histochemistry, polymerase chain reaction, electron microscopy, cytogenetics, hormone receptor analysis, etc.

On receiving the specimen, gross examination is performed and Touch Preparations (TP) are made and sections are placed on chucks. Sections of the grossed specimen are made by cryostat (Figure 1) and are submitted for H&E stain. After staining (Figure 2), the slides are reviewed by the histopathologist. The report is communicated to the requesting surgeon by phone immediately followed by the dispatch of the report. Time required for this whole procedure is approximately 25 minutes.

Some of the limitations of frozen section include the following: only a small portion of tissue can be frozen; ice crystal artifacts can be produced; special studies cannot be performed; further consultation cannot be taken as there is limited time-frame.

It is important to make TPs for analysis due to the following advantages. It is rapidly done and easy to perform with lack of freezing artifacts. In addition, large areas of tissue can be sampled providing important cytological information and avoiding contamination of cryostat with infectious agent. It is also useful when tissue is difficult to cut with cryostat, for example fat, necrotic tissue and bone.

Strict quality control measures are taken at AKUH Clinical Laboratory for maintaining the quality of reporting.

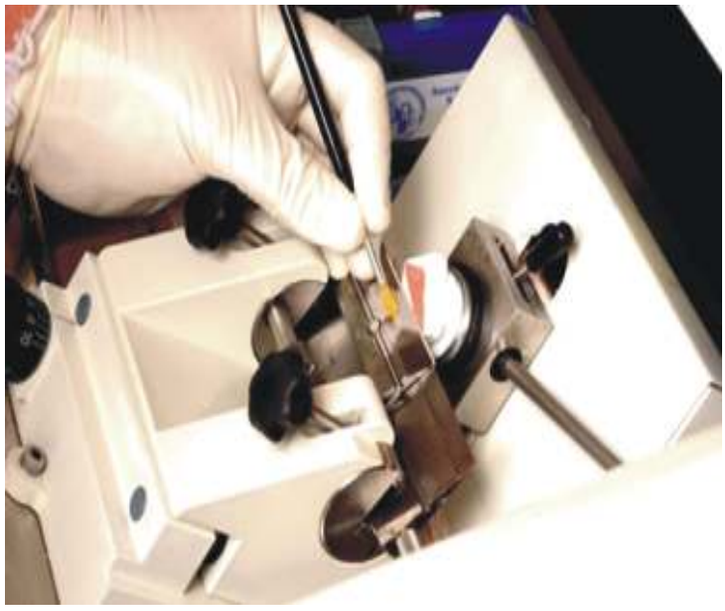

Figure 1: A technologist taking sections of tissue through microtome.

Figure 2. Process of staining slide for microscopy.

Prenatal Diagnosis of ß-Thalassaemia

Ms Toheed Kausar,
Senior Technologist, Molecular Pathology

Thalassaemia is the commonest single gene disorder in Pakistan with a carrier rate of over 6%. Over 5,000 neonates are born every year with thalassaemia major. The cost of treatment of thalassaemia is often beyond the reach of an average Pakistani family. At present the disorder can best be prevented through prenatal diagnosis. A service for prenatal diagnosis of ß- thalassaemia was introduced in Pakistan in 1994 after consultation with two renowned Islamic scholars. Accordingly, pregnancy can be terminated if the foetus is affected by serious genetic disorder, and if termination is before 120 days (17 weeks) of gestations.

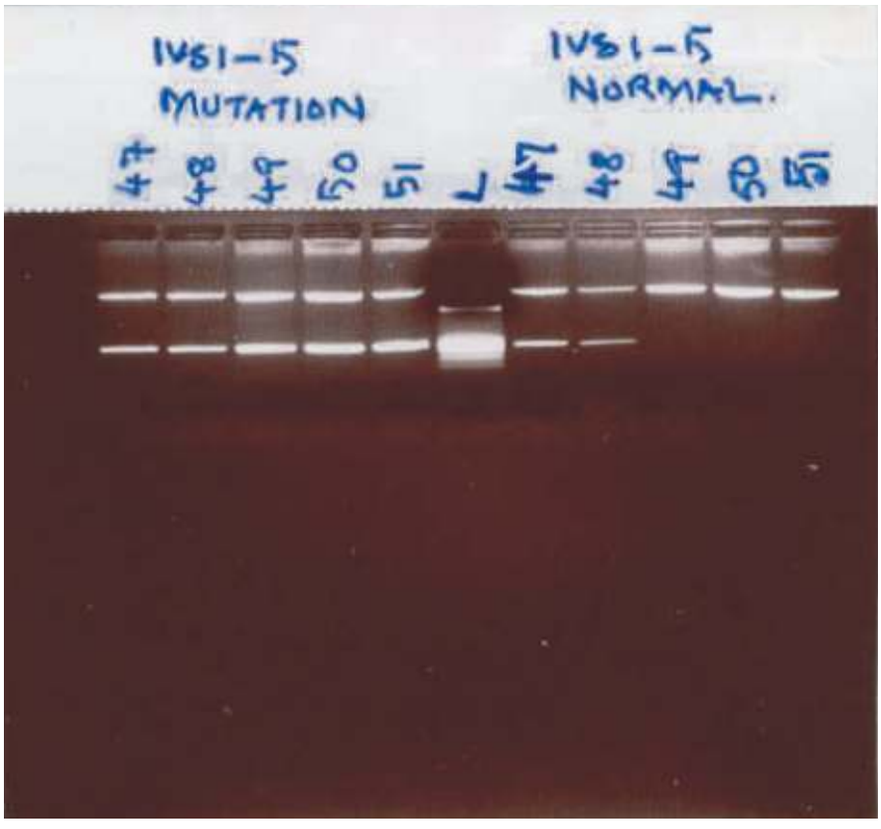

Figure 1: Gel strip showing prenatal diagnosis of ß- thalassemia.

At AKUH, the couple requesting prenatal diagnosis of ß- thalassaemia is registered for Chorionic Villous Sampling (CVS) at Department of Radiology at 10-15 weeks of gestation. At the same time, blood samples are collected from each parent to perform the mutations analysis. The CVS is dissected under a stereo microscope. Maternal tissue, if any, is carefully separated and only placental villi are collected for further DNA analysis.

The Amplification Refractory Mutation System (ARMS) is used to screen for ß- thalassaemia mutations. In the first round, the five most common ß thalassaemia mutations are tested, followed by a second or a third round for uncommon or rare mutations, when the mutation is not identified. The five most common mutations, IVSI-5(G-C), F.S 8/9 (+G), DEL 619 BP, FS 41-42 (-TTCT) and IVSI-I (G-T) constitute 82.3% of the total. Maternal contaminations and paternity is tested by VNTR (Variable Number of Tandem Repeats) analysis at various loci.

Prenatal diagnosis of ß-thalassaemia has given a new dimension to thalassaemia prevention. It is technically feasible and is particularly accepted by families who have thalassaemic children. In Pakistan there is a very strong tendency for a person to marry within their ethnic group. Another frequent custom is marriage between close relatives, especially first cousins. The situation results in an unusually high frequency of autosomal recessive disorders. This signifies the importance of public awareness of thalassaemia at grass root level.

Farewell to Administrative Director, Mr Sajid Khan

Dr Raihan Sajid,
Haematology

Mr Sajid Khan joined Aga Khan University on June 1st, 1986 as Manager of Clinical Laboratories.

He was a key member of a professional team comprising of faculty, managers and technologists in establishing AKUH Clinical laboratories as one of the top most laboratories and reference centre at national and international level.

Mr Sajid Khan retired from services as administrative director clinical laboratory on July 30th, 2009 and shall be remembered as a true gentle man, team leader and efficient manager.

The attached photograph is of his farewell. From left to right:

1st row : Ms Shahida Qureshi, Dr Rumina Hasan, Mr Sajid Khan, Dr Mohammad Khurshid, Dr Naila Kayani, Dr Farooq Ghani, Dr Imran Siddiqui, Mr Sohail Baloch.
2nd Row: Drs Tariq Moatter, Shahid Pervaiz, Bushra Moiz, Asim Beg, Erum Khan
3rd Row: Drs Aysha Habib Khan, Abrar Barakzai, Imran Siddiqui

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