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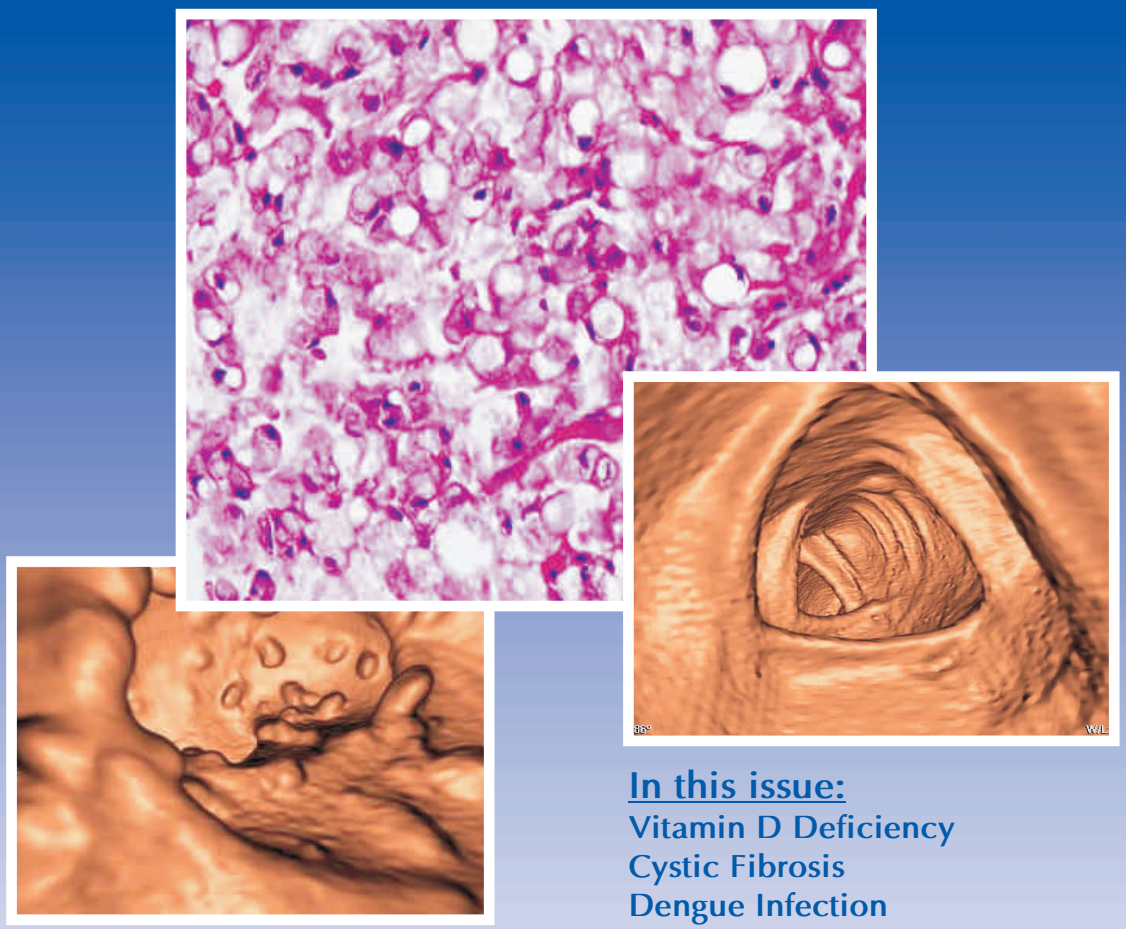
LABRAD : Vol 33, Issue 3 - September 2008

Aga Khan University Hospital, Karachi

Newsletter of Departments of Pathology and Microbiology, and Radiology

LABRAD

September 2008 Vol. 33, Issue 3



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

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Organization Accredited by Joint Commission International

September 2008, Vol. 33, Issue 3

Labrad

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

September 2008,
Volume 33, Issue 3

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Vitamin D Deficiency: Are We Still Ignoring the Evidence?

Dr Aysha Habib Khan, Consultant, Chemical Pathology

Serum 25 hydroxyvitamin D (25OHD) is the major circulating metabolite of vitamin D and reflects its overall nutritional status. Its deficiency is a recently recognized epidemic in many parts of the world. Evidence demonstrates association between low vitamin D status and risk of many chronic diseases (1).

Recently, a preclinical phase of vitamin D deficiency, known as vitamin D insufficiency is identified; when Serum 25OHD levels are between 21-29 ng/ml. This is associated with a slightly elevated serum PTH concentration and a mild increase of bone turnover, which increases the risk of fractures. Levels less than 20 ng/ml are regarded as deficient. Maintaining blood concentrations of 25OHD at least 30 ng/ml is important for maximizing intestinal calcium absorption (1).

There are sporadic reports of D deficiency and sub clinical osteomalacia from Pakistan till 2004 in pregnant & lactating women from Pakistan (2). Recently low levels have been reported in OPD patients from a public hospital in Karachi and in patients with hip fracture and from Hazara District (3-5).

One of our study showed that 92% of patients were D deficient in ambulatory care setting. 62% had severe, 24% moderate and 8% had mild deficiency. Nearly half of all these patients (including those with severe deficiency) were asymptomatic. Whereas a low serum calcium, elevated phosphate and elevated alkaline phosphatase were reflective of severe deficiency, it was only an elevated iPTH that correlated with mild to moderate deficiency (6).

The status of 25OHD in our local population has not been assessed. During the last decade, there has been a general increase in the use of vitamin D measurement in our Clinical Laboratory at Aga Khan University Hospital. Our data shows that out of 2625 cases till 2006, 36.4% had insufficient levels and 30% are D deficient by current criteria (table 1).

There is no published data regarding the prevalence of 25OHD insufficiency in adult healthy population. In another study (unpublished data) from our center to characterize the vitamin D status of healthy asymptomatic population, we found 70% of the healthy volunteers from a total of 93 cases, to be D deficient. Of more significance was the presence of elevated PTH in 28% of these individuals which is regarded as an earliest marker to indicate D deficiency. Significant associations with life style variable could not be established due to small sample size.

In spite of abundant sunshine, undiagnosed vitamin D deficiency is prevalent in our setup. There is a need to determine the vitamin D status in our community. With the magnitude of deficiency that is seen in our healthy and diseased population, fortification of food items is required. It is important to make physicians aware of the high prevalence of vitamin D deficiency in apparently healthy looking individuals. Measures for improving vitamin D status are needed to eradicate the existence of vitamin D deficiency.

Table 1

Clinical Laboratory Data of 25 OHD at AKUH (2002 – 2004) N=3099

Serum levels of 25 OHD	No of cases N=3099 (%)	Mean levels (ng/ml)
Vitamin D Deficiency	2107 (72.1)	8.04
Vitamin D Insufficiency	572 (18.5)	27.6846
Optimal	280 (9.0)	61.0544
Toxic	37 (0.1)	107.9450

Test performed by RIA by a kit from DiaSorin, USA

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Collection and Transport of Urine for Culture

Dr Joveria Farooqi, Resident Microbiology

Urine specimens can easily become contaminated with periurethral, skin, perianal and vaginal flora. This contamination can be reduced to acceptable levels, if proper technique of urine collection is followed. The methods of collection differ according to the patient's age, gender, clinical condition and history of catheterization. The common techniques of urine specimen collection are discussed below.

General Instructions

1. Preferably, an early morning sample should be submitted for culture. Urine that has remained in the bladder for at least four hours has decreased chances of false negative results.
2. Forcing fluids will dilute the bladder urine and decrease colony counts to below significant levels.
3. The sample should be processed within two hours after collection. If that is not possible, then it should be refrigerated both during storage and transport, and processed within 24 hours. If refrigeration is not possible and delay is expected, at least 3 ml of sample should be collected in a container with preservatives (boric acid-glycerol or boric acid-Na formate).
4. The submitted sample should be appropriately labeled with name, age, gender of the patient and mode and time of sample collection.
5. Fungal cultures are included in the routine urine culture. For anaerobic culture, the sample should be a suprapubic aspirate submitted in a syringe.
6. In infants, voided or bagged specimens should be discouraged.
7. Reject specimens from urinals or bedpans, bags of catheterized patients, leaky containers, unlabelled specimens, and unrefrigerated, unpreserved specimens over two hours old.

Midstream Urine by Clean Catch Method

This is the commonest technique used in adults and children (toilet trained). The patient should be conscious, able to pass urine, not catheterized and a female patient should not be menstruating.

1. Separate the labia and cleanse the urethral meatus twice with sterile sponges soaked in plain soap and water from front to back and then rinse with sterile water or saline sponges twice. The same should be done for uncircumcised males after retracting the foreskin. No preparation is necessary for circumcised males.
2. After discarding the first 10 ml, collect at least 5-10 ml of voided urine in a sterile leak-proof container by moving into the stream of urine without halting or restarting the stream. Screw

on the top of the container after making sure that there is no leakage.

Catheterized Urine

1. Indwelling catheters
   Clamp the catheter till the patient senses the urge to urinate or the bladder becomes palpable. Clean the catheter port with 70% alcohol and collect 10 ml urine using a needle and syringe. Remove the clamp.
2. Straight catheter (in and out)
   This technique is used by physician or trained health professional to collect the specimen from infants, or patients with neurogenic bladders. Urine is obtained directly from the bladder after cleansing the meatus with plain soap and water (as previously mentioned). Discard the first 15-30 ml and submit the next flow for culture.

Ileal conduit

Remove external device, cleanse the stoma with 70% alcohol, then iodine and then remove iodine with alcohol. Insert catheter tip into cleansed stoma to a depth beyond the fascial level and collect urine.

Suprapubic Aspiration

This is the preferred method of urine collection from infants and where interpretation of voided urine culture is difficult or anaerobic bacteria are suspected as cause of UTI. However this technique should be performed by physician or trained health professionals.

1. Bladder should be full and palpable. Shave and disinfect the skin over the bladder.
2. Make a small wound through the epidermis above the symphysis pubis and aspirate using a needle and syringe. Submit specimen in syringe or carefully sealed sterile container.

Reference

1- Henry D Isenberg. Clinical Microbiology Procedure Handbook, 2nd ed. Vol. 1 ASM press.

Understanding and Interpreting Serum Protein Electrophoresis

Dr Sahar Iqbal, Resident Chemical Pathology

Serum protein electrophoresis is used to identify patients with multiple myeloma and other serum protein disorders (Table1). Electrophoresis separates proteins based on their physical properties, and the subsets of these proteins are used in interpreting the results.

Table 1: Indications for Serum Protein Electrophoresis

Suspected multiple myeloma, Waldenstorm's macroglobulinemia, primary amyloidosis, or related disorder
Unexplained peripheral neuropathy (not attributed to longstanding diabetes mellitus, toxin exposure, chemotherapy, etc.)
New onset anemia associated with renal failure or insufficiency and bone pains in which multiple myeloma is suspected
Hypercalcemia attributed to possible malignancy (e.g. associated weight loss, fatigue, bone pain, abnormal bleeding)
Rouleaux formations noted on peripheral blood smear
Renal insufficiency with associated serum protein elevation Unexplained pathologic fracture or lytic lesion identified or radiograph
Bence Jones proteinuria

The pattern of serum protein electrophoresis results depend on the fractions of two major types of protein: albumin and globulins. Albumin, the major protein component of serum, is produced by the liver. Globulins comprise a much smaller fraction of the total serum protein content and consist of alpha1, alpha2, beta and gamma globulin.

Reflected in the electrophoresis gel regions; plasma protein levels display reasonably predictable changes in response to acute inflammation, malignancy, trauma, necrosis, infarction, burns, and chemical injury. The "acute-reaction protein pattern" involves increases in the different globulin components including fibrinogen, $alpha_1$-antitrypsin, haptoglobin, ceruloplasmin, $alpha_1$-acid glycoprotein and other components like CRP and C3 portion of complement. Often, there is a decrease in the albumin and transferrin levels associated with acute phase response.

Presence of monoclonal protein is characterized by the presence of a sharp, well-defined band (homogenous spike like peak on densitometer graph) with a single heavy chain and a similar band with a kappa or lambda light chain. A polyclonal gammopathy is characterized by a broad diffuse band with one or more heavy chains and kappa and lambda light chains. A monoclonal band is mostly malignant or potentially malignant, including multiple myeloma, Waldenstrom's macroglobulinemia, solitary plasmacytoma, smoldering multiple myeloma, monoclonal gammopathy of undetermined significance, plasma cell leukemia, heavy chain disease and amyloidosis. In contrast polyclonal gammopathies may be caused by any reactive or inflammatory process.

Currently recommended techniques for the evaluation of monoclonal proteins consist of high resolution electrophoresis, immunofixation and immunoselection. Immunosubstraction is a promising new technique to characterize monoclonal protein.

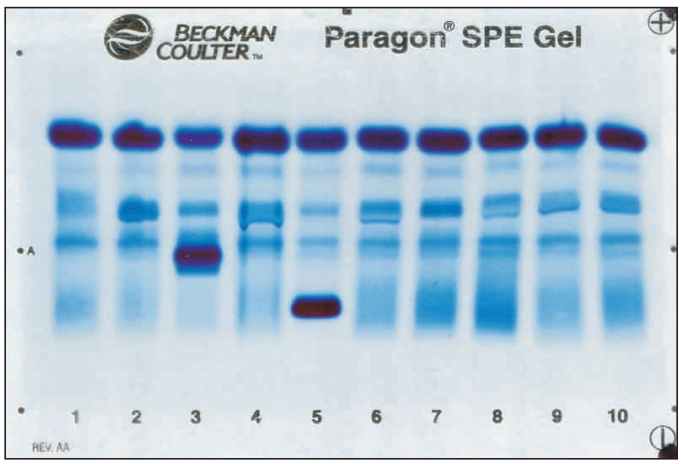


Figure 1: Serum Protein Electrophoresis on Agarose Gel
Each column of the gel is representing the single sample of the patient except the first column for the control. The monoclonal gammapathy is determined by the presence of a well defined band in the beta and gamma region as seen in 3rd and 5th columns, respectively. Similarly the polyclonal gammapathy can be seen in the gamma region on the 8th column of the gel. In the 6th column hypoalbuminemia with increase in the alpha 2 fraction can be noted which may be seen in the cases of nephrotic syndrome.

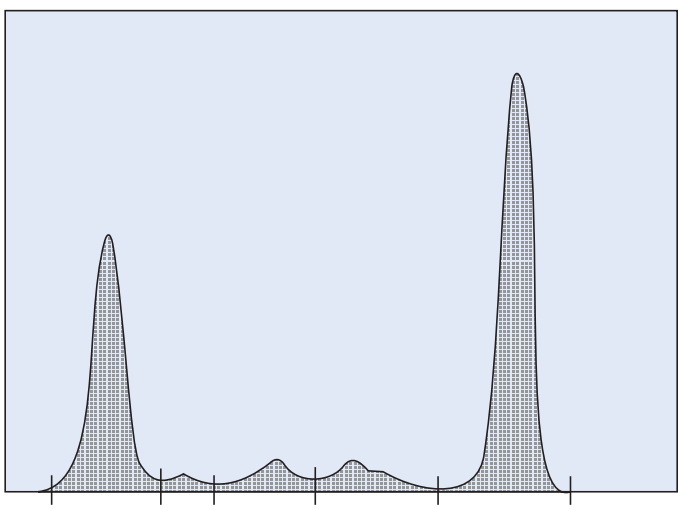


Figure 2: Monoclonal Gammopathy As Shown by Densitometer
A sharp, discrete and well defined peak is seen in the densitometer graph representing the monoclonal gammapathy.

Cystic Fibrosis at a Glance

Ms Sheeba Parveen, Technologist Molecular Pathology

Background

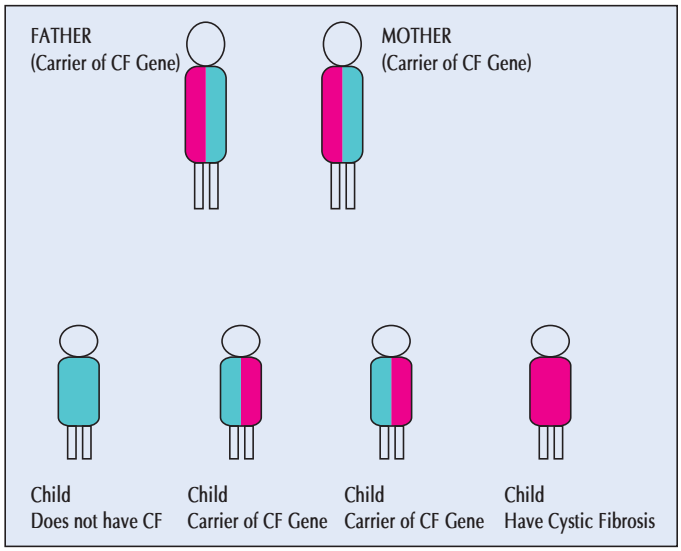
Cystic fibrosis (CF) is an inherited disease which most commonly affects the white population. Approximately, 1 in 2500 newborns is affected with this disease. It is estimated that about 7000 Britons suffer from CF in Britain; the number of cases involving CF are about 30,000 in the United States. CF was initially thought to affect only Caucasians, but there is also low incidence of CF in non-white populations.

Cystic fibrosis is an autosomal recessive disorder characterized by large number of mutations in the gene encoding the cystic fibrosis transmembrane receptor (CFTR) [1], which is located on chromosome 7q 31.2. To date more than 1600 mutations have been identified in CFTR gene. These mutations alter CFTR function with concomitant milder or severe disease manifestation. The most common mutation in CF is found in codon 508 (phenylalanine) in CFTR gene. It is commonly known as delta F508 (deletion F508). Studies have shown that 33% of Pakistani population carries this mutation. The CFTR protein functions as an ion channel and maintains the liquid volume on the epithelial surfaces by regulating the secretion and inhibition of chloride and sodium ions, respectively. This inability of the body to move salts and water in and out of cells causes the lungs and pancreas to secrete thick mucus which blocks various passageways in the body, preventing them from functioning normally.

Genetics

A baby born to parents each of whom is a carrier of the CF gene has:

- 1 in 4 chance of inheriting 2 abnormal CFTR genes and have CF.
- 1 in 4 chance of inheriting 2 normal CFTR genes and not have CF or be a carrier.
- 2 in 4 chance of inheriting one normal CFTR gene and one abnormal CFTR gene.



Symptoms

The most severe symptoms of CF occur in the gastrointestinal (digestive) system and respiratory tract, and are accompanied with the following features:

- Frequent coughing that brings up thick sputum.
- Frequent bouts of bronchitis and pneumonia which lead to inflammation and permanent lung damage.
- Dehydration.
- Infertility (mostly in men)

Diagnosis

The easiest and most accurate test for cystic fibrosis is the measurement of sweat electrolytes[2]. Patients with CF have raised sodium and chloride concentrations (>60mmole/l). When individual's sweat is 1.5-2.0 times greater than normal (40-60mmole/l) the individual has CF.

Molecular Diagnosis

Genetic testing can also be used to diagnose CF. A small blood or tissue sample is taken from the patient and analyzed to determine whether the individual's CFTR gene is normal or carries mutation(s). These mutations are screened through a procedure known as Amplification Refractory Mutation System (ARMS). In this technique three multiplex polymerase chain reactions (PCR) are run

in parallel for each individual, using a primer that enables detecting different mutations.

Newly developed techniques such as re-sequencing microarrays, multiple genotyping arrays and comparative genomic hybridization arrays allow investigators to diagnose CF robustly and quickly. A low density microarray is being developed that has the potential to identify 25 CF mutations and 6 polymorphisms simultaneously. Microarray platforms are also being developed that will facilitate detection of a larger number of CF mutations on a single chip in a cost-effective way. Additional detailed studies are required to characterize the common genetic mutations in Pakistani children with CF.

Treatment

There is still no cure for CF, but treatments useful for managing the condition have improved greatly in recent years. The key to good prognosis is early detection and timely treatment. The advantages of early detection of CF include:

- Removal of thick, sticky mucus from lungs by mucus-thinning drugs
- Prevention of airway blockage by using bronchodilators
- Nutritional benefits
- Chest physical therapy
- Oxygen therapy
- Social and Psychological
- Counselling parents for prenatal testing.

In the past, it was rare to see CF patients live beyond the age of 30. Over the last two decades, surveillance and screening programmes in different countries along with the availability of better therapies that control disease progression have significantly enhanced life spans and improved the quality of life for people with CF.

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What you need to know about Dengue Infection

Ms Samina Ghani, Molecular Pathology

Introduction

Dengue virus (DENV) belongs to the genus Flavivirus (Flaviviridae family). It is a spherical, enveloped virus that has a diameter of approximately 50 nm. It contains a single-stranded, positive-sense RNA genome. The DENV genome comprises of approximately 10,600 nucleotides. It has four closely related but antigenically distinct, virus serotyes named as DEN-1, DEN-2, DEN-3, and DEN-4.

Epidemiology of Dengue Virus

It's found during and shortly after the rainy season in tropical and subtropical areas of Africa, Southeast Asia, China, India, Middle East, Caribbean and Central America. The WHO estimates 50 million cases of dengue infection occur each year, and almost more than 100 countries are endemic to dengue infection.

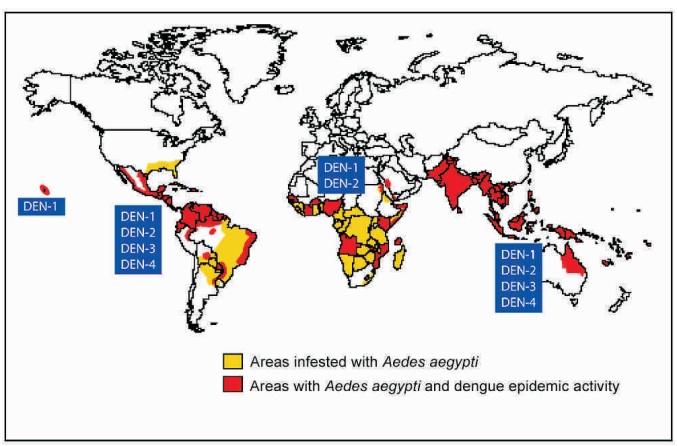

Figure 1: Worldwide Dengue Distribution

What is Dengue Fever (DF)?

It's an infectious disease carried by mosquito (Aedes.aegypti or albopitus) and caused by any of four related dengue viruses. First reported epidemics of DF occurred in 1779 in Africa and North America while first reported epidemic occurred in Karachi, Pakistan in 1994.

Who Gets Dengue fever?

Dengue fever may occur in people of all ages. Children usually have a milder disease than adults.

How Dengue Fever Spread?

Dengue fever is spread by the bite of infected Aedes mosquitoes. It cannot be spread from one person to another.

Does Past Infection with Dengue Virus Make A Person Immune?

Individuals infected with one strain maintain lifelong homotypic immunity while remaining susceptible to infections with other heterotypic strains. Interestingly, DHF/DSS is more likely to develop if an individual previously infected with one serotype is later inoculated with a different viral strain. Dengue Hemorrhagic fever (DHF) and Dengue Shock syndrome (DSS) usually occur as a second dengue infection.

Symptoms of Dengue Fever

Symptoms of typical uncomplicated dengue usually start with fever within 5 to 7 days after a person has been bitten by an infected mosquito. Typically, there is high fever up to 105˚, severe headache, retro-orbital pain, severe joint and muscular pain, nausea and vomiting and rashes caused by skin hemorrhage.

Clinical Diagnosis

Infection with dengue virus causes a broad spectrum of illnesses, ranging from asymptomatic infection to the more severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).

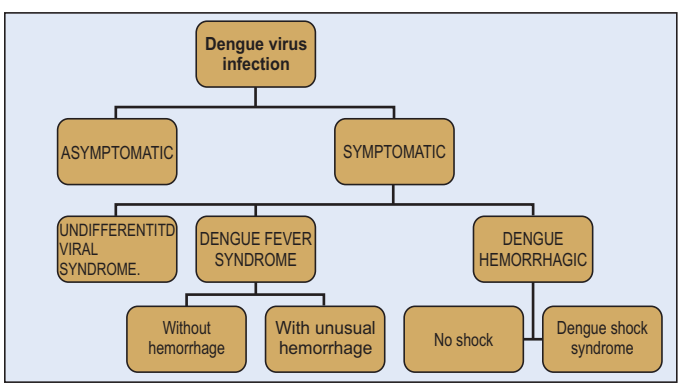

Figure 2: Clinical Presentation in Dengue Virus Infection

Manifestation of Dengue Infection

There are three main manifestations of dengue infection. Dengue fever (DF), Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). DF is characterized by fever lasting 3-5 days, headache, muscle and joint pain but usually patients recover easily.

DHF and DSS, which mainly occur in patients previously infected with the virus of different serotype, present similar symptoms to DF, but are followed by an increased vascular permeability and haemorrhagic signs leading to reduced blood pressure, hypovolemia, vascular collapse and death.

Table 1: Manifestation of Dengue Infection

Dengue Fever (DF)	Dengue hemorrhagic fever (DHF)	Dengue shock syndrome (DSS)
• FLU-like illness • Incubation: 2 to 7 days • Undifferentiated fever with rash • Mild to severe febrile syndrome with high fever • Retro-orbital pain • Rash	• Sudden onset • High fever • Facial flash • Hepatomegaly • Hemorrhagic phenomenon and circulatory failure • Recovery possible	• Occur in DHF untreated patient • Rapid and weak pulse • Circulatory failure • Without proper treatment, the patient may die within 12-24 hrs.

Laboratory Diagnosis of Dengue Infection

Serological Diagnosis

Detection of dengue virus-specific antibodies is commonly used by using ELISA plate for routine diagnosis. However, antibody appears after onset of symptoms. In primary infection, IgM and IgG rise 5 and 14 days respectively after symptoms onset. In secondary infection, IgM levels are low or undetectable while IgG rise 1-2 days after symptom onset with higher level than in primary infection.

Detection of circulating dengue non-structural protein NS1 in patient sera has been described as an alternative method for earlier diagnosis using rapid strip test following immunochromatographic principle and by ELISA assay.

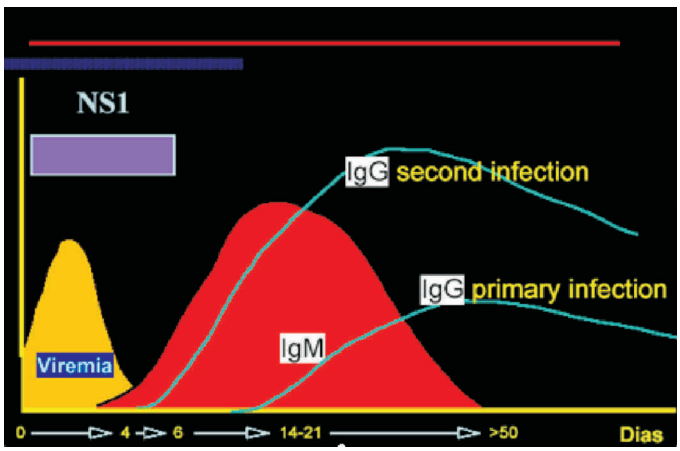

Figure 3: Important Markers in Dengue Infection

Molecular Diagnosis

At molecular level we can diagnose dengue virus using polymerase chain reaction(PCR) either by RT-PCR or using Real- time PCR.

RT-PCR is used to amplify the conserve region of dengue genome that code for capsid protein. Nested PCR with type specific primers are used to detect the four types of dengue viruses. The amplified products are detected by agarose gel electrophoresis using 2% agarose gel and visualized by UV light after staining with ethidium bromide.

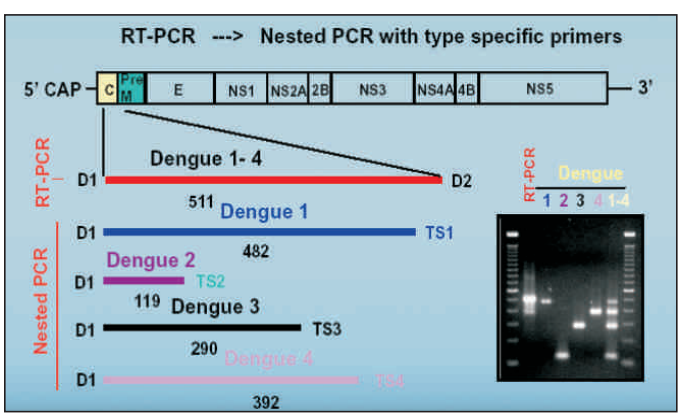

Figure 4: shows the genomic regions use to amplify by RT-PCR using type specific primers for diagnosis of four types of dengue viruses

What is the Treatment for Dengue Infection?

Treatment of Dengue fever is non-specific and supportive. There is neither specific antiviral treatment nor any vaccine available. It is advised to have plenty of bed rest, increased fluid intake and antipyretic to reduce fever.

How Can a Dengue Infection be Prevented?

Special precautions are taken to avoid contact with mosquitoes. When outdoor in an area where dengue fever has been found, it is advised to use a mosquito repellent containing DEET. Also, it is recommended to dress in protective clothing having long sleeved shirt, long pants, socks, and shoes.

Because Aedes mosquitoes usually bite during the day, precautions are taken especially during early morning hours before daybreak and in the afternoon before dark. Other precautions includes, keeping unscreened windows and doors closed, getting rid of areas where mosquitoes breed, such as standing water in flower pots or discarded tires.

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CT Colonoscopy: Introduction

Drs Zishan Haider, Ishtiaq Chishti, Dawar Burhan, Radiology

Introduction

Colorectal cancer is among the three most fatal cancers in men and women and is the second most common cause of cancer death among men aged 40 to 79 years in the World especially in the West[1].

Most colorectal cancers are believed to arise within

benign adenomatous polyps that develop slowly over the course of many years. Screening has been shown to save lives by detection and removal of pre-malignant polyps and early stage cancer. Nevertheless, despite this evidence and screening guidelines many individuals avoid screening because current tests are invasive, difficult or embarrassing and also sometime the gastroenterologist could not reached up to lesion.

Since the introduction of CT colonoscopy (CTC) or virtual colonoscopy in 1994, significant progress has occurred in the development and clinical implementation of this new technique. Currently, CT colonoscopy (CTC) is performed on an elective basis at a number of institutions around the world. A recent advance in CT colonoscopy (CTC) is the application of multi-slice CT (MSCT) technology. By combining the multi-row detector with increased gantry rotation speed, multi-slice CT (MSCT) can acquire 64 slices per second, and this number is increasing. Multi-slice CT (MSCT) makes high spatial resolution feasible at shorter acquisition times, increasing the sensitivity of the scan to smaller lesions[2].

Technical Considerations

Patient preparation consists of cleansing the patient's colon with a low residue diet, laxative bowel preparation. The accuracy of CT colonoscopy (CTC) is directly related to the adequacy of colon cleansing. Residual stool and fluid interfere with image interpretation and are a significant cause of false-positive and false-negative interpretations. Other tools such as fecal tagging with Barium and electronic cleansing or digital stool subtraction can also be used to distinguish between fecal matter and polyps. However, optimal colonic cleansing with cathartics is required to achieve acceptable results.

A well-distended colon is mandatory for proper evaluation by CT colonoscopy (CTC) which requires insufflations of the colon with room air or carbon dioxide. CT scout image is routinely checked for the adequacy of distension before the actual CT colonoscopy (CTC) scan is performed. Thin section helical CT of the abdomen and pelvis in the prone and supine position are then performed with low dose protocol. Intravenous contrast is usually used in those cases when there is already known colonic carcinoma and staging is also required.

Following image acquisition, the CT data are sent to an off-line workstation and can be viewed using a variety of techniques using different soft wares. This enables 3-D reconstructions (Fig 1), reformatted views (in lung window setting), or endoluminal images which are interpreted by radiologist in different sequences.

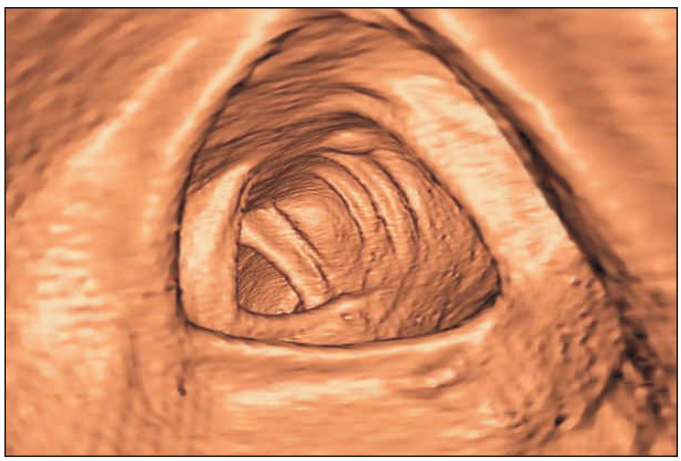

Figure 1: CT Colonographic 3-D image endoluminal view showing normal colonic folds in descending colon

Advantages

CT colonoscopy is considered to be a non-invasive tool which is considerably quicker than other techniques of colonic evaluation. It can also identify extra-colonic lesions and is helpful in staging the disease in cases of Known malignancy. The sensitivity for detection of polyps larger then 1 cm (over 90%) [3] is widely accepted (Fig 2). It is more comfortable then Barium enema examination. It can be used effectively in cases where colonoscopy could not be completed or obstructing lesion could not be negotiated.

Limitations

There are many factors which limits the sensitivity of CT colonoscopy. Most of them are related with poor patient compliance such as fecal matter, spasm

leading to lack of distension. Many artifacts are encountered such as respiratory artifacts, fluid retention, and shine through artifact. In cases of inflammatory bowel disease (IBD), especially in acute IBD or in obstructive stenosis of the colon, CT colonography is relatively contraindicated. Certain post-processing soft wares are expensive and difficult to use. Considerable experience and knowledge is required to avoid interpretation errors.

Summary

Thin-section multi–detector row computed tomographic colonography (CTC) affords increased opportunities for diagnostic imaging of the large bowel[4]. Currently colonography is establishing itself as a powerful tool for the detection and classification of colonic lesions.

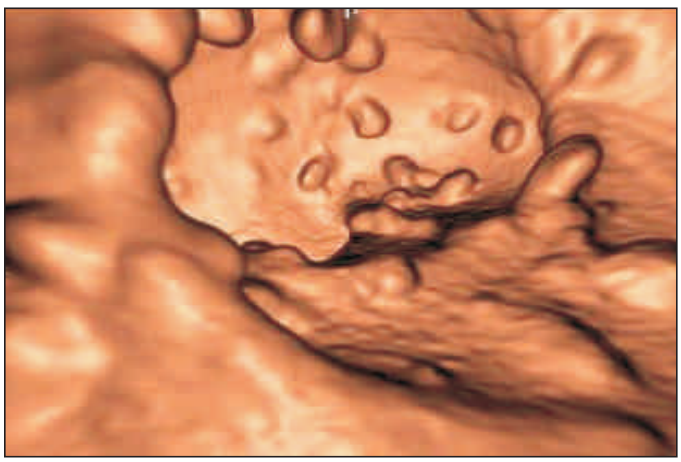

Figure 2: CT Colonographic 3-D endo-luminal view showing numerous colonic polyps in a patient with familial adenomatous polyposis

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CT Enteroclysis: A New Technique to Evaluate Small Bowel

Drs Zishan Haider. Ishtiaq Chishti, Radiology

Introduction

Early diagnosis of small bowel diseases is a diagnostic challenge to radiologists. CT enteroclysis is a new technique coming up in literature and it has recently been started in AKUH. With increased speed and resolution of 64 slice computed tomography (CT), it is regarded as a first-line modality for the examination of small bowel disease. This is further enhanced by coronal and sagittal reformations.

Indications

Indications for CT enteroclysis include a) unexplained gastrointestinal bleeding and anemia. b) unexplained abdominal pain with no evidence of significant small bowel distention on plain-film radiographs c) evaluation of known Crohn's disease or ileocecal tuberculosis. d) Sub acute or partial small bowel obstruction e) suspected small bowel tumor, such as, carcinoid.

Contraindications

Contraindications include pregnancy, hypersensitivity to intravenous contrast material and complete small bowel obstruction.

Advantages and Disadvantages

CT enteroclysis combines the advantages of conventional CT and barium enteroclysis into one method of examination. CT enteroclysis differs from routine abdomen CT in that it makes use of thin sections and large volumes of enteric contrast material to better display the small bowel lumen and wall (fig 1). The use of neutral enteric contrast agents, such as methyl cellulose, permits excellent assessment of hypervascular lesions (fig 2) and hyperenhancing segments. Compared with the

traditional small bowel follow-through examination, CT enteroclysis has several advantages: (a) it displays the entire thickness of the

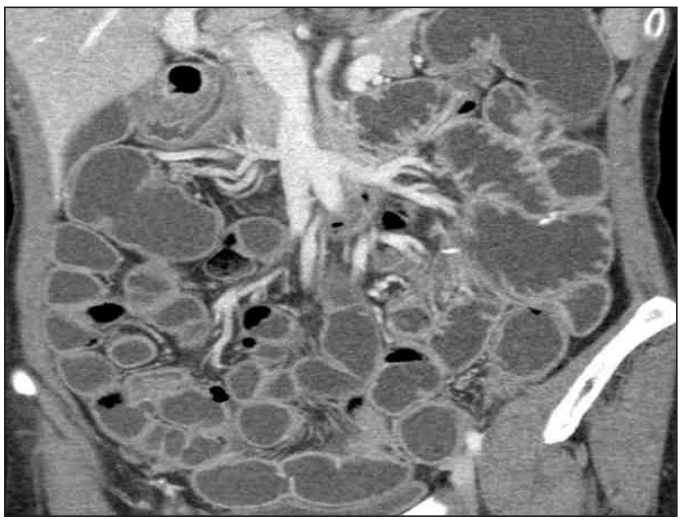

Figure 1: Normal CT enteroclysis showing good distention of small bowel loops in coronal reformation. Normal enhancement of bowel and mesenteric vessels are seen.

bowel wall, (b) it allows examination of deep ileal loops in the pelvis without superimposition, and (c) it permits evaluation of the surrounding mesentery and surrounding fat. CT enteroclysis also allows assessment of solid organs and provides a global overview of the abdomen. The disadvantages include radiation, discomfort secondary to intubation and abdominal distention.

Technique

The technical aspects of CT enteroclysis include intubation of 8Fr tube from nose which is negotiated down to duodenojejunal flexure under fluoroscopic guidance with a guide wire. This is followed by rapid injection of 0.5% methylcellulose of approximately 2 liters at a rate 75ml/min. Injection Buscopan 20mg iv usually administered just before scanning. This distends the small bowel well and after injection of IV non ionic contrast multislice CT examination is done through the abdomen and pelvis.

In those patients who can not tolerate intubation. CT enteroclysis can be performed in a tubeless method in which methyl cellulose has to be taken orally. The distention of bowel is relatively suboptimal; however it can still be useful.

Conclusion

CT enteroclysis is strongly proving its role in the evaluation of small bowel disease. Adequate luminal distention can usually be achieved with intubation and methyl cellulose injection. In the evaluation of diseases affecting the mucosa and bowel wall, CT enteroclysis is making loops more conspicuous with transparent enema so that the capacity to accurately determine the severity and extent of small bowel disease is greatly enhanced.

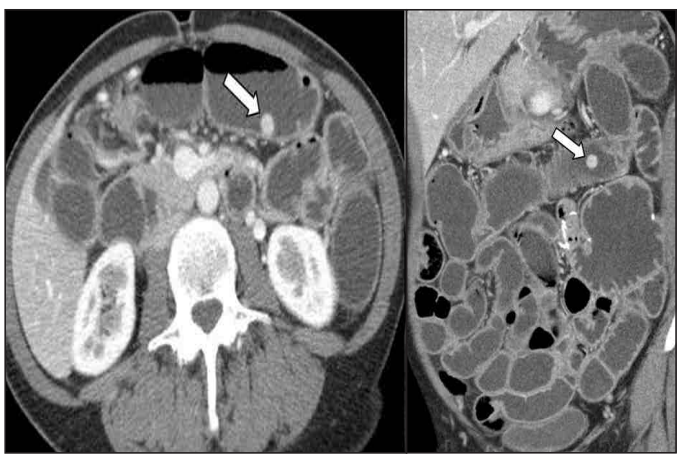

Figure 2: CT enterolysis is showing detection of small hypervascular polypoidal leison in proxinal small bowel

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CSF-DR: An Important Diagnostic Tool

Ms Roohi Noman, Haematology

Cerebrospinal fluid (CSF) is a clear fluid that circulates in the sub arachnoid space surrounding the spinal cord and brain. It cushions and protects the brain and spinal cord.
CSF collection can be a diagnostic test for many neurological disorders particularly infections and brain/spinal damage.

Formation of CSF

About 70% of CSF is formed in the ventricular choroid plexuses by a combined process of active transport and ultra filtration from plasma. 30% of CSF is formed by interstitial fluid elaborated within intercellular space of brain and spinal cord.

Rate of CSF formation in adults is about 500ml/day or 20ml/hour. Rate of formation is independent of pressure while rate of reabsorption is dependent on the pressure gradient between CSF and venous blood in the dural sinuses.

CSF concentration of some substances are regulated within narrow limits e.g., potassium, hydrogen, magnesium and calcium. Glucose, Urea and Creatinine diffuse freely and require several hours for equilibration. Proteins diffuse slowly across a concentration gradient from plasma to CSF, rate of diffusion decreases with increasing molecular size. Water and chloride diffuse rapidly across the blood CSF barrier.

Sample Collection

Lumbar puncture (spinal tap) is the most common means of collecting a specimen of CSF by inserting spinal needle usually between the 3rd and 4th lumbar vertebrae. Once the needle is properly positioned in the subarachnoid space, pressure can be measured and fluid can be collected for testing. Sample is usually collected in plain tube but preferably in the tube containing EDTA

Gross Examination

Appearance

Normally it is clear and comparable to water.

- **Cloudy CSF** may be due to leukocytosis, erythrocytosis, bacteria, fungi, amoebas.
- **Clot formation** due to traumatic tap, tuberculosis, meningitis
- **Xanthochromia** refers to pale yellow or orange color in the supernatant of centrifuged CSF. Xanthochromia may be due to
  1. Billirubin from lysed red cells.
  2. CSF protein level over 150 mg/dl.
  3. Oxyhemoglobin from lysed erythrocytes in CSF.

Pressure

Normal pressure is 50 to180 mm H_20. It increases with trauma or infection and decreases due to obstruction to the flow of CSF, shock, fainting, diabetes and coma.

Microscopy

CSF microscopy should be performed within two hours because 40% leukocytes may lyse after that. Manual cell count is performed using Neubaur's chamber. If the WBC count is more than 5, then differential leukocyte count should be performed through microscopy by staining slide with Leishmann's stain. WBCs increase in acute meningitis, acute infection, tumor, abscess, brain infarction. Normally red blood cells are not present in CSF and the finding of red blood cells may indicate a hemorrhage.

Chemical Analysis

CSF total protein, glucose and chloride ion are analyzed by automated method which has become popular and shown good results.

The normal CSF glucose level is 40 to 80 mg/dl. Glucose enters into CSF from plasma. Elevated glucose level is an evidence of hyperglycemia while decreased level is due to impairment of transport, tuberculosis, viral or amebic meningitis.

Total protein in CSF is 15 to 45 mg/dl. Hypercelluler CSF shows increased level of proteins as seen in some types of meningitis, blood in CSF, diabetes mellitus, polyneuritis and tumor while decreased protein level occurs due to rapid CSF production. Gamma globulin is the type of protein which increases in CSF in multiple sclerosis.

Chloride and other chemical tests help to differentiate disorders that affect the nerves such as poliomyelitis.

Microbiological Studies

In bacterial meningitis, the most valuable examination is the gram's staining. Reported sensitivity of this procedure is about 70 to 80% in the identification of pus cells or presence of microorganisms such as bacteria, viruses, tuberculosis, and fungal meningitis.

CSF fluid Findings in Disease States

Lab Finding	Appearance	WBC'S	Total Protein	Glucose (mg/dl)
Normal	Clear	0-5 lymph	15-45	40-80
Bacterial	Cloudy, Large clot	>500PMN	+++	Reduced
Viral	Clear, No clot	10-200 lymph	++	0
T.B	Slightly cloudy	200-500 lymph	+++	Reduced
Fungal	Clear, Not cloudy	0-5 lymph	0	Reduced
Brain Tumor	Clear, No clot	0-5 lymph	+	+
CSF Haem-morhage	Xanthochromia	0-5 lymph	+++	Reduced

Lymph: Lymphocytes, PMN: Polymorphonuclear neutrophils, T. B: Tuberculosis

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G6PD Quantification

Ms Shamina Sadaf, Haematology

Glucose-6-phosphate dehydrogenase or G6PD is one of the many enzymes that affect red cell metabolism. G6PD catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6-phosphate to 6 phosphogluconate and reducing NADP to NADPH (figure 1).

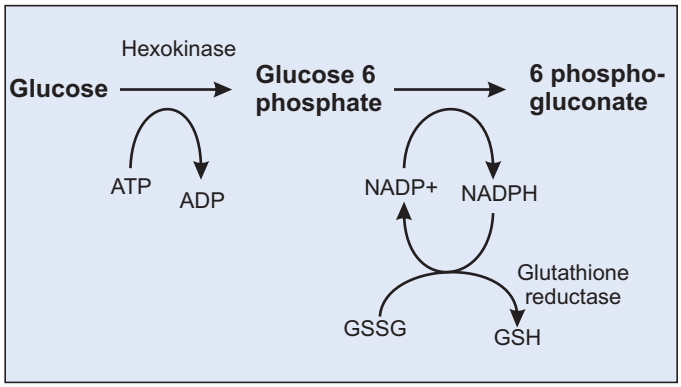

Figure 1: Reactions showing generation of reduced glutathione

NADPH then provides the reducing power that converts oxidized glutathione to reduced glutathione. Reduced glutathione so generated protects against oxidant injury by catalyzing the breakdown of oxidant compounds.

G6PD Deficiency

G6PD deficiency is an inherited X-linked disorder that occurs with increased frequency throughout Africa, Asia, Mediterranean region, and Middle East. Researchers have found evidence that the parasite which causes malaria does not survive well in G6PD deficient cells.

G6PD deficiency is characterized by variable degrees of jaundice and anemia which occurs by exposure to agents that can lead to oxidative stress and damage; particularly infections, certain drugs (table 1) or ingestion of fava beans. The four syndromes associated with G6PD deficiency include drug induced acute intravascular hemolysis, favism, chronic non spherocytic hemolytic anemia and neonatal jaundice.

- Antimalarials e.g Primaquine, Fansidar, Chloroquine
- Sulphonamides and sulphones
- Antibacterial agents e.g. nitrofurantoin, chloramphenicol
- Analgesics e.g Phenacetin
- Vitamin K Analogs
- Naphthalene balls

Table 1: Drugs commonly associated with haemolytic anaemia in G6PD deficiency

Peripheral blood film is characterized by the presence of blister cells and bite cells as is shown in figure 2.

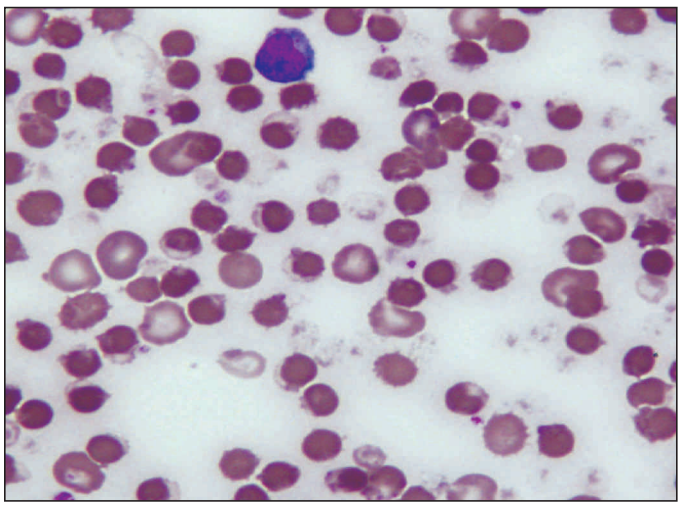

Figure 2: Peripheral blood film (Mag x 40) showing blister cells

Principle

The rate of formation of NADPH is proportional to the G6PD activity and is measured spectrophotometrically as an increase in absorbance at 340nm.

The enzyme activity below 2.5U/g hemoglobin is compatible with profound deficiency and activity between 2.5 and 6.0 U/g hemoglobin is consistent with partial deficiency.

Importance of G6PD Quantification

The diagnosis of G6PD deficiency is usually made by a rapid qualitative fluorescent spot test. The test is positive if the blood spot fails to fluoresce under ultraviolet light. However this qualitative test has limitations. When the residual enzyme activity is more than 20%, the individual will be classified as normal by qualitative assay. This is important in heterozygous females who can have enzyme activity between 20 to 60% and have a risk of hematological problems from exposure to oxidizing agents. Quantification of G6PD is important to resolve this problem.

In patients with acute haemolysis testing for G6PD deficiency may be falsely negative because older erythrocytes with enzyme deficiency have been haemolyzed and young erythrocytes and reticulocytes have normal or near-normal enzyme activity. So it is advisable that the testing for G6PD should be delayed until the hemolytic episode has resolved.

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Quality Assurance

Ms Lubna Khaleeq, Haematology

Quality assurance is a comprehensive set of policies, procedures and practice used to monitor the laboratory's entire testing process so that the results provided to the patients are reliable. The key to achieve this reliability is precision and accuracy. Accuracy refers to the closeness of the estimated value to that considered to be true while precision refers to the reproducibility of a result.

A quality assurance program includes internal quality control, external quality assessment and standardization. Quality assurance also ensures adequate control of pre analytic and post analytic stages. Pre analytic stage includes proper storage and transportation of the sample to the concerned laboratory while the timely reporting of an informative and reader friendly report is included in the post analytic stage.

Internal Quality Assurance

This program is developed according to the requirements of a specific laboratory. Both in house and commercially prepared controls are used. These controls are analyzed along with the patient's specimens and the data are then plotted on a control chart known as Levy Jennings chart (LJ chart) as shown in figure. The mean value and standard deviation (SD) of these controls are calculated. In LJ chart, mean is represented by a horizontal line and +2 SD and -2 SD are drawn above and below the mean line. If the test is satisfactory, these control values will revolve around the mean and less then 5 % of the results will fall out side 2 SD.

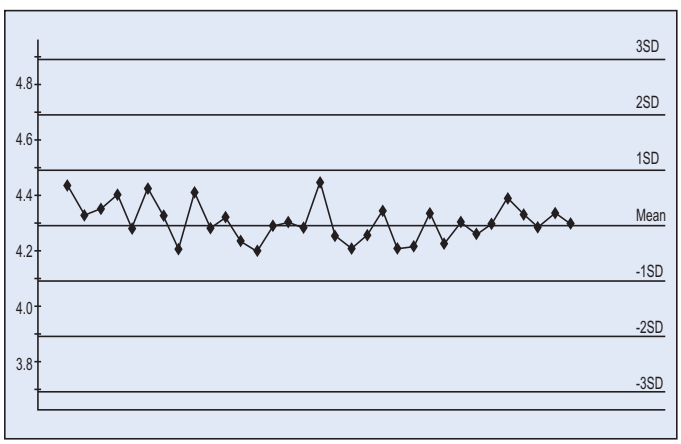

Figure: LJ chart of RBC count showing results which revolve around the mean

External Quality Assessment (EQA)

EQA acts as a complement to internal quality control. The objective is to achieve inter laboratory harmony and comparability.

EQA is the evaluation of the performance of laboratory tests by an outside agency on specially supplied samples. There are different external quality assessment agencies like CAP, NEQAS, etc. Samples are sent form these agencies at regular intervals and after analyzing, results are sent back to these agencies. The results are then interpreted. A laboratory can compare its performance in the survey with that of other laboratories and with its own previous performance from the deviation index or Z-score.

Deviation index (DI) is calculated as the difference between the individual laboratory result and the median or mean relative to the standard deviation. A DI score of less than 0.5 denotes excellent performance, a score between 0.5 and 1.0 is satisfactory, and a score between 1.0 and 2.0 is acceptable. A score above 2.0 suggests that the analyzer calibration should be checked while a score above 3.0 indicates serious defect requiring attention.

Standardization

Standardization refers to both materials and methods. A reference material or standard material is used to calibrate analytic instruments and to assign a quantitative value to calibrators. In reference materials, controls and calibrators are included. Controls can be used for internal or external quality control as they have values which fall in specific range while calibrators are international standards which have fixed values and they are used for calibrating the instruments. Reference method is a defined technique which provides accurate and precise data by which the validity of other methods can be assessed.

Quality is never due to an accident; it is the result of concerted planned activities. The objective of quality assurance is to control every variable that could possibly affect the quality of the test results.

Clinical Laboratory Continuing Medical Education (CME) Seminars

Reported by Ms Seema Vaqar,

In May 2008, Clinical Laboratory arranged its Continuing Medical Education Seminars at Quetta and Larkana for updating health care professionals with the current trends in laboratory medicine.

The CME in Quetta was held on May 10th which was attended by clinicians and pathologists of the city. Dr Farooq Ghani, Associate Professor and Consultant Chemical Pathologist at AKU welcomed the guests. In his presentation, he highlighted the "Role of BNP (B-Type Natriuretic Peptide) in diagnosis and management of heart Failure".

Dr Shahid Pervez, Professor and Head, Section of Histopathology, delivered his talk titled "Critical role of histopathologists as diagnostician and prognostician in the management of cancer." He highlighted that besides the indispensible role of histopathologists in the diagnosis of cancer, a new role as prognostician is also becoming important as newer targeted cancer therapies are being introduced and pathologists have to play a major role to identify cases where these therapies will most benefit. Some recent examples include Her-2/neu status in breast cancer for Tamoxifen therapy and CD 20 status in patients of B-cell Lymphoma for Rituximeb (Anti-CD20) therapy.

CME audience at Quetta

In addition to the speakers from AKUH, Dr Niala Ehsan, Professor of Gynaecology and Obstetrics, Bolan Medical College gave presentation on anaemia in pregnancy and its management and Dr Ghulam Haider Khalid, Professor and Head of Medicine Department, Bolan Medical College, presented on "Current concepts in the management of hepatitis."

The CME in Larkana was held on May 20th at Chandka Medical College. It was heavily attended by consultants, general practitioners, trainee doctors and laboratory officials, belonging to Larkana, Shahdad Kot, Shikarpur and other peripheral towns. Professor Sikander Ali Shaikh, Principle, Chandka Medical College, offered the welcome address. Assistant Professor Riaz Shaikh of Chandka Medical College introduced the speakers to the audience.

Dr Abrar Barakzai, Senior Instructor from Histopathology at AKU, elaborated on the "Role of histopathologists in cancer management: A Science behind the cure." He stressed on a close liaison between histopathologists and surgeons in order to assess the degree and depth of invasion of the tumor in addition to providing a diagnosis. In Lymphomas, it is the duty of the histopathologist to completely classify them either as precursor B-cell or precursor T-cell lymphomas or mature B or T cell type which helps in the treatment of the patients. The patients who have CD20 positive status in B-cell NHL are eligible to get Anti-CD20 therapy (Rituximab). He also discussed the role of immunohistochemistry in the diagnosis as well as prognosis of tumours. In breast cancers, estrogen and progesterone receptors positive patients receive Tamoxifen treatment and Her-2/neu(c-ErbB-2) positive patients get treated by Trastuzumab (Herceptin) which is also a prognostic marker Dr Salman Arain, Senior Pathologist and Consultant Haematologist, Hyderabad STAT lab, focused on basic techniques applied in "Interpretation of haematogram." He briefly talked about factors affecting the CBC interpretation like preanalytical, analytical and post analytical factors

Dr Afia Zafar delivering lecture at Chandka Medical College Larkana

Dr Afia Zafar, Associate professor and consultant Microbiologist at AKU, talked about "Diagnosis of Hepatitis B, C and D and interpretation of serological and PCR Tests." She discussed various markers important for the diagnosis of acute and chronic Hepatitis. She also highlighted the role of qualitative and quantitative PCR in the management of and care of hepatitis.

Dr Salman Arain & Dr Abrar Barakzai among audience at Larkana CME

Dr Hakim Ali Abro, Assistant Professor and Consultant, CMC, Larkana, enlightened the guest about various treatment regimes in the "Management of chronic hepatitis C"

Vice Principal, Chandka Medical College, Sikander Ali Shaikh presented the vote of thanks.

Both the seminars were a great success. The audience took keen interest with vibrant question answer sessions at the end.

Meeting Reports

Fifteenth International Society of Radiographers and Radiological Technologists World Congress

Reported by Ms Nida Husain, Assistant Manager, Radiology

Extremely successful "15th International Society of Radiographers and Radiological Technologists World Congress" was held at the International Conventional Center Durban, South Africa from the 24th to the 27th April 2008. Mr Habib Khan, Ms Nida Husain and Dr Farhan Ahmed from the Department of Radiology attended this meeting.

The "International Society of Radiographers and Radiological Technologists" was formed in 1962 and currently there are 80 member societies. The objectives of the Congress were to assist the education of radiographers and to support the development of medical radiation technology world wide.

Choir at the inaugural ceremony of the 15th International Society of Radiographers and Radiological Technologists World Congress

Core values of Radiology are to attain the highest level of achievable standards of professionalism, excellence and dedication. Our vision is to be the leading international organization offering structured Radiographers training programme with international standards of academic and clinical practices. Our plan is to liaise with international organizations for human development, quality patient care, education and research.

Keeping in view the vision of Radiology Department to promote exchange of information and enhance the learning curve of the Radiographers; a meeting

was organized with the key members; Mr Charles A. Shields, CEO, Canadian Association of Medical Radiation Technologists (CAMRT); Ms Meena Amlani and Ms Rida, British Columbia Institute of Technology (BCIT); and Ms Cynthia Cowling, Director of Education International Society of Radiographers and Radiological Technologists.

Left to Right: Row 1: Mr. Charles A. Shields, Ms Rida, Ms Meena Amlani, Row 2: Ms Fiona, Ms Nida Husain, Mr. Muhammad Habib Khan Row 3: Ms Cynthia Collins, Dr Farhan Ahmad

The objectives of the meeting were:

1. To explore options for getting an international accreditation of the Radiographers Training Programme at Department of Radiology, Aga Khan University.
2. Review of the existing curriculum and course outlines. To ensure proper dissemination of knowledge and experiences in Radiological sciences amongst radiographers and radiologists and other medical professionals.
3. To advance the science of radiography by the promotion of improved standards of education and research and to enhance the knowledge base of radiographers in techniques and patient care approaches.
4. To act as a catalyst for participants to come up with a programme for continuous professional development and enhance standards in education and training of radiography services which, in turn, will add value to the existing radiography programme.

The meeting was very productive and it was concluded that core members of the Advisory Committee of the Radiographers Training Programme and members of ISRRT, BCIT and CAMRT will work together to review and develop necessary documents for Radiology to pursue its accreditation.

Pakistan Society of Haematology Meeting

Dr Adnan Qureshi, Haematology

The tenth annual conference of Pakistan Society of Haematology (PSH) was held at the Pakistan Institute of Medical Sciences (PIMS), Islamabad from 14th to 16th March 2008.

The pre-conference workshops covered the various domains of haematology and included the updated knowledge of investigation of bleeding & coagulation disorders, diagnosis of genetic haemoglobin disorders, blood & bone marrow morphology, and basic & advanced blood banking techniques.

The conference was inaugurated on the eve of March 14, followed by the Ibn-e-Sina Lecture delivered by Brig. Dr. Suhaib Ahmed on 'Molecular Haematology – The Pakistani Perspective'. In his talk, he highlighted the advances made in the rapidly growing field of molecular haematology and emphasized upon the importance and utility of molecular techniques in diagnostic and therapeutic arenas with relevance to common haematological disorders in Pakistan.

Dr. Salman Adil delivering a talk on Autologous stem cell transplantation

The second and third days of the conference included seminars on bone marrow failure syndrome, blood transfusion, thalassemia and stem

cell transplantation. Eminent hematologists from all over Pakistan presented plenary and state-of-the-art lectures. Educational lectures which covered a range of topics from hematology and transfusion medicine were delivered by the guest speakers, especially for the benefit of post-graduate trainees. These forums saw wide participation from the audience and generated some exciting discussions.

Two parallel symposia also took place on second and third day of the conference which included the oral and poster presentations chosen by peer reviewers from abstracts submitted prior to the meeting. An exhibition of equipments, soft wares, consumables and other supplies of the latest hematology, coagulation and blood transfusion products were also displayed during the conference. On the eve of March 15, a banquet dinner was held followed by a cultural program. The conference ended on March 16.

With the expansion of laboratory and clinical haematology services in the country, PSH conferences now see greater participation not only from haematologists but also from other mainstream medical specialists. The conference promoted

Dr Bushra Moiz co-chaired session on "Thalassemia" in PSH Conference 2008

awareness and knowledge of new developments and incorporated evaluation of the current literature and changing practices in the field of hematology. This year's meeting seemed to be a great success and provided relevant and convenient quality learning opportunities to haematologists in Pakistan and continued the tradition of excellent medical education. PSH is committed to promote and foster the exchange of information relating to haematology, coagulation & blood transfusion and is aimed to serve as a forum for bringing together haematologists across the Pakistan.

AKU Delegate attended PSH conference 2008 at Islamabad

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