9-2011

LABRAD : Vol 37, Issue 2 - September 2011

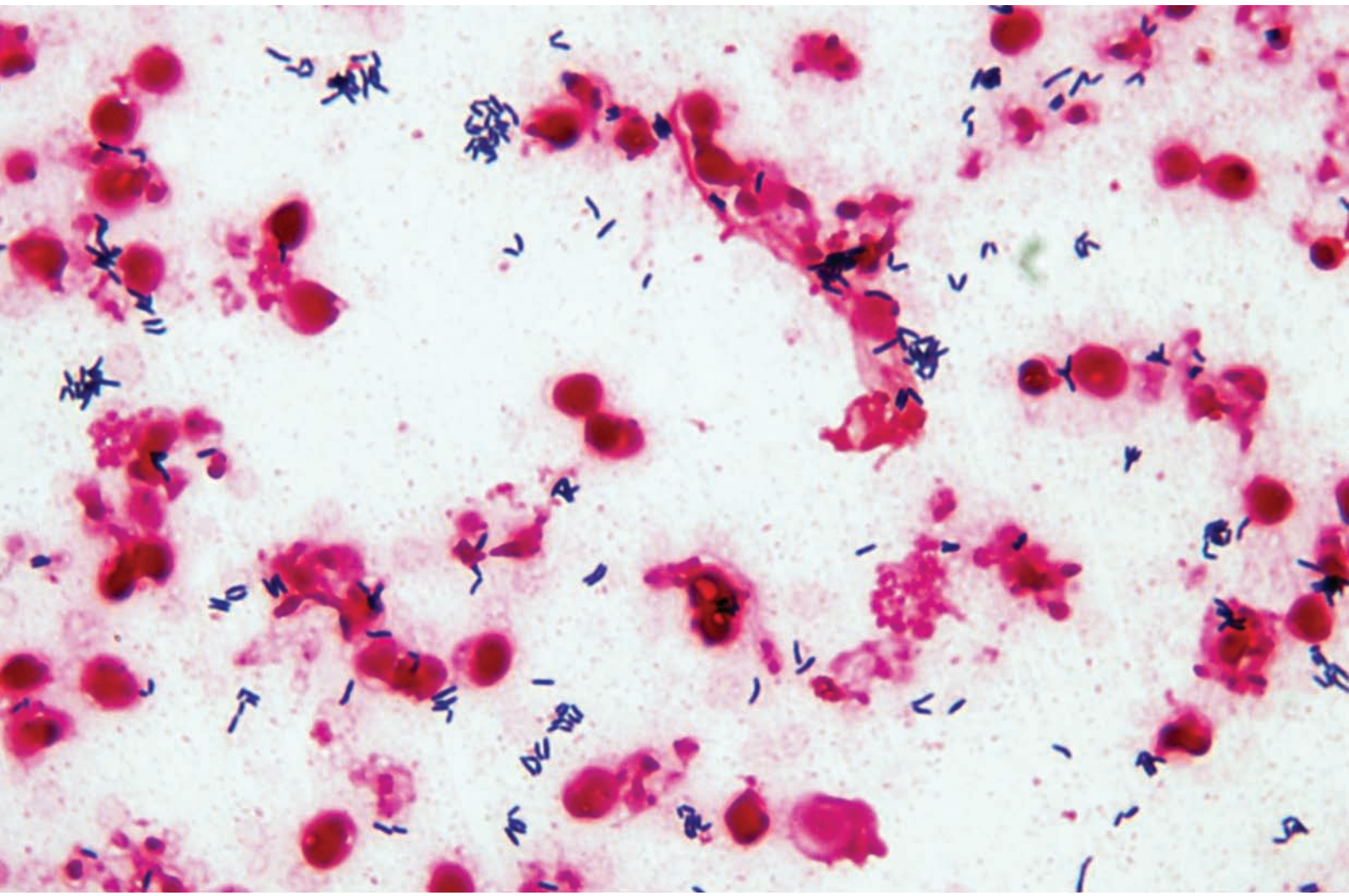
Aga Khan University Hospital, Karachi

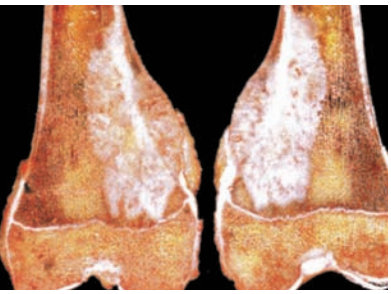
NEWSLETTER OF DEPARTMENTS OF PATHOLOGY, MICROBIOLOGY, AND RADIOLOGY

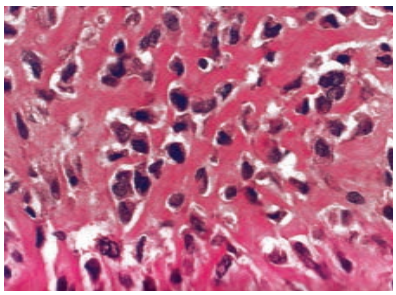
LABRAD

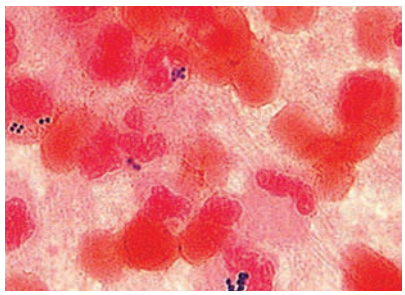
SEPTEMBER 2011

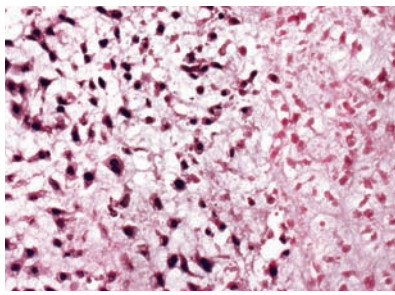
VOL. 37, ISSUE 2











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The Aga Khan University Hospital, Karachi

LABRAD

A Quarterly Publication of the Departments of Pathology, Microbiology, and Radiology

September 2011
Volume 37, Issue 2

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From the Editor's Desk

The Department of Pathology and Microbiology is committed to providing high quality diagnostic services for physicians and hospitals nationwide.

One of the functions of the Department is to update physicians throughout Pakistan about the advancement in laboratory sciences and the services available at the Clinical Laboratory for disease diagnosis, through our Laboratory Updates and this newsletter, LABRAD.

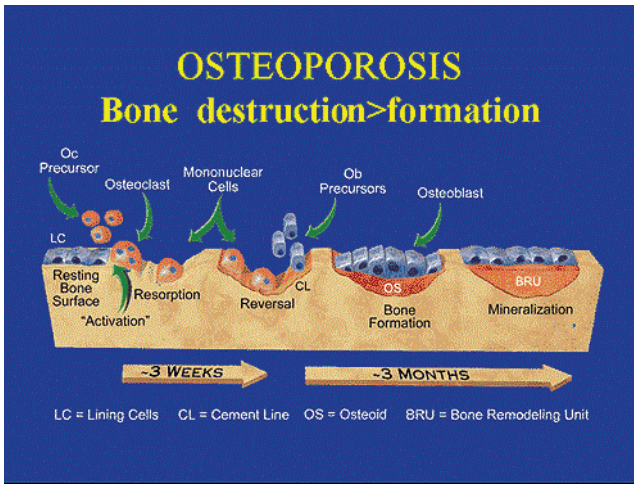
The current issue of the LABRAD focuses on measurements related to bone disorders in an attempt to give readers a better understanding of the diagnostic modalities available at Aga Khan University Hospital Clinical Laboratories.

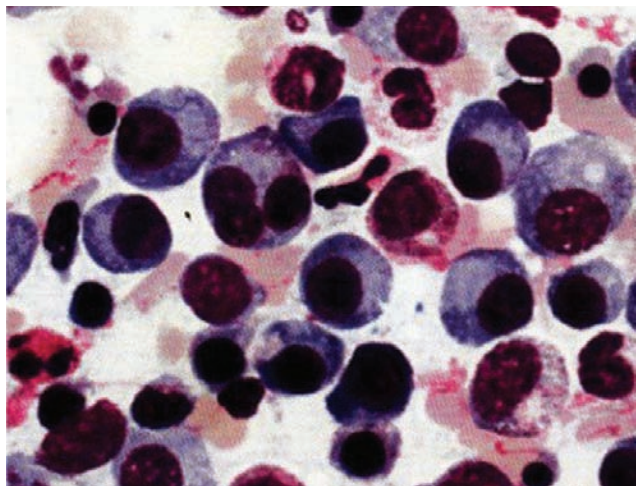
Bone disease is a vast topic which interests a diverse group of medical professionals such as: internists, rheumatologist, orthopaedics, endocrinologist, gynaecologists, paediatricians and geriatrics, researchers in anatomy, physiology, nutrition, biochemistry, pathology and imaging. Diversity of interest is due to a combination of problems ranging from malnutrition in the developing world to the aging populations of the developed world.

Several factors may influence the choice of a method for the measurement of bone disease. A major limiting factor in establishment of a service for measurement of bone diseases is the local availability of equipment and methodology, and also the availability of the required expertise and knowledge for the interpretation of results. Choice of the tests also depends on the type of information required from investigating a patient suspected of having a bone disease and achieving this aim as economically as possible in less time and money.

This issue concentrates on common bone related clinical problems; focusing on practical usefulness of various tests available for diagnosis and management of bone diseases in the field. Biochemical bone profiles have been developed in the section of chemical pathology to help the physician in evaluating a patient suspected of having metabolic bone diseases. Combination of information on different microbiological tools for facilitating management of bone infection along with diagnostic tools available for identifying disorder like ankylosing spondylitis are also presented. On the whole this is a broad sweep of information collected through individual disciplines and we hope it will improve as our newsletter evolves through a cyclic process of learning and sharing with our readers.

We hope this edition would help increase your knowledge banks and would answer many of your questions. We are hopeful for making this issue a useful document for future reference in the bone disease area. Happy reading!





Biochemical Bone Profiles Available at Clinical Laboratory of Aga Khan University Hospital

Dr Farhan Javed Dar
Chemical Pathology

The utility of bone profiles in screening and diagnosis of bone diseases have a profound effect upon bone health. Choosing any screening profile helps to rule out metabolic bone diseases such as vitamin D deficiency, osteoporosis. Panels are made to make clinical interpretation easy for physicians and economical for patients.

Following profiles are available at Clinical Laboratory AKU for diagnosis and monitoring of bone disorders. Only 5 ml of blood is sufficient for any one of the panel as shown in the table.

when we are young will help prevent problems in the future.

Commonly encountered bone diseases include those related to vitamin D deficiency (rickets in children and osteomalacia in adults), osteoporosis, osteogenesis, imperfecta and Paget's disease of bone. Bone disease can lead to fragile and brittle bones, which break easily.

AKUH clinical laboratory is now providing Bone Health Panel, (please refer to the table below) which helps in assessment of bone health. This profile is

BIOCHEMICAL BONE PROFILE

Bone Health Panel	Osteoporosis Panel
Calcium	Calcium
Phosphorus	Phosphorus
Albumin	Albumin
Magnesium	Alkaline phosphatase (ALP)
Alkaline phosphatase (ALP)	25-hydroxy vitamin D (25-OHD)
Creatinine	Intact parathyroid hormone (iPTH)
Intact parathyroid hormone (iPTH)	N-telopeptide of type I collagen (NTx)
25-hydroxy vitamin D (25-OHD)	

Bone Health Panel

Bone health is important throughout the lifetime of an individual. Bones protect our internal organs from damage, they are the factory of our blood cells and are the storehouse for minerals and nutrients (such as calcium and phosphorus) needed in the body. With all of these critical functions for our health, understanding how to maintain and build healthy bones is an important part of investigating for a healthy future.

It is especially important to build strong and healthy bones during childhood and teen years to avoid osteoporosis and other bone problems later in life. After the mid-30s, bone loss slowly begins to occur. Women lose bone quickly after menopause. Importantly, healthy habits can help to limit the bone loss that occurs. Taking care of our bones

intended to screen, investigate and monitor patients with disorder of calcium and bone metabolism. It provides answer to the potential clinical questions that a physician seeks when investigating for metabolic bone diseases for example; does my patient have hypocalcaemia or hypercalcaemia? If there is hypercalcaemia, is it due to hyperparathyroidism or a parathyroid hormone independent cause? If it is hypocalcaemia, is it due to parathyroid failure, vitamin D deficiency or renal failure? Does my patient have hypomagnesemia? If the patient has aches and pains or unexpected fracture then is it due to metabolic bone disease?

Osteoporosis Panel

Osteoporosis is a systemic skeletal disorder characterised by low bone mass and micro architecture

deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture.

According to WHO by 2025, almost 3 million of the global population will suffer from osteoporosis which will be expected to rise to 6.3 million by 2050. About 75 percent of this 6.3 million hip fractures will occur in the developing countries, mainly due to projected large increase of aged population. Osteoporosis is a costly disease due to its chronic nature, severity of its complications and means required to treat it. Osteoporosis is diagnosed late as patient remains asymptomatic until fracture occurs. AKUH Clinical Laboratory is providing Osteoporosis Panel, mentioned in the above table, which helps in assessment of osteoporosis.

It is to be noted that diagnosis should be made in correlation with clinical picture and Dual-energy X-ray absorptionetry (DXA) results.

Ionized Calcium Determination in Clinical Labortory

Dr Lena Jafri
Chemical Pathology

Plasma calcium exists in three forms ionized (iCa) (45 to 50 per cent), protein bound (40 per cent) and calcium complexed with anions (10-15 per cent). Although all the forms are in equilibrium with each other, only the plasma iCa has been reported to be active at cellular level. This parameter has also been called 'free' or 'ionic' calcium. It is considered the best indicator of calcium status because it is biologically active and tightly regulated by parathyroid hormone (PTH) and 1, 25 dihydroxy vitamin D. Total calcium level does not give an indication of what is available at the cellular level. Only disturbances in iCa are physiologically relevant.

Analysis of iCa is technically demanding. The sound analytical performance of today's iCa analyzers using ion selective electrode (ISE) technology have made measurements accurate and precise. iCa recently introduced in Aga Khan University Clinical Laboratory after thorough research and understanding of Clinical Laboratory Standard Institute Guidelines (CLSI) for sample handling, storage and transportation. Table 1 shows the precautions to be taken prior to blood sampling for iCa determination and collection.

The clinical usefulness of measuring iCa rather than total calcium is more in disorders with plasma protein abnormalities like liver failure, protein losing

Precollection Variables and Recommendations	Collection Techniques
Have the patient rest for 5-10 min before collecting blood	If a series of tubes must be collected, fill gel tube for iCa first
Ensure that the patient has not eaten for at least 4 hours	Do not leave the tourniquet on for more than 3 minutes
Collect specimens under consistent conditions ideally the patient should be seated	Do not allow the patient to exercise the forearm or make a fist
	Fill gel tubes completely
	Handle specimen anaerobically (do not open the tube until analysis)

Table 1. Recommendations for specimen collection of iCa as per CLSI guidelines

nephropathy, burns, cardiac failure or malnutrition. iCa determination is more useful in those undergoing major surgery who have received citrated blood/ platelets, heparin, or intravenous calcium.

It should be the test of choice when neonatal hypocalcemia is suspected, especially if bicarbonate is given to neonates with hypocalcemia.

Rapid measurement of iCa in intensive care units is helpful in cases with sepsis and acid base disturbances. It has been documented that the use of total calcium is unreliable in cases where there is a change in the protein-calcium binding characteristics as in patients with hypergammaglobulinemia or a decrease/increase in pH. An increase in blood pH is associated with a decrease in iCa a decreased blood pH is associated with an increased iCa.

Ionized calcium is also useful in cardiopulmonary bypass and during hemodialysis where maintenance of good cardiac function is essential. During dialysis monitoring a slight positive calcium balance is important for maintaining good cardiac contractility. Ionized calcium is the best means to monitor this.

Literature shows that iCa rather than total calcium is more useful in malignancies, hypo/ hyperparathyroidism and pancreatitis.

Parathyroid Hormone Disorders and Issues of Testing

Dr Syed Talha Naeem
Chemical Pathology

Parathyroid hormone (PTH) is secreted by the parathyroid glands and regulates serum calcium (Ca) through its effects on the bone, kidney and intestine. PTH secretion is stimulated by decrease in serum Ca and magnesium and an increase in serum phosphate, which in turn raises serum ionized Ca levels through direct action on bone and the kidneys. Long-term regulation of total body Ca by PTH occurs through its stimulation of vitamin D metabolism.

PTH can be measured in the blood in several different forms: intact PTH; N-terminal PTH; mid-molecule PTH, and C-terminal PTH. An intact PTH (iPTH) provides a better index of parathyroid function and is the test available in AKUH clinical laboratory for clinical use.

PTH is secreted in episodic or pulsatile fashion with an overall circadian rhythm characterised by a nocturnal rise. Measurement of iPTH on more than one occasion should minimise the effect of episodic secretion and circadian rhythm. Because of the nocturnal rise in iPTH levels, samples should be collected in the morning, preferably after an overnight fast.

Determination of iPTH is useful in the differential diagnosis of disorders of bone and mineral metabolism, including hypocalcaemia and hypercalcaemia, renal failure and secondary hyperparathyroidism; when PTH increases long before Ca becomes abnormally low.

PTH is elevated in the majority of patients with primary hyperparathyroidism. It is below normal or in the lower half of the reference interval in most patients with nonparathyroid hypercalcemia including hypercalcemia-associated malignancy. In cases with hypercalcemia, PTH estimation should be performed before initiating any therapy to reduce serum Ca; as PTH secretion may be stimulated by declining but still elevated levels of serum Ca. This can complicate the differential diagnosis of hypercalcaemia. In the majority of patients with hypercalcaemia associated with malignancy, iPTH is suppressed to levels below normal or at the lower end of the normal reference interval. Elevated levels of iPTH in patients with hypercalcemia and malignancy suggest coexisting hyperparathyroidism and malignancy, because ectopic PTH production appears to be extremely rare.

In secondary hyperparathyroidism, iPTH is increased before total or free calcium becomes abnormally low, a consequence of homeostatic mechanisms

for maintenance of serum Ca. Consequently, PTH is more sensitive than Ca for identifying secondary hyperparathyroidism. Subnormal or normal PTH is observed in the majority of patients with hypoparathyroidism; such concentrations are inappropriately low for patients with hypocalcemia.

In patients with end-stage renal disease, measurement of iPTH is helpful in assessing parathyroid function, in estimating bone turnover, and in improving management. Patients with high turnover bone disease because of secondary hyperparathyroidism (advanced osteitis fibrosa) have the highest concentrations of PTH, whereas patients with low-turnover, adynamic bone disease, including osteomalacia, have the lowest concentrations. Intermediate levels are found in patients with low-turnover adynamic (aplastic) disease and early osteitis fibrosa. Considerable overlap in iPTH levels is apparent between the various forms of renal osteodystrophy.

iPTH is also useful intraoperatively, for assessing the completeness of parathyroidectomy and facilitating minimally invasive parathyroid surgery, thereby improving cost-effectiveness and cosmetic outcomes.

PTH levels may be altered in some patients with hyperthyroidism and hypothyroidism and may increase after treatment with lithium carbonate. PTH concentrations are inversely correlated with T3 levels in hyperthyroid patients, increase in patients who become hypothyroid after radioactive iodine treatment, and decrease with replacement therapy; changes apparently mediated by serum Ca. Chronic lithium carbonate therapy has been reported to increase parathyroid gland size and circulating intact PTH. Drugs that may increase PTH levels include phosphates, anticonvulsants, steroids, isoniazid, lithium, and rifampicin.

Because of the physiological relationship between circulating Ca and PTH, it is always important to interpret PTH results in the light of total or ionized Ca levels. Indices of renal function, measurements of albumin, as an adjunct to measurement of total calcium levels and determinations of phosphorus, chloride, and magnesium levels may also aid in the interpretation of PTH and Ca results. It should also be remembered that hypercalcemia and hypocalcemia may be secondary to disordered vitamin D metabolism. For diagnostic purposes PTH results should always be used in combination with the clinical examination, patient medical history, and other findings.

Tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR)

Dr Noreen Sherazi
Chemical Pathology

Phosphate filters entirely through the glomeruli but is then largely reabsorbed in the proximal part of the proximal renal tubule. Several factors influence the tubular reabsorption of phosphate (Table 1) by acting on the sodium/phosphate co transporters.

Tubular reabsorption of phosphate depends on plasma phosphate and glomerular filtration rate and is not a satisfactory indicator of tubular phosphate handling. This has led to increasing use of 'tubular maximum for phosphate corrected for GFR (TmP/GFR)', a factor independent of plasma phosphate and renal functions for assessment of renal phosphate handling. TmP/GFR (Normal 2.8-4.4 mg/dL) is an index of renal threshold for phosphate which can be determined directly by the formula TmP/GFR = Plasma phosphate - (urine phosphate x serum creatinine)/urine creatinine.

Assessing renal reabsorption of phosphorus is needed in a variety of pathological conditions associated with hypophosphatemia including hypophosphatemic rickets, tumour-induced osteomalacia and tumoral calcinosis. It is also used in adjusting phosphate replacement therapy in severe deficiency states; like conditions that cause phosphate redistribution (e.g. glucose infusion, respiratory alkalosis), so if hypophosphataemia persists, a low (fasting) TmP/GFR indicates the

Factors that decrease renal phosphate absorption	Factors that increase renal phosphate absorption
Phosphate-free diet	High dietary phosphate intake
Respiratory acidosis, metabolic alkalosis	Respiratory alkalosis, metabolic acidosis
PTH, PTHrP, EGF, glucocorticoids, catecholamines, calcitonin, 1,25$(OH)_2$ D_3	GH, IGF1, thyroid hormones (T3), insulin
Diuretics	
Phosphatonins	

Table 1. Factors affecting tubular reabsorption of phosphate

need for phosphate replacement. In the treatment of severe phosphate deficiency, TmP/GFR can be used as an indicator of intracellular repletion.

The measurement of TmP/GFR along with other parameters is required for diagnosis of X-linked hypophosphataemic rickets and hereditary hypophosphataemic rickets with hypercalciuria. Measurement of TmP/GFR is central to the diagnosis of oncogenic osteomalacia in addition to other parameters

We, at AKUH clinical laboratory, are starting this test which will required at least 6-8 hours fasting serum sample for phosphate and creatinine and simultaneously taken spot urine sample for phosphate and creatinine. TmP/GFR will then be calculated from above mentioned parameters.

LABRAD Quiz

Dr Hafsa Majid
Chemical Pathology

Question #1

A three-week-old girl was brought to the emergency room because of intermittent twitching of her left limbs for 4 days. The girl had been born normally at 39 weeks of gestation and was being breast-fed. Examination was unremarkable. Her biochemical investigations were:

Na^+ : 125mmol/l (135-145 mmol/l)
Cl^- : 100mmol/l (101-111 mmol/l)
K^+ : 2.8mmol/l (3.6-5.0 mmol/l)
HCO^3 : 30mmol/l (23-28 mmol/l)

Ca^+ : 4.5mg/dl (8.4-10.2mg/dl)
Albumin : 2g/dl (3.2-5.5g/dl)
PO_4 : 10mg/dl (2.5-4.6 mg/dl)
PTH : <4pg/l (16-87pg/ml)

1.1: What is the diagnosis?

1.2: Which other bone mineral is required for optimal parathyroid function?

1.3: How would this be treated?

Question #2

A 60-year-old female came to ER due to fracture neck of femur after a minor fall. Her past complaints were of generalised body aches and bone pains with difficulty in rising from sitting position since two year. She was a known case of hypertension for the past 20 years and taking antihypertensive since then. She was diagnosed to be osteoporotic and was considered for bisphosphonate therapy.

2.1: What is the most likely cause of body aches in the patient?

2.2: What biochemical test can be performed to confirm diagnosis?

2.3: How will you monitor the response to bisphosphonate therapy?

Renal Osteodystrophy: A Disturbed Metabolic Aspect of Renal Failure

Dr Sahar Iqbal
Chemical Pathology

Renal osteodystrophy is a common complication of chronic kidney disease (CKD). It is part of a broad spectrum of disorders of mineral and bone metabolism that develop in this clinical setting and result in both skeletal and extra skeletal consequences. The term CKD-Mineral and Bone Disorder (CKD-MBD) has been recommended to be used to describe a broader clinical syndrome that develops as a systemic disorder of mineral and bone metabolism in CKD. The manifestations include abnormalities of calcium, phosphorous, parathyroid hormone (PTH) or vitamin D metabolism; abnormalities in bone turnover, mineralisation, volume, linear growth or strength; and vascular or other soft tissue calcification.

The term renal osteodystrophy should be used exclusively to define alterations in the bone morphology associated with CKD. This is a single measure of the skeletal component of the systemic disorder of CKD-MBD that is quantifiable by histomorphometry of bone biopsy. The abnormalities of the bone in the setting of CKD may manifest as high turnover bone disease (ostietis fibrosa or secondary hyperparathyroidism), dynamic bone disease, osteomalacia and mixed renal osteodystrophy.

Biochemical markers for diagnosing renal osteodystrophy are intact PTH (iPTH), vitamin D (25OHD), blood calcium and phosphorus levels and bone turn over markers such as alkaline phosphatase and N-telopeptide of type I Collagen (NTx).

The pathogenesis of osteodystrophy is complex. The cycle starts with the renal failure and retention of phosphate, inhibiting calcitriol (1,25 dihydroxy vitamin D) synthesis and decrease in calcium through a reduction in intestinal calcium absorption. Hypocalcaemia stimulates PTH secretion causing high turnover renal osteodystrophy.

The biochemical picture of high turnover renal osteodystrophy usually is an increase in blood PTH, bone alkaline phosphatase and phosphate concentration and low calcium and 25OHD levels.

Low turnover bone disease is commonly observed in dialysis patients, but has been described in some cases even before dialysis. Adynamic bone disease is associated with the over suppression of parathyroid gland activity due to high calcium intake (from diet, dialysate or calcium-containing phosphorus binders) and/or administration of vitamin D analogs in excess.

Patients with adynamic bone disease have lower blood concentrations of iPTH that may fall below the recommended target range of 150-300 pg/ml of iPTH, resulting in a bone turnover rate that is below normal. In these circumstances, the bone may not take up calcium for incorporation into new bone and any excess calcium may predispose to calcification in soft tissues. Currently in the United States, approximately 25 per cent of patients undergoing dialysis have a iPTH concentration above the target range (high-turnover disease), approximately 25 per cent have a PTH concentration within the target range and 50 per cent have a PTH concentration below the target range (adynamic bone disease).

The ultimate goal of drug therapy in the management of CKD is to prevent complications of CKD, specifically bone disease and extra skeletal calcification. Need for the evaluation, treatment and monitoring of bone metabolism is to prevent secondary hyperparathyroidism. At stage 3 CKD, serum calcium, phosphorus and iPTH concentrations should be evaluated. After initial assessment, routine monitoring should be performed (see table 1). Appropriate management requires a balanced diet, phosphorus binders and active vitamin D analogs. The recommended ranges for corrected calcium, phosphorus and iPTH for different stages of CKD for kidney diseases outcomes quality initiative are shown in Table 2.

Stage of CKD	Measurement of iPTH phosphorus	Measurement of calcium and
3 (30-59 ml/min/1.73 m^2	Every 12 months	Every 12 months
4 (15-29 ml/min/1.73 m^2	Every 3 months	Every 3 months
5 (<15ml/min/1.73 m^2	Every 3 months	Every month

Table 1. Monitoring of frequency of calcium, phosphorus and intact PTH in different stages of CKD kidney diseases
National kidney foundation. Clinical practice guidelines for bone metabolism and disease. Am J Kidney Dis 2003; 42(4 supp 3): S12

Stage of CKD	Phosphorus (mg/dl)	Corrected calcium (mg/dl)	Serum PTH (pg/ml)
3 (30-59 ml/min/1.73 m^2	2.7-4.6	Normal range	35-70
4 (15-29 ml/min/1.73 m^2	2.7-4.6	Normal range	70-110
5 (<15ml/min/1.73 m^2	3.5-5.5	8.9-9.5	150-300

Table 2. Recommended ranges for phosphorus, corrected calcium and PTH for different stages of CKD.
National kidney foundation. Clinical practice guidelines for bone metabolism and disease. Am J Kidney Dis 2003; 42(4 supp 3): S12

Osteopenia and Osteoporosis in Beta Thalassemia Major

Dr Huma Mansoori and Dr Shabina Sikandar
Haematology

Beta thalassemia major (TM) is an inherited autosomal recessive disorder in which synthesis of beta globin chains is compromised due to mutation of beta globin gene culminating in ineffective erythropoiesis.

The anaemia manifests at 6 to 9 months after birth as haemoglobin switches from HbF to HbA with features like failure to thrive, poor feeding, recurrent infections, pallor and enlarged spleen. In untransfused patients haemoglobin level ranges from 3 to 6 gm/dl. However, regular blood transfusion and compliance with prompt iron chelation therapy has markedly improved life expectancy of thalassemics, but morbidity due to its chronic complications like osteopenia and osteoporosis remains there.

Osteopenia refers to bone mineral density (BMD) that is lower than normal peak BMD but not low enough to be classified as osteoporosis while osteoporosis is characterised by low bone mass and disruption of bone architecture, resulting in decreased bone strength with an increased risk of fracture.

The frequency of osteopenia or osteoporosis in well-treated patients of TM is approximately 40-50 per cent which clearly depicts that despite the normalisation of haemoglobin levels, adequate hormone replacement and effective iron chelation; thalassemics continue to show an unbalanced bone turnover and an increased resorption resulting in seriously diminished BMD. Bone disease in thalassemia is manifested by diffuse bone pain, spinal deformities like scoliosis, nerve compression and various degrees of osteopenia, osteoporosis and spontaneous fractures.

Factors contributing to osteoporosis and osteopenia in thalassemics include iron overload resulting in endocrinopathies; which directly or indirectly leads to decreased bone mass. There is marrow expansion due to increased erythropoiesis causing mechanical interruption of bone formation leading to cortical thinning of bones. In addition, osteoblasts are also affected by direct iron toxicity as well as due to liver disease. Chelation therapy with desferrioxamine inhibits DNA synthesis, osteoblast and fibroblast proliferation, osteoblast precursor's differentiation and collagen formation, whereas in high doses it enhances osteoblast apoptosis.

The serum biochemistry is usually unhelpful in diagnosis of osteoporosis because the levels of calcium, phosphate and PTH are generally normal. However, markers of bone turnover such as serum alkaline phosphatase, serum osteocalcin, and N telopeptide of Type I Collagen (NTx) may be raised in osteoporosis in TM. Dual energy x-ray absorption (DXA scan) is also commonly used to assess bone mineral density but it should be kept in mind that it may fail to provide accurate and precise information on osteoporosis in thalassemics as they have spinal degenerative skeletal changes, which can be detected only by MRI and is likely to interfere with BMD values, resulting in false diagnosis of bone disease. Vitamin D deficiency can also co-exist.

Following therapeutic strategies should be undertaken to prevent and treat osteopenia/osteoporosis in TM.

- lifestyle measures should be encouraged such as physical activity and smoking quitting.
- calcium and vitamin D intake during skeletal development increases bone mass in adult life with the final goal to prevent bone loss and fractures.
- induction of puberty at a proper age and treatment of hypogonadism with hormone replacement therapy shows quite promising results to prevent osteoporosis and other bone deformities.
- calcitonin, a potent inhibitor of osteoclasts, in combination with calcium and vitamin D has shown to decrease bone pain and radiological signs of osteoporosis.
- alendronate, pamidronate, and zoledronic acid have shown efficacy in osteoporotic patients with TM either with normal or impaired gonadal function but further research in the therapeutic trials with bisphosphonates is needed to allow definite conclusions especially in our setting where vitamin D deficiency is widely prevalent. However, they may be used as a second line treatment if the above strategies have failed.

Role of β_2 Microglobulin in Multiple Myeloma

Dr Mehreen Imran
Haematology

Multiple myeloma is a B cell malignancy characterised by monoclonal accumulation of abnormal plasma cells in the bone marrow (Fig. 1). The clinical signs and symptoms of myeloma are heterogeneous and include bone complications, symptoms of impaired formation of blood cells and hyperviscosity, renal dysfunction, infections, peripheral neuropathy and extra medullary disease.

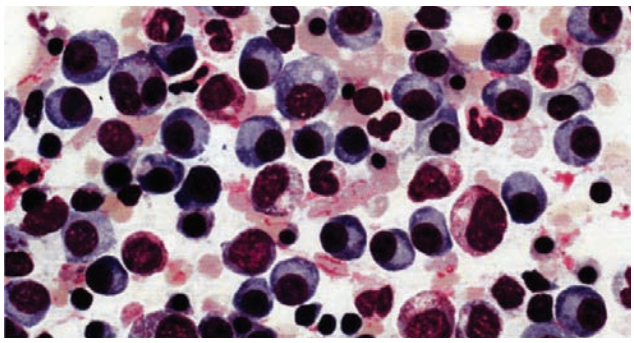


Fig. 1. Bone marrow aspirate demonstrating plasma cells of multiple myeloma

Multiple myeloma constitutes about 15 per cent of the haematological malignancy. The disease develops in one to four per 100,000 people per year. It is more common in men, and is twice as common in blacks as it is in whites. With conventional treatment, the prognosis is three to four years, which may be extended to five to seven years or longer with advanced treatments.

Diagnostic work up for myeloma is shown in Table 1. One of the laboratory parameter is β_2 microglobulin

Laboratory Tests

Full blood count, peripheral film and ESR

Evaluation of kidney function, serum calcium, CRP, β_2 microglobulin, LDH, uric acid levels and liver function tests

Protein electrophoresis and paraprotein quantification

Quantitative analysis of normal immunoglobulins, 24-hours urine collection for light chain (Bence Jones protein) excretion, coagulation screen

Bone marrow aspiration and trephine biopsy for morphology, immunophenotyping (CD138, CD79a, kappa, lambda, CD20) and cytogenetic

Radiological Tests

Complete skeletal survey

Computerised tomography (CT)

Magnetic resonance imaging (MRI)

Table 1. Laboratory and radiological work ups for multiple mayeloma available at AKUH

(also known as β_2M) is a component of MHC class I molecules, which are present on all white blood cells (including plasma cells). In humans, the β_2 microglobulin protein is encoded by the β_2 microglobulin gene (Fig. 2). Normal urinary excretion of β_2 microglobulin is less than 370 micrograms per 24 hours. Elevated serum concentration in the presence of normal glomerular filtration rate suggests increased β_2 microglobulin production or release, which is seen in lymphoproliferative diseases such as multiple myeloma.

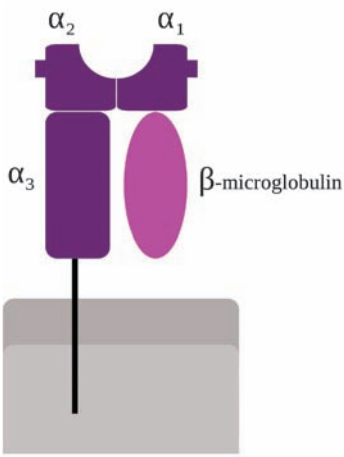


Fig. 2. Structure of MHC class I β_2 microglobulin

The increase is seen in cancers involving white blood cells, but it is more meaningful in newly diagnosed multiple myeloma patients. Increased production or destruction of plasma cells causes β_2 microglobulin level in the blood to increase. Pre-treatment values of β_2 microglobulin are thus useful in confirming tumor mass grade, and in assessing response to chemotherapy; marked reductions following chemotherapy correlated well with the onset of remission. The serum β_2 microglobulin level is one of the prognostic factors incorporated into the International staging system. Patients with high values have inferior survival.

The following levels of β_2 microglobulin correlate with stages of multiple myeloma:

Stage I multiple myeloma

- β_2 microglobulin level is lower than 3.5 mg/mL
- albumin level is 3.5 g/dL or higher

Stage II multiple myeloma

- β_2 microglobulin level is lower than 3.5 mg/mL and the albumin level is lower than 3.5 g/dl
- β_2 microglobulin level is between 3.5 and 5.5 mg/mL

Stage III multiple myeloma

- β_2 microglobulin is 5.5 mg/mL or higher.

The prognostic value of serum β_2 microglobulin level in myeloma is probably due to correlation of levels with tumour burden. High levels are also associated with renal failure, which carries an unfavourable prognosis in multiple myeloma. The reference ranges are shown in table 2.

		ng/mL			
Group	Median	2.5%ile	95%ile	97.5%ile	N
Males	1,556	604	2,157	2,284	424
Females	1,473	607	2,295	2,454	370

Table 2. Reference ranges for β_2 microglobulin as reported in AKU-Laboratory

β_2 microglobulin test is performed by a chemilumniscent assay in Clinical Laboratory AKUH. It is done on clotted blood sample of the patient; however, it can also be performed on patient's urine. Urine may be a single collection or collected throughout a 24 hour time period. The urine should be refrigerated until it is brought to the laboratory and must not become acidic.

Update on Microbiological Diagnosis of Bone and Joint Disorders

Dr Kauser Jabeen
Microbiology

Prosthetic joint infections; How to effectively use the available diagnostic modalities?

Prosthetic joint infections (PI) are associated with substantial morbidity and costs; therefore early and accurate diagnosis is crucial. The clinical presentation may be non-specific and vary between different patient populations. Effective use of the currently available diagnostic modalities for PI results in improves outcomes. PI occurring within three months of joint replacement are categorized as early and 3-12 months after surgery as delayed and >12 months as late.

A widely accepted case definition for PI includes:

- purulence around a prosthesis at arthrotomy or arthroscopy
- presence of one or more sinus tract communicating with the joint
- histological features of infection
- isolation of similar organism from at least two deep culture samples. Isolation of virulent organisms, such as Staphylococcus aureus, Escherichia coli, or Candida spp., in one deep tissue sample may be considered as significant to confirm the diagnosis.

Gram positive organisms, especially Staphylococci (commensal skin organisms), are most commonly involved and in early infection, pathogens are usually more virulent (for example, Staphylococcus aureus), whereas more indolent organisms predominate later on (for example, coagulase negative staphylococci, Propionibacterium acnes) (Table 1).

Gram positive organisms	Gram negative organisms	Fastidious organisms
Coagulase negative staphylococci	Enteric Gram negative bacilli	Anaerobes
Methicillin sensitive *Staphylococcus aureus*	*Pseudomonas spp*	Mycobacteria
Methicillin resistant *S aureus*		Fungi
Streptococcus spp		
Enterococcus spp		
Diphtheroids (*Corynebacterium* spp, *Propionibacterium* spp)		

Table 1. Organisms commonly involved in prosthetic joint infections

Laboratory investigations

Detection of inflammatory markers:
Baseline blood tests for inflammatory markers including C reactive protein (CRP), erythrocyte sedimentation rate (ESR), leukocyte count should be performed for any suspected case. These parameters may be elevated up to two weeks after surgery and cannot be relied upon in early infection. Serial measurements of these markers are recommended and persistently or progressively high inflammatory markers most likely reflect infection. It is important to note however, that a normal result does not exclude joint infection especially if the pathogen is of low virulence.

Cultures:
Blood for culture should be taken in every case before starting antibiotics as even a single dose is known to decrease the diagnostic yield. In most cases however, it is difficult to take deep cultures before starting antibiotics in patients with systemic sepsis or rapidly evolving local infection.

Superficial swabs should not be collected as they most likely reflect colonising flora and results must therefore be interpreted with caution. Deep samples of synovial fluid and tissue taken during arthrotomy/ arthroscopy, or by joint aspiration are needed for definitive diagnosis. Organisms should be identified accurately as treatment

options differ for different organisms (Fig.1 and Fig. 2). Identification of similar organisms from at least three culture samples is highly predictive of infection and

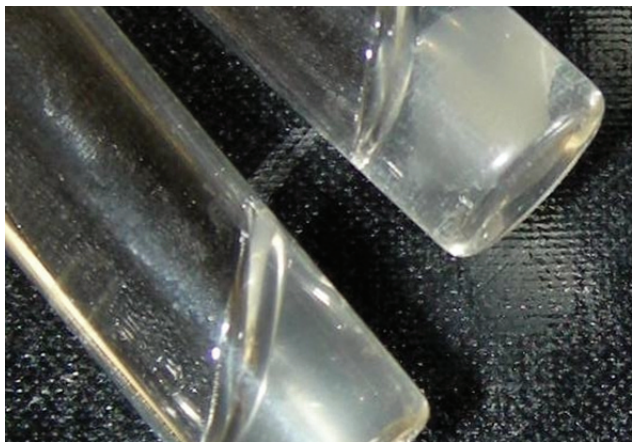


Fig. 1. Coagulase test that is required to differentiate between Staphylococcus aureus and other Staphylococcus species; two most common organisms responsible for prosthetic joint infections. Upper tube is coagulase positive confirming the identity of this organisms as Staphylococcus aureus

to increase sensitivity and specificity multiple (five to six) samples should be sent for culture. Organisms associated with prosthetic-joint infection usually form biofilms thus culture of prosthesis by vortexing and sonication than conventional peri-prosthetic tissue culture is more sensitive and specific especially in patients with prior antibiotic treatment. Cultures should also be hold for longer duration if fungi or mycobacteria are suspected as causative organisms.

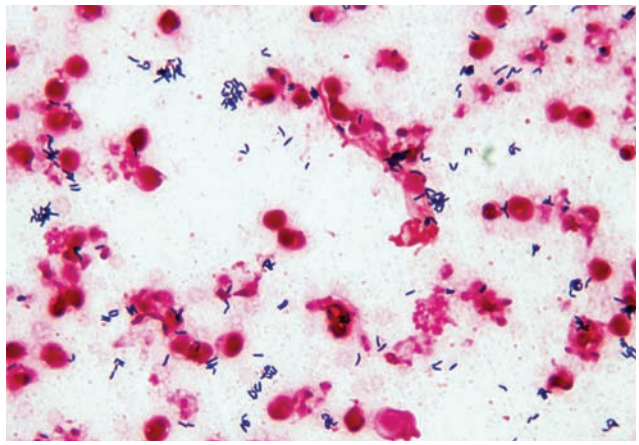


Fig. 2. Photomicrograph on the left showing Gram stain of Propionibacterium species; this organisms requires anaerobic conditions

Other diagnostic modalities:
Apart from culture histopathology is an essential adjunct to microbiology in the diagnosis of infection. An elevated white cell count and neutrophil differential of the synovial fluid are also suggestive of infection. Serial plain radiographs may help in detecting loosening or bone loss in chronic PI. Ultrasound should be performed to confirm effusion and to facilitate aseptic aspiration. Computed tomography and magnetic resonance imaging may be useful in the evaluation of complex cases, but metal inserts interfere with these tests, and abnormalities may be non-specific.

Bone and joint infections: Laboratory Diagnosis

Bone and joint infections occur as a result of haematogenous seeding, contiguous spread of infection to bone from adjacent soft tissues, or direct inoculation of infection as a result of trauma or surgery. Haematogenous spread is more common in children while contiguous spread is commoner in adults. Contiguous infection may be either polymicrobial or monomicrobial while haematogenous infection is usually monomicrobial. Staphylococcus aureus, coagulase-negative staphylococci, and aerobic gram-negative bacilli are the most common organisms; less common pathogens include streptococci, enterococci, fastidious gram negative organisms and anaerobes. Mycobacteria, Brucella spp. and fungi have also been reported in appropriate endemic and clinical settings.

Diagnosis; General Considerations

Cultures: Several factors should be considered while investigating for the diagnosis of these infections. The most important factor is specimen collection as it is extremely important to prevent contamination by normal flora. The samples should be collected as tissue or fluid rather than swabs because:

- they are more likely than tissue or fluid to be contaminated
- the amount sent for culture is often insufficient
- they may inhibit the growth of certain pathogens
- fastidious organisms survive less well in swabs than in aspirated fluid or pus
- bacteria may adhere to swabs and give a false-negative microscopy result

Ideally, culture specimens should be taken before antibiotics are commenced. In practice, however, empirical antibiotics may be commenced before culture specimens are taken.

Bone and Joint Infections: Laboratory Diagnosis

If Mycobacteria or fungi are suspected as etiologic agents; specimens should be sent for mycobacterial and fungal culture in addition to culture for routine organisms.

Histopathology: Histopathology and cytology not only provide rapid results than culture but also guide in assessing the significance of cultures. However the tissue Gram stain is an insensitive test for detecting bacteria as fixing and processing including chemical decalcification, adversely affects the Gram stain characteristics.

Nucleic acid amplification tests: There is little published information regarding the role of nucleic acid amplification based methods for skin, bone, joint, and soft-tissue infections. Therefore, routine use of nucleic acid amplification is not recommended for the diagnosis of these infections. Although these techniques may prove useful in selected cases where fastidious organisms are suspected as cause of infection.

Bone Infections

Close communication between clinician, radiologist and pathologist/microbiologist is required for optimum diagnosis. Excisional bone biopsy is required in cases in which clinical and radiographic features are not diagnostic. In all cases however, culture is essential for identification and antimicrobial susceptibility testing. For that an adequate amount of tissue should be sent to the microbiology laboratory. For chronic osteomyelitis, culture of an infected bone specimen is necessary to establish the diagnosis. Blood culture should also be sent to aid the diagnosis if the patient is septic. A positive culture precludes the need for more invasive procedures if the organism isolated from blood is a likely pathogen to cause osteomyelitis.

Cultures of superficial wounds and sinus tracts are of no value because the results do not correlate reliably with the pathogen in the underlying bone except if *Staphylococcus aureus or Salmonella spp* are isolated (Fig. 1).

Fig. 1. Swabs from superficial wounds and sinus tract should not be sent as the organisms isolated do not correlate with the pathogen in bone

Serial measurements of serum C-reactive protein levels and erythrocyte sedimentation rates are also commonly used to monitor response to therapy. The CRP is a more sensitive parameter than the ESR.

Joint Infections

Synovial fluid analysis is useful in establishing diagnosis and presence of pus suggest the possibility of infection (Fig. 2). Joint fluid should be cultured for both aerobic and anaerobic bacteria. Blood

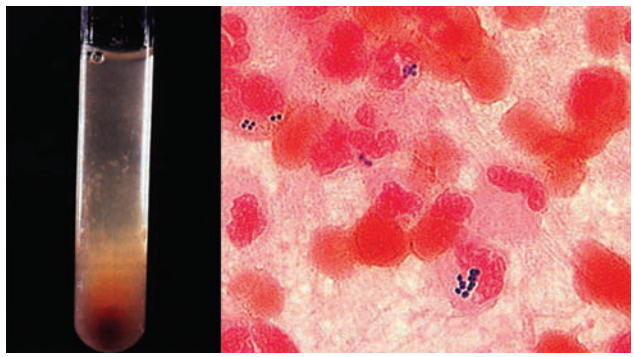


Fig. 2. Pus sample aspirated from infected knee joint highly suggestive of infection. Photomicrograph on the right is showing Gram stain of same sample revealing numerous pus cells and Gram positive cocci in clusters

culture bottles should not be used to culture joint fluid as they have not been evaluated for yield for culturing joint fluid (Fig. 3). Fluid aspirated from joints should be collected aseptically, transferred to a sterile container (or left in the syringe), and

Fig. 3. Blood culture bottles should not be used to culture joint fluid; fluid aspirated should be transferred to a sterile container

sent immediately to the laboratory. Cultures for Neisseria gonorrhoeae should be transported immediately to the laboratory. Specimens to be submitted for mycobacterial culture do not require special handling.

Mycetoma: Microbiological Diagnosis

Mycetoma is a chronic inflammation of skin and subcutaneous tissue caused by either fungi (eumycetoma) or filamentous bacteria (actinomycetoma). This infection most commonly presents as a progressive, subcutaneous swelling with development of multiple nodules. These nodules later develop into sinuses with discharging grains (Fig. 1).

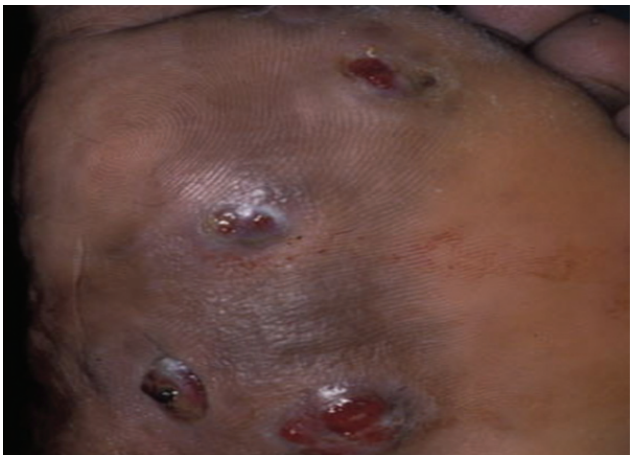


Fig. 1. Mycetoma of the foot

As the disease progresses involvement of bones and in advanced cases osteoporosis and bone destruction can occur. The organisms responsible for mycetoma are present in the soil and enter the subcutaneous tissue by traumatic inoculation. This infection is mainly seen in tropical regions and Pakistan is one of the endemic countries. Mycetoma commonly affects adults aged 20 to 40 years, predominantly males. The foot is the most commonly affected site (80 per cent of the cases).

Diagnosis

Diagnosis is usually determined clinically; however radiology is required to assess the extent of spread and involvement of bone and joints. Visual examination of colour of discharging grains is crucial for identification of possible etiologic agent (Fig. 2). In addition to that microscopic examination after crushing the grains between two slides should be performed to visualise fungal or bacterial elements (Table 1). Specimen should also be cultured for fungi and aerobic actinomycetes (Fig. 3). If granules are not present, an excisional biopsy for culture and histopathology or cytology is essential. Histopathology is also important for correlation with

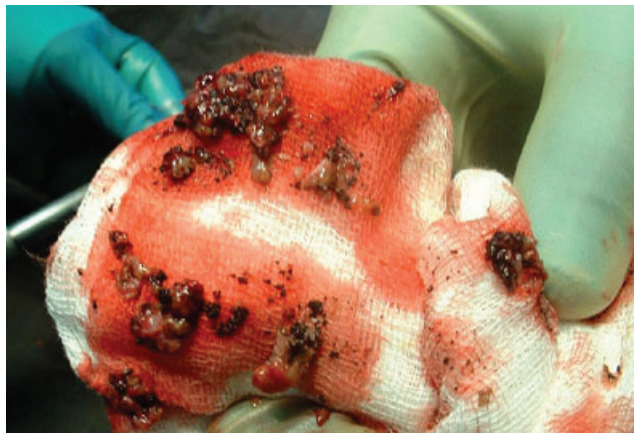
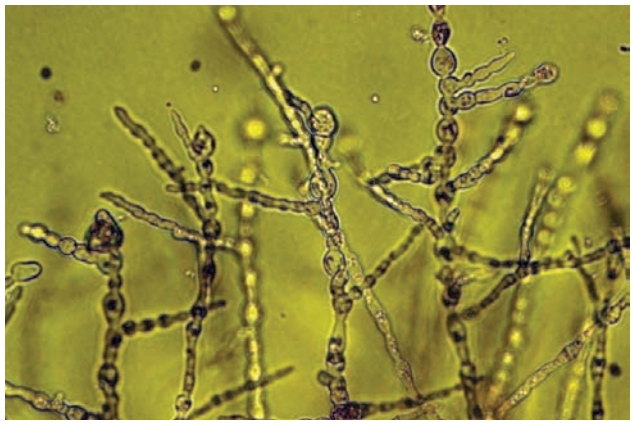


Fig. 2. Discharging grain from a case of mycetoma

cultures as the specimens may become contaminated with bacteria or fungi from skin or sinus tracts. The superficial material from sinus tracts should never be sent for culture as it will likely grow mixed flora that does not represent the actual cause of infection.



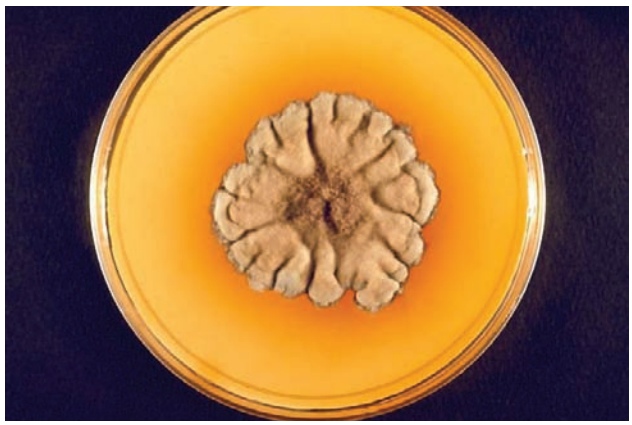


Fig. 3. Culture plate and photomicrograph showing Madurella spp.; commonest agent of eumycetoma

Etiologic agents	Colour of the grains
***Eumycetoma* species**	
Madurella mycetomatis	Black
Madurella grisea	Black
Fusarium spp	White
Acremonium spp	White
***Actinomycetoma* species**	
Nocardia brasiliensis	White
Nocardia asteroides	White
Streptomyces somaliensis	White
Actinomadura madurae	White
Actinomadura pelletieri	Red

Table 1. Common etiologic agents of mycetoma

Bone Sarcomas: Role of Histopathology in Diagnosis

Dr Fatima A Firdousi
Histopathology

Primary bone tumors are rare with relatively high incidence in children and adolescents. They are difficult to recognise as malignant by clinicians, radiologists, pathologists and leads to major diagnostic difficulties.

Table 1 shows 2002 WHO classification of malignant bone tumour. Osteosarcoma usually arises in the metaphysis of long bones, most commonly around the knee. Risk factors for osteosarcoma include previous radiation, Paget's disease of bone and germ line abnormalities (Fig. 1). Ewing sarcoma (ES) is the second most common bone cancer. About 25 per cent of the patients have ES of the pelvic bones, while 50 per cent have extremity tumours (Fig. 2). Chondrosarcoma is most frequently encountered in adults arising in the diametaphyseal region of long bones. Most of them are low grade (Fig. 3).

Fig. 1. Conventional osteosarcoma

Enchondroma and osteochondroma can also give rise to chondrosarcoma; most are solitary but can occur as multiple lesions. Other sarcomas like spindle cell sarcomas of bone, malignant fibrous histiocytoma/ fibrosarcoma, lieomyosarcoma, undifferentiated sarcoma, all present with pain and fracture.

Osteogenic tumours	Osteosarcoma, Conventional, (chondroblastic, fibroblastic, osteoblastic) Telangiectatic, Small cell, Low-grade central, Secondary, Parosteal, Periosteal, High-grade surface
Ewing Sarcoma/ PNET	Ewing sarcoma
Cartilage	Chondrosarcoma, Central, primary and secondary, Peripheral, Dedifferentiated, Mesenchymal, Clear cell
Fibrogenic Tumours	Fibrosarcoma
Fibrohistiocytic Tumours	Malignant fibrous histiocytoma
Haematopoietic Tumours	Plasma cell myeloma, Malignant lymphoma, NOS
Giant Cell Tumour	Malignancy in giant cell tumour
Notochordal Tumour	Chordoma
Vascular Tumours	Angiosarcoma
Smooth Muscle Tumours	Lieomyosarcoma
Lipogenic Tumours	Liposarcoma
Miscellaneous Tumours	Adamantinoma

Table 1. Classification of bone sarcoma

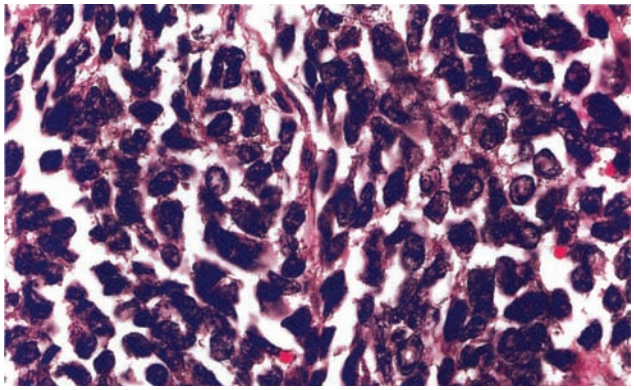


Fig. 1. B Osteoblastic osteosarcoma

The likely diagnosis of a bone tumor is related to age. Metastatic neuroblastoma or eosinophilic granuloma is seen in children below 5 years while primary bone sarcomas are found in children above 5 years of age. In adult's greater then 40 years, it is usually metastaic or myeloma.

Radiographs in 2 planes are the first investigation to confirm the diagnosis and to localise the tumour so that biopsy can be performed. CT should be used in a case of diagnostic problem or doubt. MRI of the whole bone with adjacent joints is the next step for local staging.

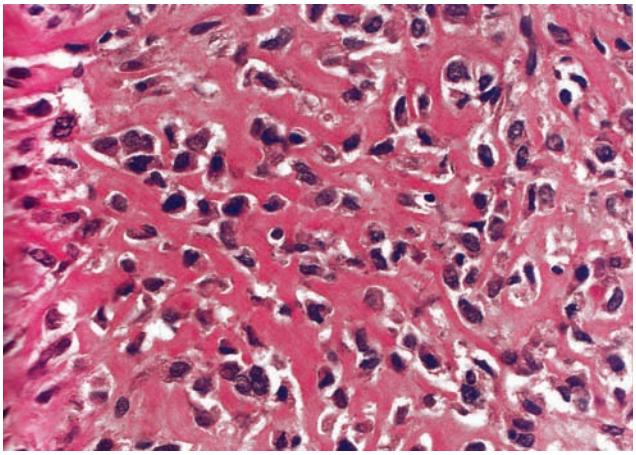


Fig. 2. Ewing sarcoma

Biopsy should be taken to prevent minimal contamination of normal tissue with the tumor cells. Samples should be taken for microbiological culture and histology. Core needle biopsy is preferred and with aid of staging studies the primary location of biopsy can be chosen. Excision biopsy is contraindicated for all cases with a possibility of aggressive and benign lesion.

The pathologist should be directly consulted by frozen section after taking the biopsy in case more material is required. In bone sarcomas, to prevent local recurrence biopsy tract should be removed with the resection specimen. Biopsy tracts should be tattooed by ink.

Material should ideally be transported to the lab within half an hour, touch preps can be taken before formalin fixation of the biopsy material. All patients should have a bone marrow biopsy and aspirate performed before starting treatment.

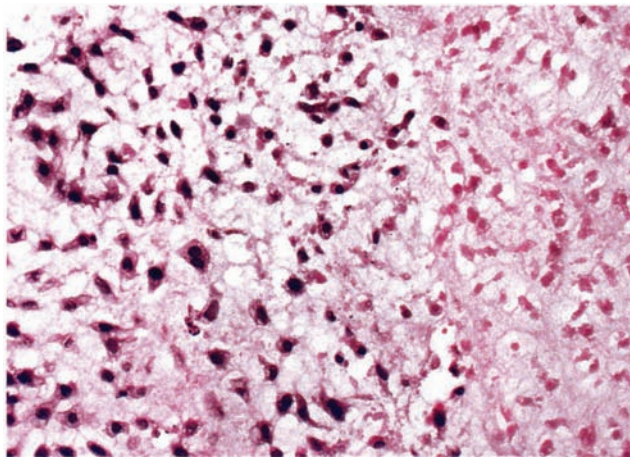


Fig. 3. Chondro sarcoma grade II

The biopsy request should carry information regarding the origin of tumour, preoperative findings and any chemotherapy received. Related information should also be provided with the biopsy material to the laboratory like nature of the bone specimen, segmental recession, curettage, needle biopsy, limb amputation or any other complex recession.

The bone tumour biopsies are decalcified in Histopathology Section at AKUH, The size of the tumour measured in 3 dimensions (in mm) with histological features and tumour type. Tumour necrosis whether less than or more than 90 per cent is included. The resection margins whether involved or clear by the tumour is also measured in mm.

Routine H&E staining is performed of the biopsy material. In ES special stain like PAS+ and immunostain CD99 (MIC 2) is also performed. The results of immunohistochemical stains using SNOMED or ICD-0 codes are recorded.

Reference:
Annals of Oncology 21 (Supplement 5): v204-v213, 2010

HLA B-27 at a Glance

Sheeba Parveen
Molecular Pathology

HLA (Human leukocyte antigen) B-27 is class I surface antigen which is encoded by genes present on short arm of chromosome 6

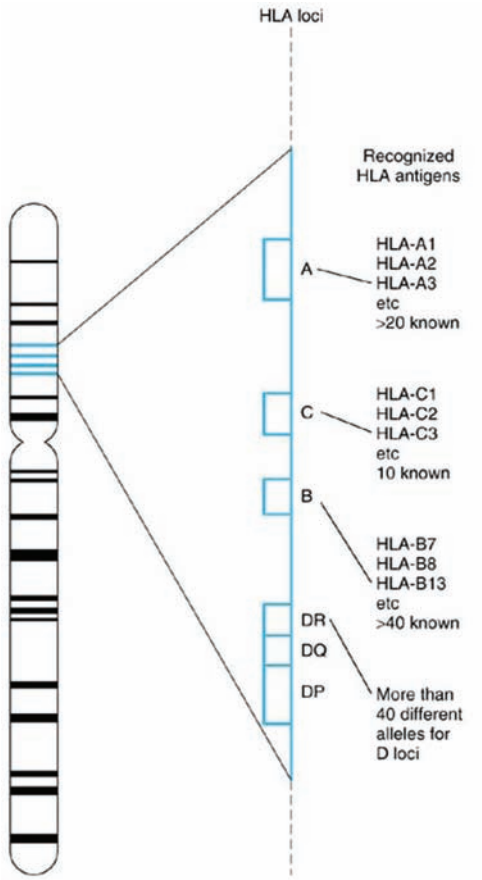


Fig.1.A. The organization of HLA genes on chromosome 6

(Fig. 1A). The HLA genes are the human versions of MHC genes that are found in most vertebrates. The proteins encoded by certain genes are also known as antigens, as a result of their historic discovery as a factor in organ transplantation. The major HLA antigens are essential elements for immune function. Different classes have different functions.

The role of HLA B-27 molecules is presentation of peptides to T-cells. The immune system uses HLAs to distinguish the body its own proteins 'self' from the proteins made by foreign invaders such as viruses and bacteria 'non-self'. HLA B-27 is strongly associated with the condition ankylosing spondylitis, which is named due to the terms 'Ankylosing' or fusing together and 'spondylitis' or inflammation of bones of spine. This and other associated inflammatory diseases are collectively referred to as 'spondyloarthritis'. It is uncertain how HLA B-27 causes increase risk of ankylosing spondylitis.

Researchers speculates that HLA B-27 may abnormally display to immune system peptides that trigger arthritis other researchers suggests that joint inflammation characteristic of this disorder may result from improper folding of HLA B-27 protein or the presence of abnormal forms of the protein on the cell surface.

Ankylosing spondylitis is two to three times more common in males than in females, and it affects all age groups including children. The most common age of onset of symptoms is the second and third decade of life. The effect of the disease is depicted in Fig. 1B Symptoms of ankylosing spondylitis include back pain and stiffness (worst at night and

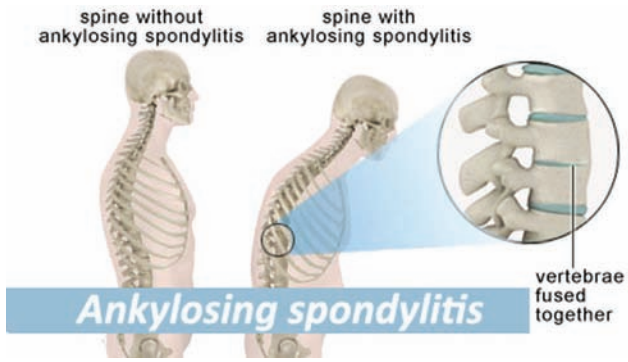


Fig.1.B. Diagrammatic representation of the effect of ankylosing spondylitis on the spine.

in morning), weight loss, feeling unwell, tiredness, pain and swelling of hips, knee or joints, plantar fasciitis (pain under heel of foot), aching in chest, around ribs. It may be associated with psoriatic, colitic and reactive arthiritis. The complications of the disease are uveitis (inflammation of eye) and issues related to heart and lungs.

For molecular diagnosis of ankylosing spondylitis a venous blood sample is required for the test. DNA is extracted from blood using the sodium dodecyl sulphate (SDS)-proteinase K lyses method and then amplified by conventional polymerase chain reaction (PCR). Using Sequence specific primer (HLA-A and HLA–B) primers. In addition, PCR for the growth hormones (GH 1 and GH 2) genes is used as an internal control. Amplified product is than analysed by agarose gel electrophoresis (Fig. 2).

A positive band of 144 kb obtained in the PCR reaction indicates the presence of the HLA-B27 allele, and based on this the result of the test is reported as a 'presence' or an 'absence' of the HLA B27 allele. An internal control is included in each PCR reaction to monitor the presence of PCR inhibitors in the specimen.

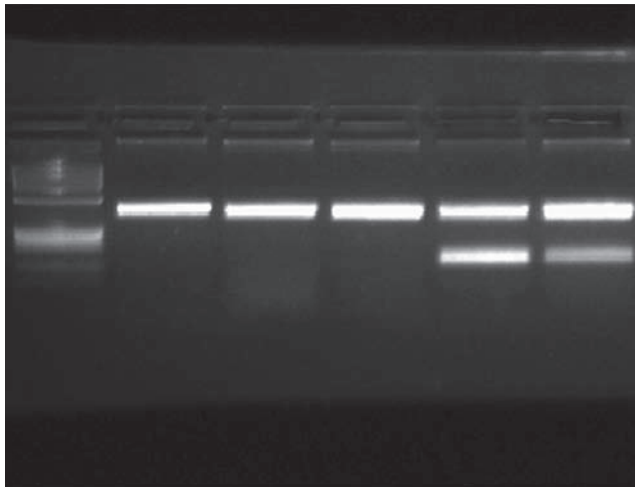


Fig. 2. Detection of the HLA B-27 all ele. The picture depicts the presence of DNA on an agarose gel stained with ethi dium bromide. Fluorescent bands indicate the presence of DNA in each lane

Meeting Reports

Seema Vaqar
Clinical Labortory

Multiple CME seminars were held at different cities in Pakistan as a part of continuing educational activities of the Department of Pathology and Microbiology, where faculty presented the latest advancements and updates in medical and laboratory sciences.

CME Seminar held at Hotel City Centre Baldia Complex, Mirpurkhas on February 09, 2011, covered broad range of topics on chronic hepatitis B and hepatitis C interpretation, diagnosis and management, vitamin D deficiency and approach to diagnosis of anaemia. It was well attended by general practitioners, physicians, surgeons, of District Civil Hospital, Muhammad Medical college Hospital Mirpurkhas and nearby periphery towns i.e. Digri, Umer Kot, and Tando Allahyar.

Dr Yasmeen Khoonharo, Assistant Professor and Consultant Gynaecologist and Obstetrician, Muhammad Medical College Mirpurkhas appreciated role of AKUH pathologists in educating as well as providing high quality diagnostic services for physicians and hospitals nationwide. Dr Mahadev Harani referred to data obtained by the AKUH Clinical Laboratories for differential diagnosis of anaemia with histogram. He emphasised diagnosis and management of anaemia by detail history and laboratory investigations.

Dr Aysha Habib Khan Consultant Chemical pathologist, highlighted the wide spread prevalence of vitamin D deficiency in Pakistan, She presented data from healthy volunteers and

Presenters and organisers of the CME at Mirpur Khas

Dr Farooq Ghani addressing the audience at CME

ambulatory care patients highlighting the need to diagnose and treat these case at an early stage. She shared that testing in form of bone health profile is available at AKUH Clinical Lab; the profile is developed to answer the potential clinical questions that a physician has, if a patient is suspected of having a metabolic bone diseases. Dr Syed Zafar Abbas Consultant Physician and Gastroenterologist, Medical Superintendent Muhammad Medical Hospital Mirpurkhas and Dr Romena Qazi Assistant Professor and Section head Molecular Pathology AKU both address the chronic issue of hepatitis B and C prevalence and management of the infection,

Dr Sara Malik receiving her shield from Prof. Sabiha Riyaz

diagnosis of Hepatitis C by PCR was emphasised and stressed by the speakers as an important step in management.

CME held on February, 9 2011 at Fatima Memorial Hospital College of Medicine and Dentistry Lahore, was well attended by health care professionals and consultants from all disciplines of Hospital.

Dr Farooq Ghani, Associate Professor and Director out reach laboratory services addressed about 'Diagnostic Approach to Genetic Disorders' and Triple Marker Screening test. He introduced advancement in technology and importance of maternal blood screening test for Down's syndrome and neural tube defects in his presentation. Dr Sara Malik explained about 'Serum Markers in Prenatal Diagnosis', where the fetus is treated as patient. She shared that approximately three per cent of all pregnancies have a genetic disorder or birth defect. Dr Ayesha Ehsan in her presentation explained about maternal blood sampling for testing for fetal DNA in maternal blood. **CME seminar was held at Thalassaemia Care Center, Badin with on 22nd February 2011.** Dr Muhammad Haroon, Pathologist at Civil

Speakers at CME Seminar on Recent Advances in Pediatrics Pathology

hospital and In-charge Thalassaemia Care Center at Badin presided over the session.

Dr Bushra Moiz, Associate Professor and Consultant Haematologist, AKUH delivered an interactive talk on 'How to investigate a bleeding patient?' She emphasised on taking a detailed history of a bleeding patient along with physical examination and recommended complete blood count with peripheral film review for correct platelet estimation, bleeding time (BT), prothrombin time (PT), activated partial thromboplastin time (APTT) and urea-clot lysis test as first line tests. The last is required to detect factor XIII deficiency

which cannot be tested by PT and APTT alone. She emphasised the review of platelet film in case of low platelets as spurious results are sometimes generated by analysers in the presence of platelet clumps and large or giant platelets. The screening tests assist in making a presumptive diagnosis in conjunction with clinical details. Based on these tests, the doctor should order more specific tests e.g if von Willebrand disease is suspected than vWAg, RiCof and factor VIII assay are required for proper evaluation. She also stressed that all such patients should be referred to haematologists for proper assessment. Ordering of all tests is neither required nor financially viable hence an appropriate understanding of underlying pathology is utmost essential for correct diagnosis and management of the bleeding disorder. AKUH laboratory offers all routine and specialized tests required for diagnosis of various bleeding disorders.

The talk was followed by a question-and-answer session where doctors showed their keen interest in management of bleeding disorders.

CME Seminar on 'Recent Advances in Pediatrics Pathology' was held at auditorium of King Edward Medical University/ Mayo Hospital Lahore on March 8th 2011

Professor Mahmood Shaoukat shared his vision about the 'Use of Stem Cells in Trauma and Burn Management. Prof Farah Asghar explained about 'Fetal anomalies screening'. She stated that congenital abnormalities account for 20-25 per cent of prenatal deaths. Dr Farooq Ghani spoke about the 'Prenatal Diagnosis of Birth Defects' and explained the techniques and methods of prenatal testing. He talked about common chromosomal and genetic disorders seen in our population and shared his experience of prenatal testing at AKU.

Dr Shahid Pervaiz, Professor Department of Pathology and Microbiology presented about the common childhood leukemias and lymphomas seen in our population and how the accurate diagnosis can impact patient management.

Answer to LABRAD Quiz

1.1: Hypoparathyroidism is the most common cause of hypocalcemia, which is causing symptoms in this patient. Her presentation soon after birth suggests presence of congenital hypoparathyroidism.

1.2: Serum magnesium levels: Magnesium is required for optimal activity of parathyroid gland. Levels are usually low in hypoparathyroidism.

1.3: The goal of treatment is to normalise the levels of calcium, magnesium and phosphorus. A treatment regimen typically includes replacing calcium by giving oral calcium carbonate or gluconate along with 1 alpha vitamin D.

2.1: Symptoms of bone pain and weakness can mean that patient have a coexistent vitamin D deficiency along with senile osteoporosis. For many people, the symptoms are subtle. Yet even without bone symptoms, inadequate vitamin D levels can pose other health risks

2.2: Biochemical tests to measure calcium, phosphorus, magnesium, vitamin D and parathyroid hormone and bone turnover markers can be performed. Depending upon the symptomatology, tests to screen for secondary osteoporosis can also be considered.

2.3: Bone tumor markers (BTM) are helpful in monitoring bisphosphonate therapy. Early changes in BTM measure the clinical efficacy of an anti-resorptive treatment and to reinforce patient compliance. At AKUH Clinical Laboratory, N-telopeptide of Type I collagen is performed.

www.aku.edu/akuh/hs/cs/pathology.shtml