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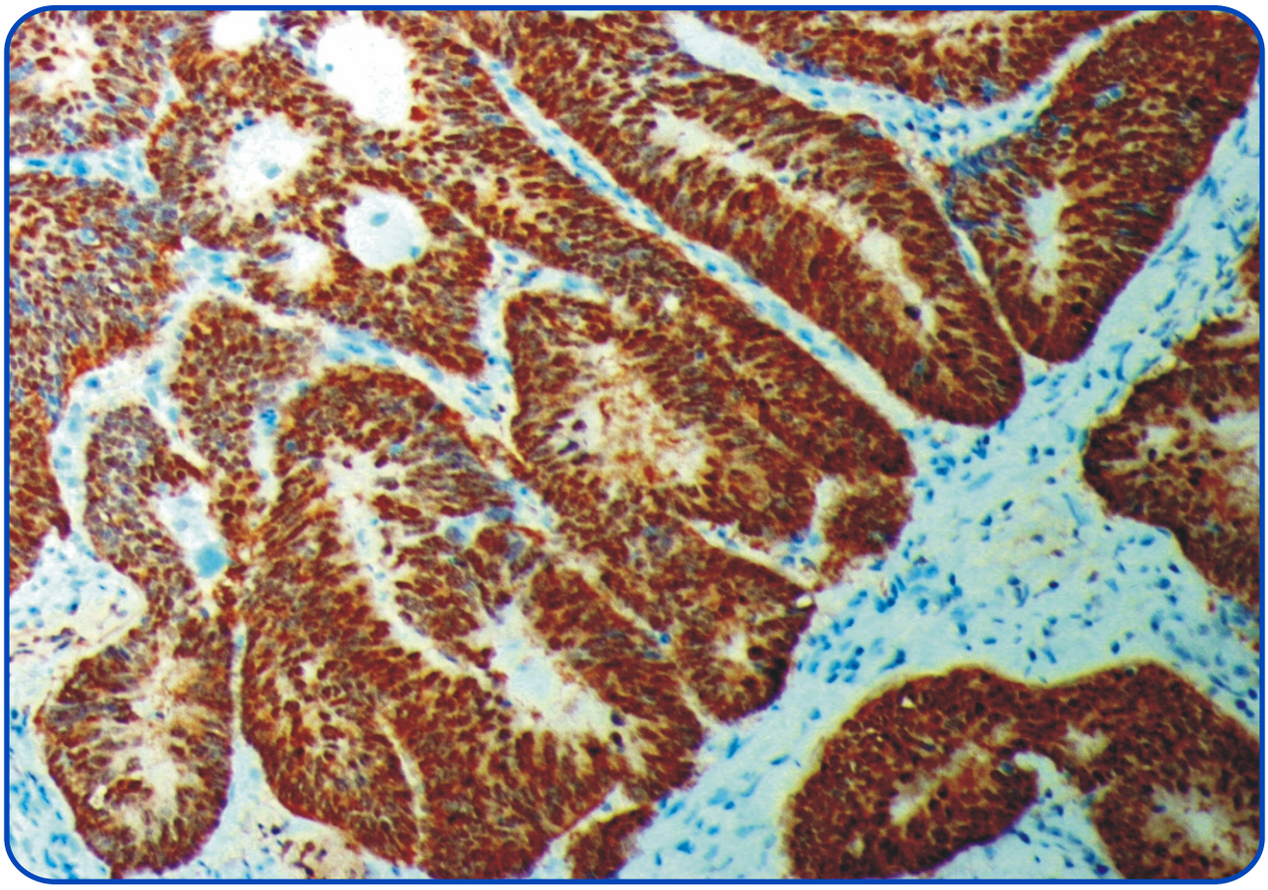
LABRAD : Vol 35, Issue 1 - January - February 2010

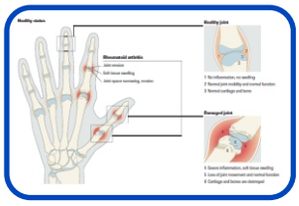
Aga Khan University Hospital, Karachi

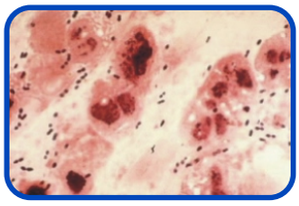
Newsletter of Departments of Pathology and Microbiology, and Radiology

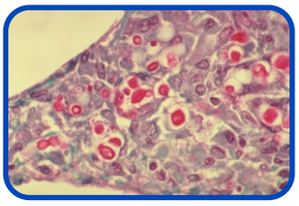
LABRAD

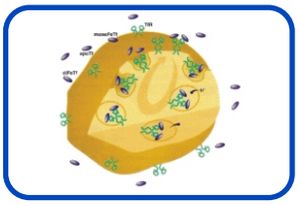
January - February 2010 Vol. 35, Issue 1











In this issue

آغا خان یونیورسٹی ہسپتال، کراچی
The Aga Khan University Hospital, Karachi

January - February 2010, Vol. 35, Issue 1

Labrad

A quarterly publication of the Departments of Pathology and Microbiology, and Radiology

January - February 2010,
Volume 35, Issue 1

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Assessment of Osteoporosis Risk by NTx- A Biochemical Marker

Dr Aysha Habib Khan
Chemical Pathology

The risk of osteoporosis depends largely upon the peak bone mass acquired during early adulthood against the rate of bone loss occurring later in life. A poor intake of calcium and vitamin D, physical inactivity and prolonged amenorrhea are among the factors that can severely diminish peak bone mass. Females having low peak bone mass are more liable to suffer from osteoporosis at the time of menopause.

A number of risk factors are known to be involved in the pathogenesis of osteoporosis (table 1). The most important risk factors include female gender, Caucasian or Asian ethnicity, early menopause, previous low trauma fracture, family history of osteoporosis in first degree relatives, current smoking and low body mass index. Additional factors include chronic illness, endocrine disorders, excessive alcohol intake and use of certain drugs notably steroids. It is important to emphasise that none of the risk factors predict bone mass with sufficient accuracy in an individual patient. The best combination of risk factors only accounts for about 20 per cent of the variability in bone mass. Clinical risk factors thus add information about the risk of osteoporosis, which is only partly explained by variation in bone mass. They should therefore preferably be used to target individuals for further investigations (which include bone densitometry and biochemical markers of bone turnover), and treatment (table 2).

The definition of osteoporosis is based on bone densitometry. Normal bone mass is defined as a bone mineral density (BMD) above or below 1 SD from the premenopausal mean value. Osteopenia is BMD between -1SD and -2.5 SD while osteoporosis is BMD below -2.5 SD. These criteria reflect the fact that bone mass accounts for 75-85 per cent of the variability in ultimate bone strength, it is considered as one of the best single predictors of fracture risk and low bone mass is an important risk factor for osteoporosis.

Table 1: Risk Factor of Osteoporosis

Age
Previous fragility fracture
Endocrine Premature menopause BMI < 20 Previous Ammenorrhea (> 6 months duration)
Genetics Low trauma fracture in first degree relative Caucasian or Asian ethnicity Female gender
Lifestyle Low level of physical activity Poor calcium intake (< 0.5 g/day) Alcohol excess (> 14 units/week) Cigarette smoking

Table 2: Use of Risk Factors in Management

Target individuals for further investigation Densitometry Bone turnover markers
Eliminate modifiable risk factors
Discuss Treatment Advantages Disadvantages

Low bone mass together with a high rate of bone loss further increases the risk of fracture. High bone turnover as estimated by biochemical markers is associated with an increased rate of bone loss and predicts the risk of fracture independently of BMD. Studies have reported odds ratio for fracture of around 2 per 2 SD increase in the biochemical marker above the premenopausal mean value. In these studies, the biochemical markers with the best predictive accuracy were urine C- and N-telopeptides of type I collagen and urine deoxy pyridinoline, all known to be biochemical markers of bone resorption.

It is significant that a bone mass in the osteoporotic range and an increased value of a biochemical marker increase the odds ratio for hip fracture to 4-5, suggesting that combined information on bone densitometry and biochemical markers improves the estimation of osteoporosis risk.

The biochemical marker of bone turnover that we have introduced in our laboratory is N-terminal telopeptide of type I collagen (NTx). It is measured by a competitive-inhibition enzyme-linked immunosorbent assay. Studies support the use of NTx to monitor the anti-resorptive effect of therapy and to determine the probability for a decrease in BMD if therapy is not initiated. The recommendation for monitoring therapy is to have baseline samples just prior to or on the day of therapy initiation. Subsequent specimens for comparison should be collected at approximately the same time of day as the baseline specimen.

Antibodies Against Cyclic Citrullinated Peptides (Anti-CCP) Assay in Rheumatoid Arthritis

Ms Shahmina Sadaf
Haematology

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting 0.5-1 per cent of the world population. This systemic disease is characterised by chronic inflammation of the synovial joints and progressive joint degeneration causing pain, joint destruction and impaired joint mobility and eventually leading to loss of function and disability of the affected individuals. Figure 1 shows differences between a healthy and damaged joint. Early and aggressive intervention with new and effective biological and targeted treatments can alter the course of the disease and improve overall quality of life. This suggests that improved markers for diagnosis and prognosis are needed to identify these patients at an earlier stage and modify the available therapeutic choices according to the individual patient's needs.

The diagnosis of RA often relies on clinical manifestations and laboratory tests such as erythrocyte sedimentation rate (ESR), rheumatoid factor (RA-Factor) and C-reactive protein (CRP).

However, RA-Factor is a non-specific marker and may be present in healthy persons or in patients with other autoimmune and infectious diseases while CRP is a non-specific inflammatory marker. A new laboratory test for diagnosing RA by measuring antibodies against cyclic citrullinated peptides (anti-CCP) has recently become available.

Anti-CCP is a quantitative determination of human IgG autoantibodies against cyclic citrullinated peptides in the human serum. It involves the IgG capture principle by utilising the electro-chemiluminescence methodology on fully automated immunoassay analysers (Figure 2). The assay has the sensitivity of 67.4 per cent and specificity 97.0 per cent using a cut-off of 17 U/mL.

The antibodies are directed against the circular peptide containing an amino acid called citrulline. Citrullination refers to the post-translational modification of the amino acid arginine to citrulline by the enzyme peptidylarginine deiminase (PAD). The physiologic role of citrullination is unclear; however it has been shown to occur during apoptosis, and is thought to play a role in the degradation of intracellular proteins by unfolding protein molecules and thereby exposing them to degradation enzymes. PAD enzymes can be found in monocytes and macrophages associated with inflammation, mostly in the synovial fluid of patients with RA. Anti-CCP, which is found in the serum, is thought to be a result of diffusion of these antibodies from the synovial fluid into the general circulation

Anti CCP-2 antibodies are more promising as a diagnostic marker of RA and can be used for the diagnosis of early RA, prediction of disease severity, differentiating RA from other forms of non-RA inflammatory or arthritic diseases and assessing treatment efficacy.

Anti-CCP antibodies have been implicated in RA pathogenesis and hold promise for the earlier and more accurate diagnosis of RA, and improved prognostic information. If the physician is to intervene optimally during a patient's window of opportunity before irreversible damage occurs, such a biomarker may be very useful when used in combination with other clinical, radiological and laboratory diagnostic features.

American College of Rheumatology, recommends the use of anti-CCP assay as it is a sensitive and more specific test than RA-Factor in early as well as fully established disease. It also predicts the eventual development into RA when found in undifferentiated arthritis. It is a marker of erosive disease in RA and may be detected in healthy individuals" years before the onset of clinical RA.

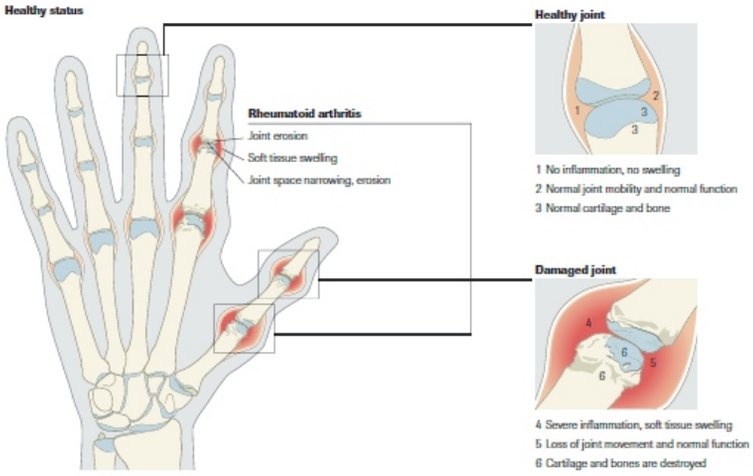

Figure 1: Comparison of a healthy and damaged joint as seen in rheumatoid arthritis

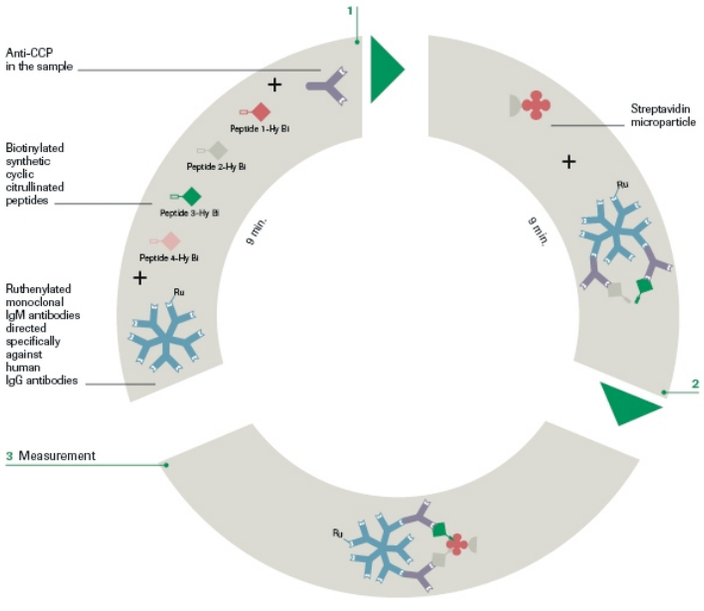

Figure 2: Principle of anti-CCP assay in an automated immunoassay analyser

A Disintegrin and Metallopeptidase with Thrombospondin Type 1 Motif, 13 (ADAMTS-13)

Dr Farheen Karim Mahar
Haemataology

ADAMTS-13 is an enzyme which is released primarily from the stellate cells of liver but is also expressed in platelets and endothelial cells. It is involved in the regulation of the size of von willebrand factor (vWF) in the plasma. ADAMTS-13 maintains primary haemostasis by proteolysing ultra-large von Willebrand Factor (ULvWF). The latter is the hyperactive form of vWF which is released from storage granules (Weibel-Palade bodies) of endothelial cells in response to inflammatory stimulation. The ULvWF has more affinity for platelets favouring platelet aggregation at sites of high shear stress with subsequent formation of micro vascular thrombi. The ULvWF relies upon ADAMTS-13 for its cleavage and its conversion into a less active form.

Deficiency of ADAMTS-13 may be congenital (Upshaw-schulman syndrome) or much more frequently acquired. An acquired deficiency is attributed to the presence of auto-antibodies against ADAMTS-13 which may either inhibit ADAMTS-13 function or may cause rapid clearance of circulating ADAMTS-13. Inherited or acquired deficiency of ADAMTS-13 impairs ULvWF cleavage. Deficient proteolysis of ULvWF results in disseminated platelet-rich thrombi in the microcirculation which in turn cause typical thrombotic micro-angiopathies resulting in end organ ischemia.

The laboratory assessment of ADAMTS-13 levels is useful since severe deficiency of ADAMTS-13 has been proposed as a specific laboratory marker of thrombotic thrombocytopenic purpura (TTP) or Upshaw-schulman syndrome. It is also seen in conditions like sepsis, disseminated intravascular coagulation (DIC) and metastatic malignancy. Mild to moderate deficiency is seen in chronic inflammation, hepatic dysfunction and pregnancy.

Given that severe secondary deficiency of ADAMTS-13 might correlate with development of widespread microvascular thrombi and end organ injury, determination of ADAMTS-13 activity at the time of hospital admission in patients with severe conditions like sepsis and DIC will help in understanding the extent of the disease. It also raises the possibility of novel supportive therapies for patients with sepsis and DIC such as ADAMTS-13 supplementation and plasma therapy because sepsis may have the same pathophysiology of severe ADAMTS-13 deficiency for thrombotic microangiopathies as idiopathic TTP.

The test is currently offered by clinical laboratories of AKUH. It is based on the principle of ELISA technique. Two to three mls of blood collected in citrate anticoagulant will be required for the test. The reference range developed at AKUH for ADAMTS-13 is 175-365 ng/dl. There is no difference in reference range between males and females or adults and children.

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Soluble Transferrin Receptor (sTfR)

Dr Muhammad Shariq Shaikh
Haematology

Anaemia associated with chronic disease and iron deficiency comprises the major bulk of all anaemias. Differentiation between these two types of anaemias and diagnosis of a patient with chronic disease having iron deficiency is not very simple as they tend to present with similar clinical and laboratory features. In recent years, soluble transferrin receptor (sTfR) and its ratio to ferritin (sTfR/ferritin) have been proposed as useful tools in this regard.

The uptake of iron by cells is mediated by a transferrin receptor (TfR) expressed on their external surface. TfR binds diferric transferrin, and the receptor-transferrin complex is internalised into an endosome, where the iron is transferred to the cytosol (Figure 1). After recycling to the cell surface, the apo-transferrin dissociates, and the receptor is free to repeat the process. Cells deficient in iron up-regulate their expression of TfR to compete more successfully for available iron. TfR is a disulfide-linked dimer of two identical 85-kDa-subunits. Each subunit has a 61-amino acid N-terminal cytoplasmic domain, a transmembrane region, and a large extracellular domain. TfR is shed from cells by proteolytic cleavage just external to the plasma membrane and just after the two interchain disulfide bonds. The product circulates in the blood as soluble TfR (sTfR), a 74-kDa monomer bound to transferrin. The amount of circulating sTfR is proportional to the total amount of cell-associated TfR.

The cellular need for more iron causes increased expression of TfR, regulated by a mechanism involving Iron-Responsive Elements (IRE) of mRNA and Iron-Binding Proteins (IBP). IBPs change conformation on binding iron; the iron-free conformation binds to IREs, modifying mRNA in ways that depend on the location of the IRE. In the case of TfR mRNA, binding of IBPs stabilizes the mRNA, thereby increasing the steady-state concentration and increasing the rate of synthesis of TfR. Thus, a low concentration of intracellular iron leads to increased expression of TfR.

Since most cellular iron utilisation (80 per cent of metabolic iron) is by erythroid precursor cells, circulating sTfR is proportional to erythroid precursor mass (i.e., rate of erythropoiesis). As a consequence, sTfR is low in patients with hypoplastic anaemias, and high in patients with hyperplastic anaemias such as RBC haemolysis or chronic blood loss. Similarly, plasma sTfR is high in patients who are iron deficient and therefore concentration of serum sTfR is useful in the diagnosis of iron deficiency, especially in patients with chronic inflammatory, infectious, or malignant disease, where the usual tests of iron status may be misleading.

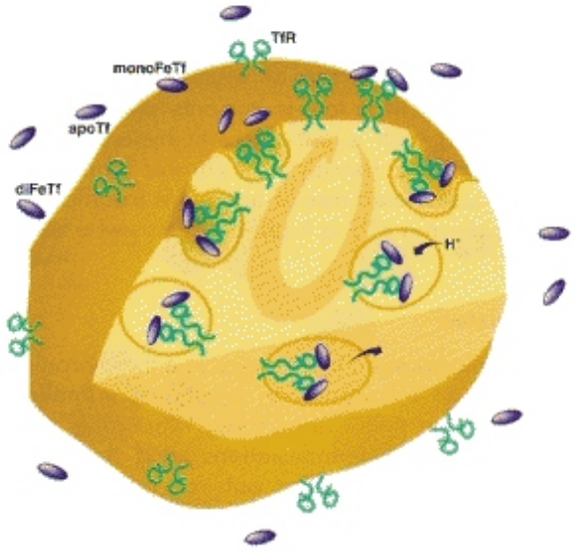

Figure 1: Schematic mechanism for the uptake of iron by a cell. TfR expressed on the cell surface preferentially binds Transferrin (Tf) that has two atoms of iron. The Tf-TfR complex is internalized into endosomes. The pH of endosomes decreases, facilitationg iron dissociation and increasing the affinity of the receptor for apoTf. The iron moves to the cytosol, and the endosome recycles to the plasma membrane with the Tf-TfR complex. At the higher pH of the extracellular fluid, apo-Tf dissociates and is replaced by diferric Tf, and the cycle repeats.

It has been established that serum or plasma sTfR is elevated by iron deficiency in patients with or without chronic disease but is not affected by chronic disease in the absence of iron deficiency. In many clinical trials, it has been demonstrated that elevated sTfR highly correlated with absence of iron in a marrow aspirate, is the "gold standard" of iron deficiency.

At AKUH the test is performed by immunoturbidimetric method on Roche Hitachi 902 method. The assay operates on the quantitative two-site immunoenzymometric (sandwich) technique. A monoclonal antibody specific for sTfR is pre-coated onto the wells of a micro plate. After the addition of sample and assay diluents to the wells, it is incubated for one hour, during which sTfR becomes bound to the immobilised antibody. After any unbound material is washed away, a second monoclonal antibody conjugated to horseradish peroxidase is added and incubated for one hour, during which the conjugate binds to the captured sTfR. After another washing away of unbound material, the amount of bound conjugate is detected by reaction for 30 minutes with a specific substrate, which yields a coloured product that is proportional to the amount of conjugate (and thus to the amount of sTfR in the sample). The colour reaction is stopped with hydrochloric acid, and the concentration of sTfR in each well is read from a calibration curve.

In addition to diagnosis of iron deficiency, serum sTfR has been used to monitor the rate of erythropoiesis. It has been used to follow marrow engraftment after bone marrow transplant and to categories anaemia as either hyperplastic or hypoplastic.

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Diagnosis of Cryptococcal Meningitis

Dr Kauser Jabeen
Microbiology

Cryptococcus neoformans is encapsulated yeast that is responsible for serious infections in immunocompromised population. The common predisposing factors include AIDS, prolonged treatment with steroids, organ transplantation, advanced malignancy, diabetes and sarcoidosis. Lungs and the CNS are the most frequently involved sites; however any organ can be involved in a severely immuno-suppressed host. Accurate management of this infection requires a high index of suspicion and prompt diagnosis.

Diagnostic Modalities

Microscopic examination by India ink: It is a rapid, cheap and effective method for diagnosis of Cryptococcal infections. Samples are mixed with a drop of India ink on a glass slide and visualised under the microscope. The microscopic examination is positive in around 80 per cent of HIV and 50 per cent of non-HIV patients. Yeast could be confused with lymphocytes surrounded

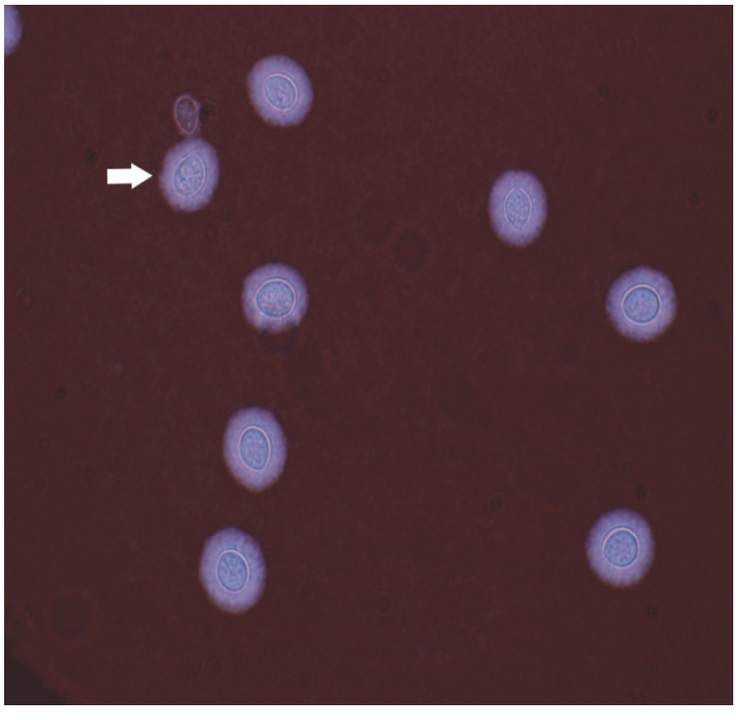

Figure 1: India ink preparation of CSF of a patient with meningitis showing multiple encapsulated yeasts with budding (white arrow).

by proteinaceous material. Therefore it is always important to visualise budding yeast cells (figure 1).

On Gram staining Cryptococcus are visualised as poorly-stained Gram positive yeasts.

Histology: Tissue biopsies and cytologies both are very helpful in the diagnosis of cryptococcosis. Cryptococcus are visualised in routine hematoxylin and eosin (H&E) stain as round yeast cells surrounded by empty spaces (figure 2). The capsule could be stained using mucicarmine and alcian blue stain. Gomori's methenamine silver (GMS) stain could also be used to visualise this narrow-based budding yeast in the tissue.

Culture: *Cryptococcus neoformans* could grow on most routine bacterial and fungal media. This yeast usually grows within 3-7 days after inoculation of specimen on the media. Most of the isolates are positive for rapid urease test. Final identification is usually performed biochemically on API yeast. DNA-based methods could also be performed to identify the isolate.

Latex particle agglutination: This test detects the presence of the polysaccharide capsule of the organism in the serum and CSF (figure 3). It is a rapid and extremely accurate test for the diagnosis of Cryptococcal infections. It has a sensitivity and specificity of greater than 90 per cent. An additional advantage of this test is the reporting of polysaccharide antigen titer. This information could be used as a general prognostic indicator as a high titer of 1:1024 reflects greater chance of therapeutic failure. However it should be noted that these titers should not be used to make therapeutic decisions and the serial polysaccharide antigen titers are often imprecise.

All of the above modalities for the diagnosis of Cryptococcal infections are available at the clinical microbiology laboratory of Aga Khan University.

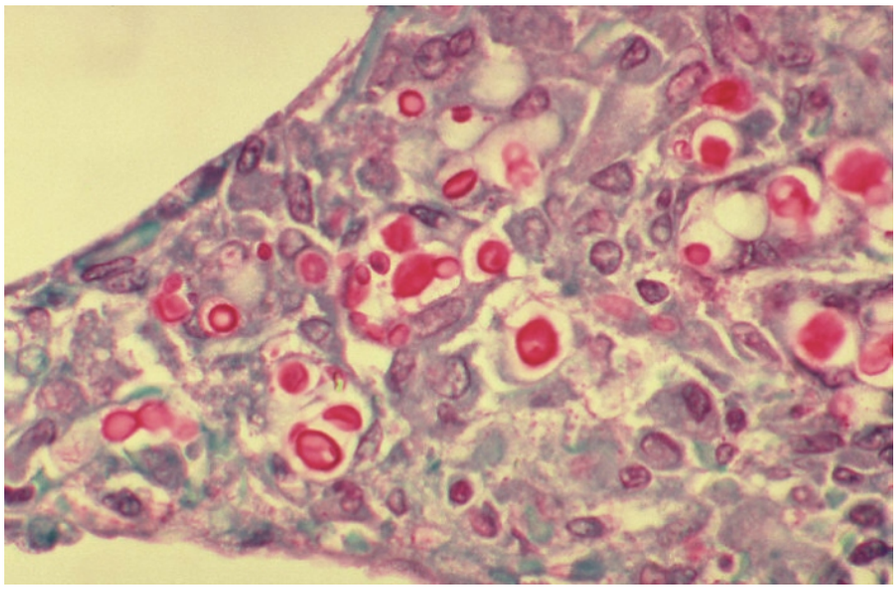

Figure 2: Histology of lung of a patient showing multiple pink rounded yeast cells with empty spaces suggestive of Cryptococcal infection

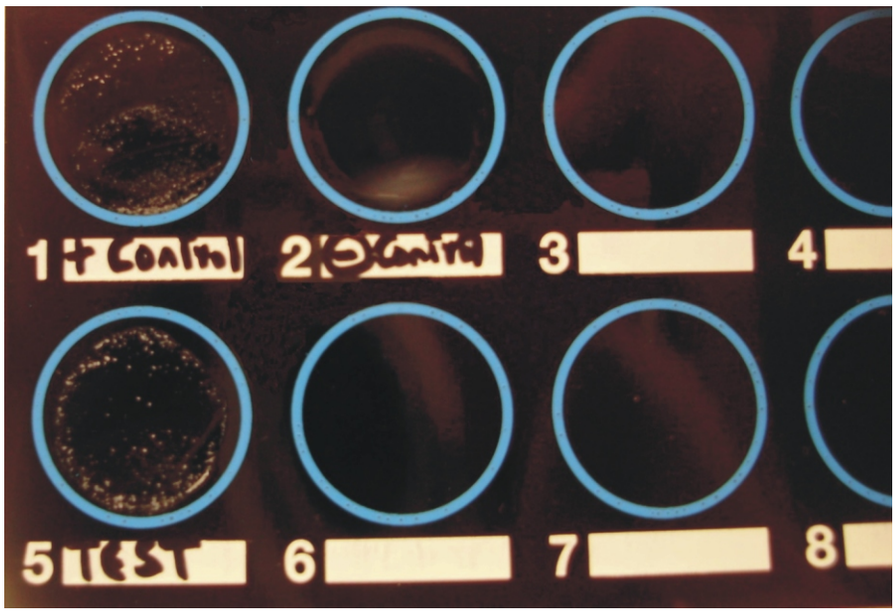

Figure3: Latex particle agglutination test for the detection of Cryptococcal capsular antigen.
Well 5 is showing a positive test with positive and negative controls in Well 1 and 2 respectively.

Infections in Thalassaemia

Dr Sadia Omer
Microbiology

Thalassaemia is a heterogeneous group of genetic disorders of hemoglobin synthesis characterised by a disturbance in the production of globin chains, leading to anaemia, ineffective erythropoiesis, and premature destruction of red blood cells.

Clinical severity varies widely ranging from asymptomatic forms to severe or even fatal entities. In the severe forms of thalassaemia such as, Cooley's anaemia or thalassaemia major, the abnormal red blood cells processed by the spleen and its haematopoietic response to the anaemia lead to massive splenomegaly and consequent manifestations of hypersplenism, ultimately requiring splenectomy.

Infections are the predominant cause of death in these patients accounting for 12-46 per cent of overall patient deaths. It is estimated that about 9,000 children with beta thalassaemia are born every year, although no documentary registry is available in Pakistan. The estimated carrier rate is 5-7 per cent, accounting for 9.8 million carriers in the total population.

Factors predisposing to infections in these patients include:

1. Immune abnormalities in thalassaemic patients including increased number and activity of CD8 suppressor cells, decreased CD4/CD8 ratio, C3 and C4 levels along with defective neutrophils, macrophages and natural killer cell function.

2. Splenectomy-induced immune abnormalities including reduced immune clearance along with lower specific antibody responses.

3. Iron overload and bacterial infections: Microbial pathogens must obtain growth-essential iron from healthy hosts. Some potential pathogens however are more dangerous in hosts with iron overload. Iron overload causes decreased phagocytosis by the monocyte/macrophage system and polymorphonuclear leucocytes due to the deleterious effects of ferritin associated with iron and impairment in immunoglobulin secretion along with suppression of complement system function. Organisms such as *Yersinia enterocolitica*, *Klebsiella* species, *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Legionella pneumophila* have increased virulence in vitro in the presence of excess iron. Iron overload can also have adverse effects on the outcome of viral infections.

Unfortunately, desferrioxamine therapy predisposes to infections by *Yersinia* spp. The virulence enhancing effect of desferrioxamine is caused by either due to FoxA receptor that acts as a receptor for its active metabolite too or due to its partial immunosuppressant effect. Furthermore the rate of progression of HIV-1 disease is faster in patients with thalassaemia major on low doses of desferrioxamine and high serum ferritin concentrations and with high bone marrow macrophage iron.

Specific infectious agents involved in infection include:

Yersinia enterocolitica: *Y.enterocolitica* is an uncommon cause of severe infections in thalassaemic patients in the East unlike the West. *Y. enterocolitica* has been associated with septicemia, mesenteric lymphadenitis, liver and splenic abscesses, and an acute appendicitis like syndrome in these patients.

Microbiologic culture remains the standard for diagnosis of yersiniosis. Stool is the preferred clinical specimen for patients with intestinal symptoms. Other specimens which should be considered for culture include blood, throat, wounds, and specimens obtained at surgery such as the appendix or lymph nodes. Serologic tests are also widely used for the diagnosis of yersiniosis.

***Klebsiella* spp:** High ferritin level and deranged liver function in thalassaemia appears to be the risk factors of infections due to *Klebsiella* spp. The diagnosis of *K. pneumoniae* infection is confirmed by culture of blood, sputum, urine, or aspirated body fluid.

Klebsiella spp. typically appears as short, plump,

gram-negative bacilli, which are usually surrounded by a capsule that appears as a clear space. They have no specific growth requirement and could grow on ordinary media. They are lactose fermenters and this property is used for identification in microbiology laboratories.

***Salmonella* spp.:** Salmonellae are motile Gram-negative bacilli. Salmonella are oxidase negative and virtually all are non-lactose fermenters. Most laboratories identify Salmonellae by a combination of antigenic and biochemical reactions. Suspicious colonies are agglutinated using antisera directed against specific O (lipopolysaccharide) and H (flagellar) antigens that allow identification of the serogroup

Escherichia coli: *E. coli* is Gram-negative, facultative anaerobic, non lactose fermenters and non-sporulating bacilli. Typically diagnosis is done by culturing on MacConkey medium and biochemical reactions. Other methods for detecting *E. coli* include ELISA tests, colony immunoblots, and direct immunofluorescence microscopy of filters, immunocapture techniques and PCR.

Post-splenectomy infections: The incidence of severe infections (for example, meningitis, pneumonia, sepsis) caused by encapsulated bacteria (*S pneumoniae*, *H influenzae* type B, *N meningitidis*) is six per cent higher among splenectomised patients with thalassaemia compared with those individuals splenectomised for trauma. Children appear to be at particularly high risk. Other pathogens responsible for post-splenectomy infections include bacteria such as *E coli*, *P aeruginosa*, group B beta hemolytic streptococci, *Enterococcus* spp, *Ehrlichia* spp, and *Plasmodium* spp. Blood culture is routinely done for the diagnosis of these infections. Several rapid diagnostic tests have been developed to aid in the diagnosis along with PCR.

Streptococcus pneumoniae has characteristic lancet-shaped gram positive diplococci. Like *Klebsiella spp.,* they have no specific growth requirement. Complementary method to detect *S.pneumoniae* is urinary antigen assays. The test has high sensitivity compared to blood cultures and sputum studies in patients with bacteremia. Other tests including PCR tests for detecting pneumococcal autolysin or pneumolysin have been developed.

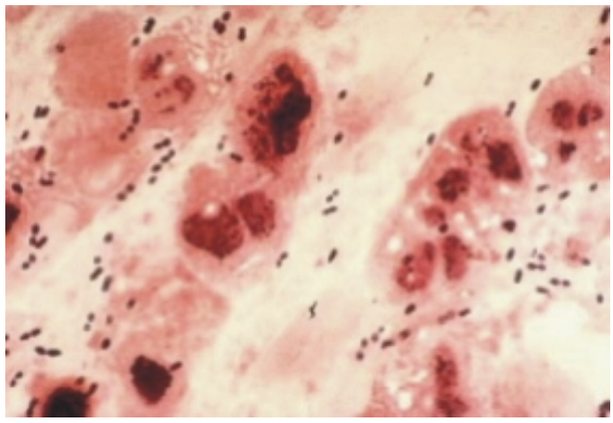

Figure 1: Lancet-shaped gram positive diplococci suggestive of *Streptococcus pneumonia* in a sputum smear

Effective vaccines are available against each of these pathogens. It is mandatory for every patient undergoing elective splenectomy to have Pneumovax, optimally at about 1 month pre-surgery. In addition to this immunoprophylaxis other measures include chemoprophylaxis with prolonged use of antibiotics and patient education to attend seriously to any impending fever or infection.

Hepatitis B and C virus

Hepatitis B (HBV) and C virus (HCV) infections are a major problem for thalassaemic patients because of repeated blood transfusions. Infection with these blood borne viruses is associated with characteristic changes in the serum levels of viral antigens and antibodies. Diagnostic qualitative and quantitative PCR tests for HBV and HCV in serum have been developed to assess in diagnosing viral replication.

The availability of vaccines and the introduction of the screening of transfused blood units for hepatitis Bs antigen (HBsAg) and HCV antibodies have reduced the incidence of these infections.

Malaria and α-thalassaemia

Both α-and β- thalassaemia have been shown to afford some protection against *P. falciparum* malaria in keeping with their geographical distribution, but the mechanism of protection remains unknown. However in case of infection, detection of parasites on Giemsa-stained blood smears by light microscopy remains the gold standard for diagnosis of malaria. Rapid diagnostic tests for detection of *Plasmodium spp.* have been developed. However desirable characteristics for RDTs vary depending

on the epidemiology of infection and goals for control in the region where the test is used. Molecular technologies are also emerging in assisting the diagnosis of malaria.

Early recognition with prompt treatment and prevention of predisposing factors are essential in the treatment of patients with thalassaemia and infection.

CDX-2, A Reliable Diagnostic Marker for Carcinomas of Colorectal Origin

Dr Arsalan Ahmed and Dr M. Abrar Barakzai
Histopathology

CDX-2 is a caudal-related homeobox transcription factor whose expression in adults is normally restricted to the intestinal epithelium. CDX-2 protein is responsible for regulating the proliferation and differentiation of intestinal epithelial cells and therefore it is expressed predominantly, as a nuclear marker of epithelial cells, lining the villi and crypts in the small and large intestines. Hence, CDX-2 protein is a useful marker for intestinal adenocarcinomas, especially colorectal adenocarcinomas.

The differentiation of secondary from primary adenocarcinomas of the colorectum is important because their clinical management and prognosis are different. CDX-2 immunostaining may be useful in discriminating primary and metastatic colorectal carcinomas from frequent types of adenocarcinomas of nongastrointestinal origin, since CDX-2 is consistently expressed in more than 90 per cent of colorectal carcinomas, while its expression is rare in extraintestinal adenocarcinomass like breast, lung, thyroid, kidney and endometrial adenocarcinomas. However, CDX-2 is expressed in 10-30 per cent of other gastrointestinal malignancies, 20-65 per cent of mucinous ovarian tumors and 50-100 per cent of urinary bladder carcinomas. Compared with villin, a previously described marker of gastrointestinal adenocarcinoma, CDX-2 demonstrates superior sensitivity and comparable specificity.

Another important aspect of CDX-2 expression in colon carcinomas is that CDX-2 expression is inversely related to the degree of anaplasia, therefore loss of CDX-2 protein expression has been correlated with loss of differentiation in colorectal cancers and hence poorly differentiated colorectal carcinomas demonstrate a lower frequency of CDX-2 staining compared to well and moderately differentiated tumors. Thus, it may have both diagnostic and prognostic uses.

CDX-2 is a reliable, specific, and sensitive immunohistochemical marker of normal and neoplastic intestinal epithelium and can be easily applied to routine histologic and cytologic material. It may have both diagnostic and prognostic uses, suggesting including of CDX2 in any antibody panel to distinguish between primary and secondary epithelial colorectal malignancies, as well as targeting metastasis from an unknown primary. AKUH Laboratory has recently added this marker in the immunohistochemistry panel.

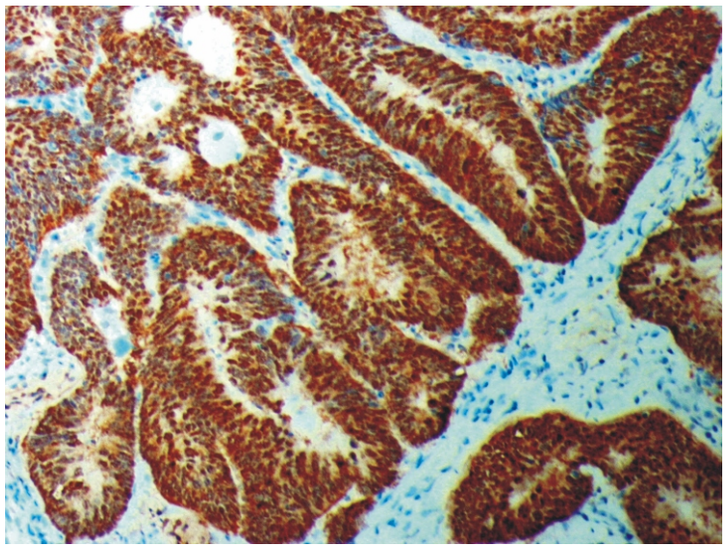

Figure 1: Colorectal Carcinoma showing diffuse nuclear positivity to CDX-2

Radiopaque Metallic Marker Insertion in Breast Cancers Prior to Neoadjuvant/ Preoperative Chemotherapy

Dr Shaista Afzal, Dr Gulnaz Shafqat, Dr Imrana Masroor
Radiology

Over the last two decades neoadjuvant chemotherapy or hormonal manipulation has been increasingly used as a treatment option in the management of patients with large operable breast cancer. The advantage of neoadjuvant chemotherapy over adjuvant chemotherapy is that large cancers can be down-staged thereby increasing the possibility of breast conserving surgery and the response to the treatment can also be monitored. There are a number of studies which have reported remarkable radiological and pathological response with little or no residual tumor following the treatment. Such excellent response creates problems in localisation and excision of the residual tumor and so imposes a challenge for accurate surgery. Complete response at imaging does not necessarily implicate a complete pathological response and it is not a safe practice to plan surgery based on radiological findings alone. It is in these cases that localisation of the tumor bed with radiopaque markers prior to the institution of chemotherapy may facilitate breast conservation surgery with complete excision of tumor bed to ensure removal of microscopic disease.

The procedure of radiopaque marker insertion is very simple and can be carried out by a radiologist under mammographic or ultrasound guidance. After routine prepping and local anesthesia a 16-18 gauge lumbar puncture needle is inserted into the lesion ideally in the centre and radiopaque metallic clip is inserted and the trocar is used to push the marker in position. After the insertion of the clip unilateral mammogram is obtained to confirm the position of the marker (figure 1)

On completion of chemotherapy (figure 2) the surgeons reassess to determine the feasibility of breast conservation surgery and if the tumor shows a complete response to chemotherapy or is impalpable pre-operative guide wire localisation for the metallic markers is carried out for marking the tumor bed.

In summary implantation of radiopaque clips in or adjacent to carcinoma of breast is useful in tagging the tumor bed prior to breast conservation surgery, especially in patients with complete radiological and clinical tumor regression following neoadjuvant chemotherapy.

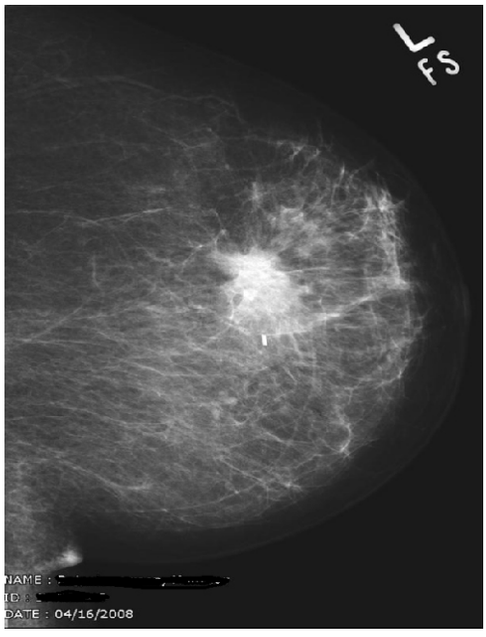

Figure 1: Mammogram before the commencement of neoadjuvant chemotherapy Showing the radiopaque clip

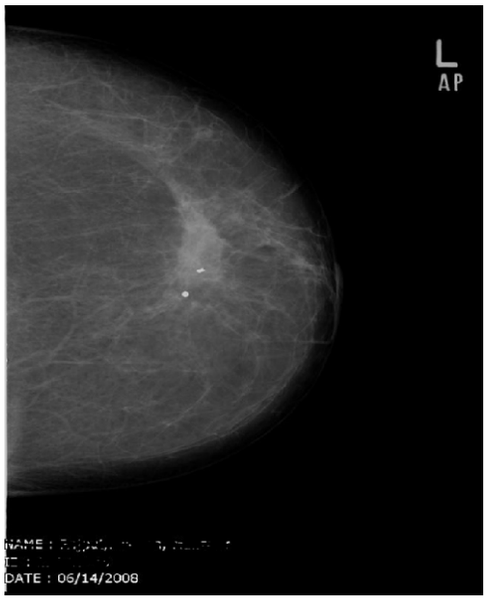

Figure 2: Mammogram after the completion of neoadjuvant chemotherapy Showing the radiopaque clip and the tumour showing partial response

Meeting Reports: 10TH International Radiological Conference 2009

Dr Azieka Imdad, Muhammad Bilal Ahmad
Radiology

The 10th International Radiological Conference was held from November 7-9, 2009 at the Sheraton Hotel in Karachi. The theme of the conference was 'achieving excellence in radiology practice.' It was organised by the Radiological Society of Pakistan (RSP).

Participants from all over Pakistan as well as from USA, UK, Canada and India attended the conference. Papers presented during the conference covered a diverse range of topics with discussion of recent advancements and methods.

Very informative and interactive pre-conference workshops were arranged for the radiologists, trainees and radiographers, covering many important aspects of radiology training and advancements. On day one several workshops were held.

A basic MRI course was held at the Sheraton hotel. The chief organiser, Dr Zafar Sajjad, Associate Professor and Interim Chair, Department of Radiology, AKUH conducted it with excellence. It focused on providing the participants about basic knowledge of MRI physics, contrast usage and MR imaging of the shoulder, knee, spine and brain.

A hands-on workshop of obstetric/gynae ultrasound was organised at Jinnah Post Graduate Medical Center (JPMC). It was a very successful and helpful workshop that allowed participating radiologists to enhance their practical skills, regarding foetal doppler ultrasound and placental evaluation.

Another useful pre-conference workshop showed participants how to initiate a radiology research project. Facilitated by Dr Wasim Akhtar, Assistant Professor, Department of Radiology, AKUH, it was very appreciated as a number of radiologists needed guidance on the initiation of research projects. It proved to enhance the basic know-how of the participants about radiology research, literature research, biostatistics and common errors in paper writing.

A workshop session on radiographic critique was held at AKUH: it was organized for radiographers, regarding quality control, emergency and trauma radiography and radiation protection.

On day two, the pre-conference workshops were continued and Multi-Detector CT (MDCT) as an advanced imaging modality was discussed, during the first session. It was focused on orienting the participants about MDCT image formation, image manipulation and contrast delivery. An interpretation session followed the workshop.

Hands-on practice was given to the participants during Doppler and Musculoskeletal (MSK) ultrasound workshop session, which focused on the carotid, peripheral, renal and visceral Doppler, as well as providing basic knowledge about MSK ultrasound and interpretation.

A workshop on Breast imaging facilitated by Dr Imrana Masroor, Associate Professor, Department of Radiology, AKUH, and her team highlighted on breast ultrasound, intervention and MRI and mammograms. The workshop was interactive and helped the participants to understand the basics of breast imaging.

A full-day workshop on Interventional Radiology was held at AKUH; Dr Imran Syed, Consultant Radiologist, from Queen's Hospital, UK conducted the workshop and gave a live demonstration of interventional procedures like biliary interventions, Peripherally Inserted Central Catheter (PICC) and central venous line placement and Trans Arterial Chemoembolisation (TACE). Hands-on was also given for US guided procedures.

Dr Naila Nadeem, Assistant Professor, Department of Radiology, AKUH organised a workshop on Assessment and feedback to trainees during work in radiology. Its main focus was assessment of radiology residents during training and role of faculty and residents on feedback system.

The session of video conferencing between UK and Pakistan was highly interactive and mainly focused on the musculoskeletal imaging, also served as a bridge between the Pakistani and the UK-based Pakistani consultants.

AKUH radiology actively participated in the conference and won prizes. The first prize for best paper presentation was won by Dr Zainab Hussain, Resident, Department of Radiology, AKUH while the first prize for best poster was won by Mr Mansoor Naqvi, Physicist, Department of Radiology, AKUH and second prize by Dr Rana Shoaib, Fellow of Radiology, Department of Radiology, AKUH. The winners of the conference quiz were Dr Fahd Haroon and Dr Mohammed Zeeshan, Resident, Department of Radiology, AKUH who jointly received the first prize.

Another highlight of the conference was the cultural evening and banquet dinner held on November 9. It was well attended and was very refreshing for the participants of the conference, with music served as a food for the soul.

The conference concluded on November 9 with the distribution of certificates and souvenirs among the organisers and participants. Efforts of scientific committee were commendable under the leadership of Dr Tanveer-ul-Haq who was fully supported by president of the RSP, Dr. M. Nadeem Ahmad and Dr Zafar Sajjad.

Continuing Medical Education Seminar Organised by Clinical Laboratory at AKUH

Ms Seema Vaqar

On December 9, the Clinical Laboratory arranged a Continuous Medical Education Seminar at AKU auditorium on "Assessing and Managing Osteoporosis". It was the first time at AKU that this CME was seen by all AKHSP via Elluminate Live! The seminar was a unique experience and a successful one as questions and answers were exchanged between AKHSP audience and speakers.

Dr Farooq Ghani, Associate Professor and Consultant Pathologist, Department of Pathology & Microbiology, AKUH, gave the opening statement followed by a presentation by Dr Aysha Habib Khan, Assistant Professor and Consultant Pathologist, Department of Pathology and Microbiology, AKUH, on osteoporosis and its assessment. She highlighted the risk factors leading to osteoporosis and showed concerns about patients with fractures not being evaluated for osteoporosis. Dr Romaina Iqbal, Assistant Professor and Consultant Nutritionist, Department of Community Health Sciences and Medicine, AKUH, enlightened the audience about taking healthy food to avoid calcium deficiency. Dr Jaweed Akhtar, Associate Professor and Consultant Endocrinologist, Department of Medicine, AKUH, talked about pharmacological management of osteoporosis, and discussed different therapies for osteoporosis with their pros and cons. Mr Asim Mahmood, Physiotherapist, Department of Physiotherapy, AKUH, emphasised on different exercises which helps in correcting postures.

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