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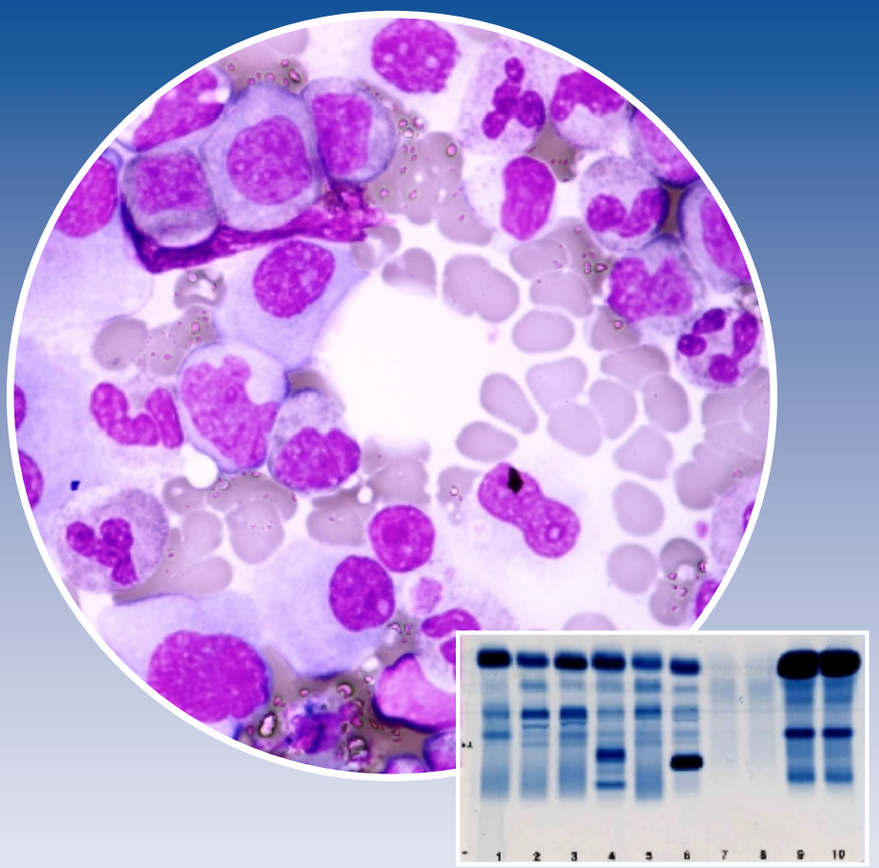

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Contents Page No.

Transcription Mediated Amplification for the Diagnosis of HCV RNA

Ms Saima Javed,
Technologist, Molecular Pathology

What is TMA?

TMA, Transcription Mediated amplification is an isothermal (single temperature), autocatalytic (self starting) nucleic acid target amplification system that can produce more than a billion RNA copies of target DNA or RNA in an hour or less.

How TMA Detects HCV RNA?

TMA can detect HCV RNA in human plasma and serum. It has three main steps which are **Sample preparation, target amplification and amplicon detection**. Sample preparation involves detergent lysis of virus followed by hybridization of free viral nucleic acid probes oligonucleotide complimentary to the 5' untranslated region (conserved region) of HCV genome. An internal control is hybridized in the same manner. The hybridized targets are then captured on to magnetic particles which firmly hold captured targets and allow thorough washing to remove potential inhibitory substances.

TMA starts with the addition of primers, nucleotides, reverse transcriptase (RT) and RNA polymerase (RP). Figure 1 depicts TMA cycle.

Detection of Amplified Products

Identification of amplicons is accomplished by hybridization protection assay (HPA) and dual kinetic assay (DKA) as explained in figure 2. In HPA single stranded DNA probes are labelled with two different chemiluminescent acridinium ester (AE) molecules. One is specific for internal control RNA(IC RNA) and another is specific for HCV amplicon. AE molecules are chemically modified and have different kinetics for duration of light emission. AE molecules labelled probes bind specifically with amplicons and are protected from hydrolysis while non-specifically bound and unbound AE molecules are hydrolyzed after addition of selection reagent and then auto detect reagent is added which causes the protected AE molecules on annealed probes to emit chemiluminescent signals. The dual kinetic nature of these AE molecules results in shorter "flasher signal" and longer "glower signal". Light emission is measured and expressed numerically in relative light units (RLUs). Results are reported by TMA data reduction software.

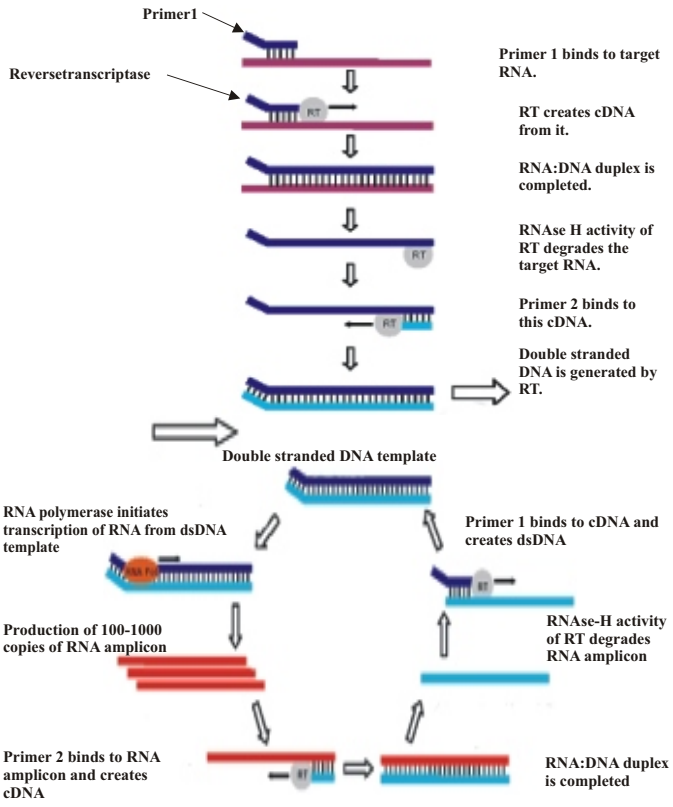


Figure1:
Steps Involved in Transcription Mediated Amplification

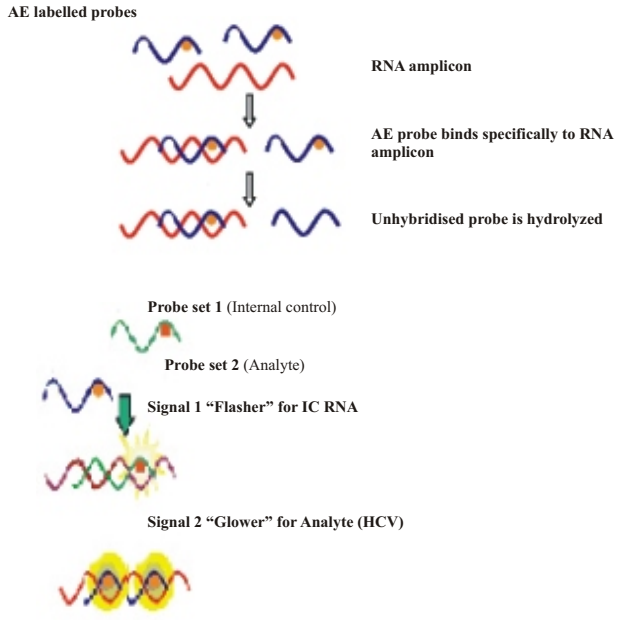


Figure 2:
Schematic Diagram of HPA and DKA

Result Interpretation

TMA Data Reduction Software calculates two cut offs. Analyte cut off for Analyte signal and IC cut off for IC signal. Sample is considered negative when analyte signal is less than the Analyte cut off (i.e.Analyte S/CO‹1) and an IC signal is greater than or equal to IC cut off. Sample result is considered positive when Analyte signal is greater than or equal to Analyte cut off (i.e. Analyte S/CO=1) and IC signal is less than or equal to 475,000 RLUs.

Advantages of TMA

- It is a single tube assay with few steps, which makes it fast.
- TMA produces RNA amplicons which are unstable in lab environment and are easily inactivated by deactivation fluid.
- HPA reaction eliminates washing steps which minimise the risk of carry over contamination.

Why TMA is Preferred Over PCR Based Assay?

- **TMA Does Not Require Special Equipments:** **TMA** is isothermal; a water bath or heat block is used instead of a thermal cycler.
- **TMA is Rapid: PCR** produces two copies per cycle whereas TMA produces 100-1000 of RNA amplicons per cycle that results in 10 billion fold increase within about 15-30 min.
- **TMA has higher sensitivity:** It has potential to detect less than 50 HCV RNA copies per ml and residual viremia while PCR based assay has lower detection limit of 100-1000 HCV RNA copies per ml and is not suitable for detecting residual viremia in HCV patient.

Conclusion

Amplification by TMA is relatively simple, rapid and extremely sensitive when compared with PCR. It is FDA approved and provides good clinical performance especially when detecting response to therapy for viral clearance. Hence, TMA can be incorporated in routine laboratory diagnosis of hepatitis C virus.

Vanillyl Mandelic Acid

Dr Sahar Iqbal
Resident, Chemical Pathology

Introduction

The catecholamines (Epinephrine and nor-epinephrine) are secreted from the Adrenal Medulla. Most of the catecholamines are metabolised by mono-amino oxidase and chtechol-o-methyl transferase (COMT) and produce major urinary metabolite vanillyl mandelic acid (VMA). The others metabolites are metanephrin and nor-metanephrin.

Pheochromocytoma is the catecholamine producing tumour of adrenal medulla, which can be diagnosed by assessing the urinary level of VMA. In addition, neuroblastoma, a common malignant tumour of childhood, can also be diagnosed by urinary VMA. About 75 per cent of the patients with neuroblastoma show increased urinary level of VMA, however in healthy children at least up to age of 15 years urinary VMA and metanephrin tend to be high and variable than adults.

Significance

Urinary VMA measurements are commonly used to screen for pheochromocytoma. It is more specific but less sensitive than metanephrine measurement. It helps in early diagnosis of pheochromocytoma, as undiagnosed pheochromocytoma is a potentially lethal condition.

Collection and Storage

Patient is instructed to collect a 24 hours urine specimen. VMA is stable at room temperature upto five days. Hydrochloric acid is used to preserve the specimen for longer than five days before analysis.

Reference Range

In adults, reference value for VMA in 24 hours urine collected specimen is approximately 7.0 mg (range 1.9 - 9.8 mg/24 hour). False positive results (more than reference range) may be seen in the patients taking salicylate, caffeine, phenothiazines and antihypertensive agents. Coffee, tea, chocolate, fruit (bananas) and vanilla containing substances should be avoided for 72 hours prior to collection.

Diagnosis of *Pneumocystis jiroveci* Infections in Microbiology Laboratory

Dr Summiya Nizamuddin
Resident, Microbiology

Introduction

Pneumocystis jiroveci previously known as *Pneumocystis carinii* is one of the most common opportunistic pathogens in HIV infected patients. Currently it is classified as a fungus, with a tropism for growth on respiratory surfaces leading to pneumonia. The organism is found in three distinct morphological stages. The trophozoite or trophic form, the sporozoite, and cyst, which contains intracystic bodies also known as spores.

Diagnosis

P jiroveci cannot be propagated continuously in host cell free culture systems. Hence, its diagnosis at present is dependent on the morphological identification of the organisms on microscopy. Various respiratory specimens (sputum, induced sputum, tracheal aspirate fluid, bronchoalveolar lavage (BAL) fluid, tissue obtained by transbronchial biopsy, cellular material obtained by bronchial brush, pleural fluid, and tissue obtained by open thorax lung biopsy) could be sent to laboratory for detection.

The diagnostic yield of the various specimens is critically dependent on the underlying diseases of the patient, the expertise of the staff evaluating the patient and obtaining the specimen, and the expertise of the laboratory staff for processing and examining the specimen. Generally, the more invasive the procedure, the better is the diagnostic yield which ranges from 50-90 per cent for induced sputum to >90 per cent for BAL. Moreover sensitivities are also higher in AIDS patients than in other immunocompromised patients owing to the higher burden of these organisms.

By use of rapid processing and staining techniques, a laboratory diagnosis of PCP can usually be made within a few hours.

Stains

A variety of stains have been used for the identification of Pneumocystis. Commonly used fungal stains are periodic acid Schiff (PAS), toluidine blue, calcofluor, Giemsa, toluidine blue, Gomori methenamine silver, immunofluorescent and Gram-Weigert. All these methods are highly efficient in the hands of experienced microscopists.

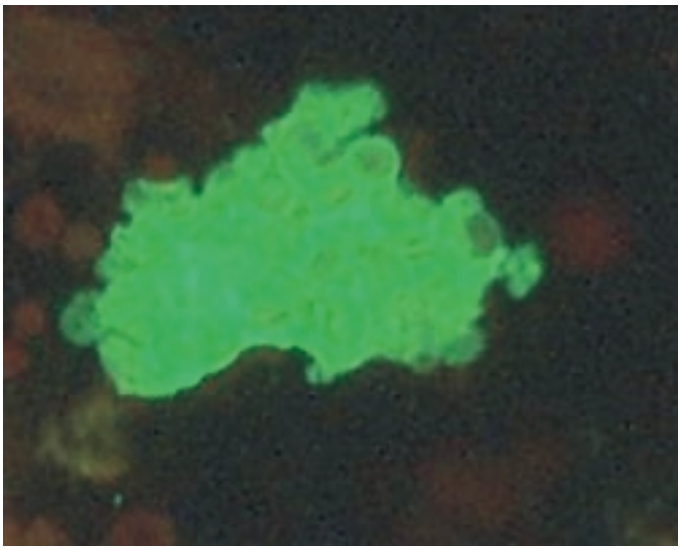
One of the most widely used methods is immunofluorescence using direct fluorescein conjugated monoclonal anti Pneumocystis antibodies which stains and fluoresces the walls of the trophic forms and cysts, apple green. This technique is available at the Department of Microbiology, AKUH.

Other Diagnostic Modalities

Serological assays detecting Pneumocystis antibodies and antigens have not been clinically useful. PCR based methods, although more sensitive are costly, labour intensive and are not widely available.

Conclusion

Pneumocystosis should be considered in any immunocompromised patient presenting with fever, respiratory symptoms and an abnormal chest radiograph. Current microscopy based methods have produced results concordant with clinical diagnosis. They therefore remain as a mainstay for diagnosis.



Stain : immunofluorescence test (IFA)
Magnification: x 400
Round to oval shaped cysts and extracellular matrix seen.
Trophozoites and sporozoites may be embedded in extracellular matrix and fluoresce apple green.

Laboratory Diagnosis of Metabolic Bone Diseases

Dr Aysha Habib Khan
Assistant Professor and Consultant Chemical Pathologist

The principal differential diagnosis in patients presenting with bone fractures, bone pain and reduced bone mineral density is osteoporosis versus osteomalacia. This distinction is made from the history, physical examination and a combination of laboratory and radiological studies.

For an uncomplicated patient with osteomalacia and osteoporosis, a lab workup includes serum calcium, phosphate, creatinine, magnesium, alkaline phosphatase, vitamin D (25OHD) Parathyroid hormone (PHT), Thyroid stimulating hormone (TSH) and 24-hour urine calcium. Males should have testosterone measured.

The main purpose of laboratory tests is to check for abnormalities of calcium/phosphate metabolism and secondary causes of osteoporosis such as renal or hepatic failure, acidosis and hypercalciuria. Secondary causes of osteoporosis often go undetected in part because of the lack of specific guidelines for laboratory evaluation in newly diagnosed cases.

Measurement of serum total calcium and phosphate concentrations, although readily available tests; are of limited value in the assessment of osteoporosis. However, low concentrations of serum phosphate and calcium are indicative of osteomalacia. Alkaline phosphatase is an inexpensive method of checking for osteoblastic activity. The 24-hour urine calcium measurement is frequently ignored but it is a valuable and inexpensive test. High levels are seen in idiopathic hypercalciuria, and low levels suggest malabsorption, vitamin D deficiency, etc. The test should be done on a patient's customary calcium intake.

The measurement of vitamin D is becoming increasingly important in the management of patients with various disorders of calcium metabolism associated with rickets and osteomalacia, neonatal hypocalcaemia, pregnancy, nutritional and renal osteodystrophy, hypoparathyroidism and hyperparathyroidism and osteoporosis.

Mild vitamin D deficiency frequently occurs in the absence of hypocalcaemia, especially in Asians. Secondary hyperparathyroidism may occur with normal calcium; most of these cases are detected by low calcium, low 25OHD levels or decreased renal function. In patients with abnormal serum calcium or with unusually severe bone disease, the 25-OH D and PTH levels should always be measured. The 25 OH D provides more useful information than the 1, 25 $(OH)_2$ vitamin D level.

Protein electrophoresis should be done whenever a patient presents with new fractures. Corticosteroid excess that causes osteoporosis can usually be detected clinically by cushingoid features. A urine cortisol can be helpful in puzzling cases.

Gonadal hormones are very important causes of osteoporosis. In females who are postmenopausal, it is not helpful to measure levels of estrogens or gonadotropins. In males, however, testosterone levels should be measured because there is much greater variability in the prevalence of hypogonadism. Also, men may have low testosterone without other clinical symptoms. If testosterone is low, then further work-up is needed.

In conditions where renal wasting of phosphate is in question, then measurement of urinary phosphate excretion should be helpful. Phosphate excretion is measured by calculation of the fractional excretion of filtered phosphate from a random urine specimen by measuring serum and urinary phosphate and serum and urinary creatinine.

To conclude, laboratory testing plays an important role in the early diagnosis and management of metabolic bone diseases. It is important that appropriate investigations are requested after establishing a differential diagnosis. The relation of the lab results to the disease may be complex. At times, a close liaison between laboratory and physician provides a better judgment.

24 Hours Urinary Specimen Collection

Dr Ayaz Baig
Resident, Chemical Pathology

The urine specimen has been referred to as a liquid tissue biopsy of the urinary tract that is painlessly and easily obtained. Urine yields a great amount of valuable information quickly and economically, but as for all other human specimens used in laboratory, the urine must be carefully collected, preserved, and processed for the information to be regarded as reliable.

Types of Urine Collection

1. Random: these specimens usually do not require any preservative
2. Timed specimen: usually 24 hour sample is preferred for most of these specimens and this requires a suitable preservative

Methods for Preserving Urine Samples

Various methods of preserving urine are available, most of which inhibit the growth of bacteria, thus preventing many alterations from occurring. The best method is immediate refrigeration during and after collection. The specimen may be kept 6 to 8 hours under refrigeration with no gross alterations and without any preservative. There are several chemicals available for preservation.

Toluene is one chemical preservative which is in liquid form and inhibits growth of bacteria. It forms a thin layer which covers surface of urine. It is the best preservative as it does not interfere with various tests done in routine urinalysis.

Thymol, a crystalline substance, inhibits the growth of bacteria but it may interfere with protein and bilirubin estimation.

Formalin, a liquid preservative acts by fixing the formed elements in urine sediment, however it interferes with reduction tests for urine sugars and may form precipitate with urea that interfere with microscopic examination of sediment.

Boric acid is used to preserve creatinine, uric acid and proteins

Hydrochloric acid is used to preserve calcium, phosphate and oxalate

When a 24 hour sample is sent to the laboratory it should be ascertained that it has been properly collected. A preservative has been added at the beginning of collection and the correct collection time is used.

Instructions for the Patients

The patient is carefully instructed about details of collection process. The bladder is emptied at the start of collection (e.g. in the morning at 8:00 AM) and this time is noted on collection container. This urine is discarded and not put into container. All subsequent voiding are collected and put into container up to and including urine at 8 AM the following morning. This urine specimen will complete the 24 hours collection.

At AKUH Clinical Laboratories

At Clinical Laboratories and all its collection centres, patient information regarding collection of urine is available. In addition, bottles for 24 hour urine collection are also available at the receptions. These bottles do not contain any preservative.

Genetic Analysis of Duchenne Muscular Dystrophy

Ms Asma Hanif
Assistant Technologist, Molecular Pathology

Duchenne Muscular Dystrophy (DMD) was first described in 1986 by Guillaume Benjamin Amand Duchenne.

What is Dystrophy?

Dystrophy is basically a group of disorders that affect body muscles. DMD occurs when a particular gene on X chromosome fails to encode correct message for a protein known as dystrophin. The gene is located on Xp21 and has 79 exons spanning 2.5 megabases. It produces mRNA of 14.6 kilobases and a protein of over 3500 amino acid residues. Dystrophin connects cytoskeleton of muscle fibers to the surrounding extracellular matrix through the cell membrane. Dystrophin associated muscular dystrophies range from milder Becker muscular dystrophy to severe DMD. Almost 70 per cent of the cases occur because of inheriting a defective gene while 30 per cent result from new mutations. Although the majority of deletion mutations are found at 3' end, the point mutation can occur along entire gene. Point mutations create stop codons that produce severely reduced or absent levels of dystrophin which links actin filaments

to a complex of transmembrane proteins and hence to the extracellular matrix (Figure 1).

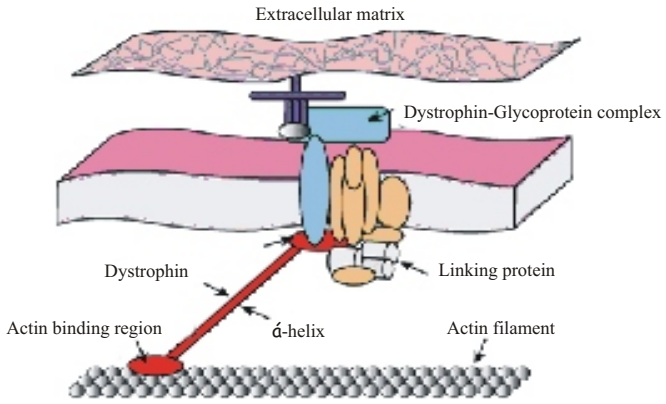


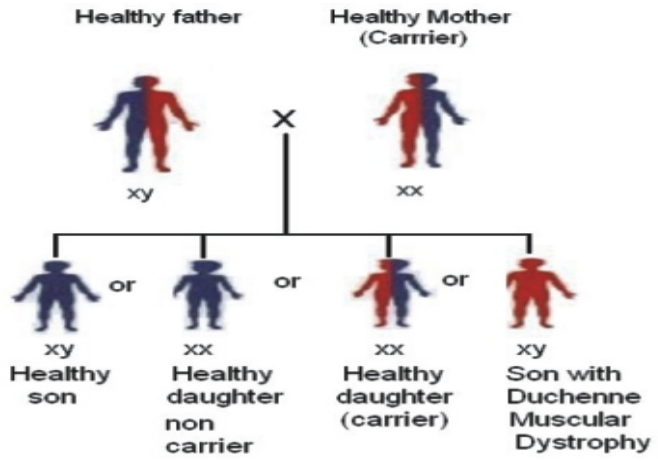
Figure: 1

Symptoms

The symptoms of the disease become apparent between three to five years of age resulting in enlargement of calf muscles, weakness of arm that progressively loses strength in shoulders. In DMD, creatine kinase leaks out of the muscle fibres and is therefore found in greatly increased amounts in the serum. In young boys with DMD, the serum creatinine kinase level is at least five times as high as the maximum for unaffected people and sometimes it is 50 to 100 times as high. As the disease progresses, failure of heart muscles and malfunction of organ systems ultimately leads to death.

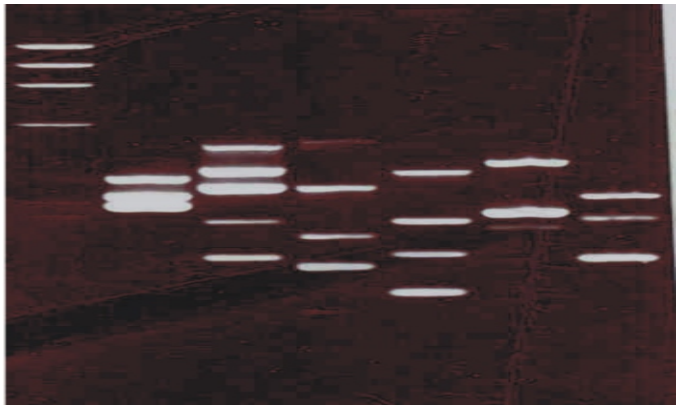
Inheritance

Male offspring have a 50:50 chance of inheriting the affected chromosome and thereby developing DMD as it is an X-linked recessive disorder (Figure 2). Females tend to be mildly affected compared to males because females have a second X chromosome to compensate for the defective gene.



Diagnosis

DNA is isolated from peripheral blood leukocytes using standard salting out procedure. Polymerase chain reaction assay of dystrophin gene is carried out using primers specific for various exons and promoter region. PCR products are then separated on an agarose gel containing ethidium bromide and bands are visualised under UV light. Dystrophin gene is amplified in 6 sets of PCR reactions. Each set includes 3-5 exons; details of exons are listed in the following table.



MWM 1 2 3 4 5 6

Figure 3:
Results of gel electrophoresis in a patient with DMD

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
45	48	Pm	3	49	PB
19	17	43	50	16	41
51	8	13	6	32	42
42	44	47	60	34	
46	4	52			

Figure3: Indicate deletion of exons 45 and 46 in lane 1, exon 52 in lane 3 and 34 in lane 6 or 5 thus confirming the diagnosis of DMD.
MWM=Molecular Weight Marker 100bp.

Conclusion

Multiplex PCR is a useful assay for the diagnosis of deletion in DMD gene, when compared with Southern blotting it is more sensitive and rapid. Presently, preventive measures or treatment is not available for these disorders but molecular techniques such as multiplex PCR can be used for their early identification and better management.

Hybrid of Cystic Adenomatoid Malformation with Pulmonary Sequestration

Ms Fatima Mubarik, Naila Nadeem, Zishan Haider, Gulnaz Shafqat, Shaista Afzal,
Radiology

Abstract

This is a case report of full term baby boy who was antenatally diagnosed of having an echogenic lung mass. The neonate under went CT scan and consequently thoracotomy and was found to have hybrid form of cystic adenomatoid malformation (CCAM). The case report reminds us that cystic adenomatoid malformation and pulmonary sequestration are included in spectrum of bronchopulmonary foregut malformation and if we detect one pathology we should look for other pathologies as these can coexist.

Introduction

CCAM and pulmonary sequestration are congenital lesions that have distinct embryology, pathology and natural history. Seventeen cases of intralobar pulmonary sequestration with CCAM and 19 cases of extra lobar pulmonary sequestration with CCAM have been described since 1949(1).

Case Report

Patient was a baby boy born through emergency lower segment caesarian section due to foetal distress at 40th week of gestation. Birth weight of 3.3 kg, Apgar score was 8 at 0/min, baby was tachypenic with respiratory rate of 80/min and had sub costal recession.

Antenatal ultrasound showed echogenic lung mass with cystic changes in left chest, it was interpreted as adenoid cystic malformation. On postnatal examination, child was found to have displaced apex beat, chest X-ray revealed air space shadowing with cystic changes in left lower lobe with contra lateral mediastinal shift.

Enhanced CT chest confirmed X-ray findings. In addition, an aberrant vessel from thoracic aorta directed toward affected region was visualised. By virtue of its position and appearance, diagnosis of sequestered lung was suggested. On third postnatal day, posteriolateral thoracotomy through 5th intercostals space was carried out, intralobar pulmonary sequestration was found in left lower lobe with aberrant blood supply from descending thoracic aorta.

Sample was sent for histopathology. Features were consistent with congenital cystic adenomatoid malformation type II (multiple small cysts < 1cm in diameter). Child recovered well after surgery and was discharged.

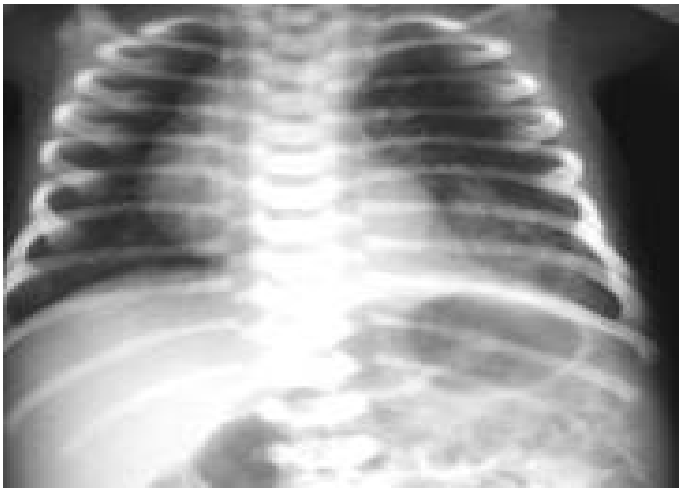


Figure 1:
Chest X-ray Showing Increased Density with Cystic Changes in the Left Lower Lung Zone.

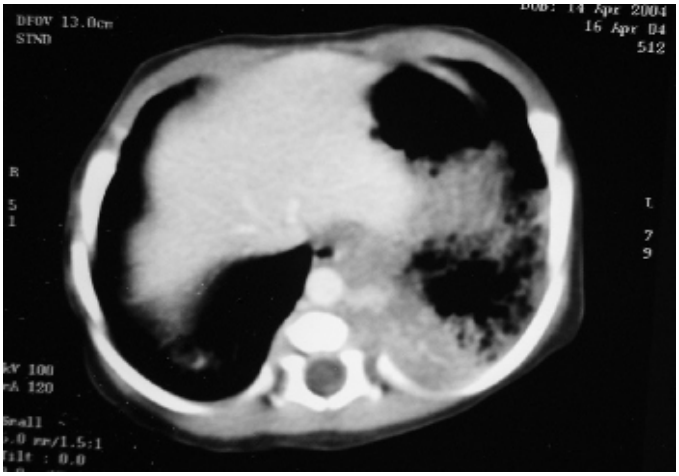


Figure 2:
CT Scan Chest Lung Parenchyma Producing a Solid Density in the Posteromedial Segment of Left Lower Lung

Discussion

Pulmonary sequestration are regions of lung parenchyma which lack normal connection to tracheobronchial tree and possess an anomalous systemic blood supply usually from aorta or its major branches (2). Its incidence is 0.15per cent -1.7per cent in general population. It was first described by Rokitansky and Rektorzik in 1861 (1).

There are two types of sequestration, intralobar and extralobar sequestration (3). CCAM is a developmental hamartomatous abnormality of lung with adenomatoid proliferation of cysts resembling bronchioles (4). Chin and Tang first described CCAM as a distinct entity in 1949 (5). CCAM represent approximately 25 per cent of all congenital lung lesions. CCAM communicate with bronchial tree and derive their blood supply from pulmonary circulation. The review of hybrid lesions by Cass et al suggested that prognosis in hybrid CAMS appears to be more favourable than CAM without a systemic feeding vessel (6).

Intra abdominal extralobar pulmonary sequestration /CCAM seem to have a good prognosis. Complications such as respiratory distress, infection, intrathoracic bleeding, haemoptysis, cardiac failure can occur. The potential risk of malignancy necessitates surgical excision of this congenital pulmonary lesion (7).

Conclusion

Ultrasonic evaluation of lung lesion done antenatally with good postnatal imaging algorithm has a strong impact on patient management and prognosis in pulmonary sequestration /CCAM.

Reference

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How Can We Achieve Good Response From Trans-Arterial Chemo-Embolisation (TACE)?

Ishtiaq Ahmed Chishty, Zishan Haider, Dawar Khan, Tanveer ul Haq, Radiology

Viral hepatitis is becoming common in Pakistan. Hepatoma is a lethal sequelae of chronic liver disease. Hepatoma is among the leading causes of death worldwide as well. The prognosis of hepatoma is grim particularly when the tumour is unresectable or when orthotropic liver transplantation is contraindicated. This tumour receives most (90-100 per cent) of their supply from hepatic artery, therefore Transarterial Chemoembolisation (TACE) is the most common technique used to treat unresectable HCC. TACE can reduce viable tumour tissue and improves survival.

Patient selection for TACE is a difficult task, because treatment of hepatoma can be influenced by liver function, vascular invasion, tumour size and vascularity. Clinically, if the patient has massive ascites and encephalopathy, the response of TACE is poor. Inclusion and exclusion criteria for TACE are mentioned below:

Inclusion Criteria

- Unresectable tumors
- Patent portal vein
- Satisfactory Liver function (Normal alkaline phosphatase and aspartate transaminase level)
- Serum bilirubin level < 2mg/dl (34 micromol/l)
- No major contraindication to angiography (e.g. normal coagulation and renal function)

Exclusion Criteria

- Clinically apparent jaundice
- Hepatic encephalopathy
- Occluded portal vein
- Hepatofugal portal vein flow
- Extra-hepatic tumours
- Other medical conditions likely to be life threatening within three months
- Liver rupture or tumour invasion of liver capsule
- Poor liver function
- (Coagulopathy is not correctable with Vitamin K, Lactate dehydrogenase level >425 IU/L, elevated alkaline phosphate level)
- Serum bilirubin level > 5mg/dL (85ìmol/l)

- Biliary Obstruction
- Serum creatinine > 2mg/dl (177µmol/l)
- Haemoglobin Level < 8 g/dl (80 g/l)
- White blood cell count $< 2.5\times10^{3}/\mu l$ ($2.5\times10^{9}/l$)
- Platelet count $< 60\times10^{3}/\mu l$ ($60\times10^{9}/l$)
- Pregnancy
- Liver involvement > 50per cent
- Albumin < 35g/L
- Aspartate aminotransferase level >100 IU/L

In our institute, we are using Child-Pugh classification for the selection of patient for TACE. The Child-Pugh Classification system which relies on following biochemical and clinical criteria,

a. Bilirubin level
b. Albumin level
c. Prothrombin time
d. Degree of Ascites
e. Hepatic encephalopathy

These criteria have been successfully used for many years to provide an accurate reflection of patient's liver function. We do not perform procedure in Child-Pugh Class C patients. Table 1 is showing scoring system of Child-Pugh Classification.

Table 1: Child-Pugh Classification of Liver Function

Score	1	2	3
Ascites	Absent	Slight to moderate	Severe
Encephalopathy	Absent	Slight to moderate	Severe
Serum Albumin	>3.5 g/dl	2.8-3.8 g/dl	<2.8 g/dl
Serum Bilirubin	<2 mg/dl	2-3 mg/dl	>3 mg/dl
PT* Prolongation	<4 second	4-6 second	>6 second

*PT=Prothrombin Time
Score of 5-6 corresponds to Child-Pugh Class **A**
Score of 7-9 corresponds to Child-Pugh Class **B**
Score of 10-15 corresponds to Child-Pugh Class **C**

Portal vein occlusion is not an absolute contraindication for TACE. To achieve substantial tumour necrosis, delaying progression to liver failure, chemoembolisation is a well established procedure for patients with unresectable hepatoma. TACE is an effective way to improve survival and quality of life. We can achieve good response from trans-arterial chemoembolisation by proper selection of patients (Fig 1, 2, 3).

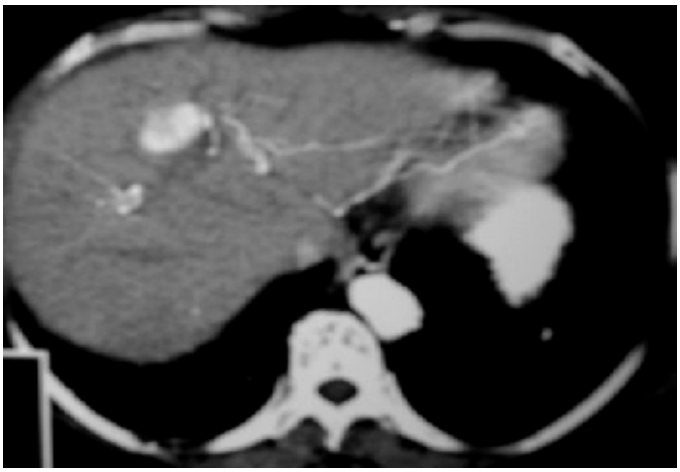

Figure1:
Pre-TACE contrast enhanced -CT scan shows enhancing hepatoma in segment IV of liver

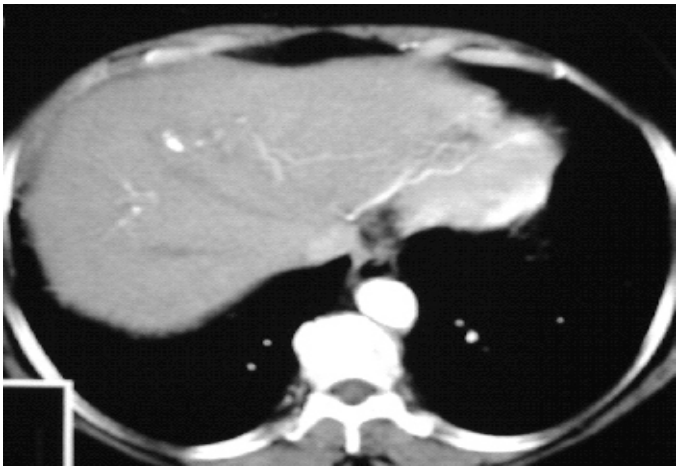

Figure2:
Same patient after three sessions of TACE: Contrast enhanced -CT scan is showing minimal residual vascularity in treated tumour

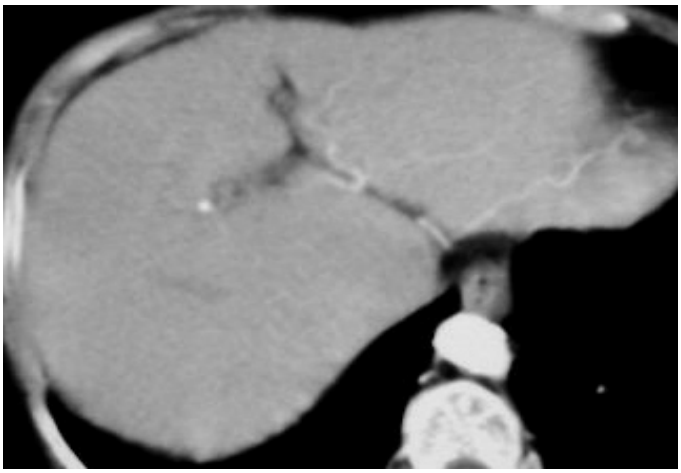

Figure3:
Same patient after three years: There is no appreciable tumour vascularity on contrast enhanced CT scan.

Anti-factor Xa Assay

Natasha Ali,
Resident, Haematology

Heparin is used widely for the prevention and treatment of thromboembolic diseases and is also utilised during procedures of haemodialysis and cardiopulmonary bypass surgeries. Two types of heparin preparations widely in use today are unfractionated heparin (UFH) and low molecular weight heparin (LMWH). The inconvenience and limited precision of monitoring of (UFH) therapy has contributed to the increasing use of LMWH preparations. The pharmacokinetic properties of LMWH are different from (UFH) and allows for easily monitored anticoagulant effects of a given dose. Monitoring is, however recommended in the following clinical settings like renal insufficiency, obesity, paediatric patients and those on prophylactic anticoagulant therapy in conditions like malignancy or antiphospholipid syndrome. Anti factor Xa assay is used to monitor LMWH therapy.

Principle of anti- factor Xa is based on the fact that both factor Xa and antithrombin III are added in excess amounts to the test plasma and the residual factor Xa activity is inversely proportional to the heparin concentration. It is assumed that the patient has a normal concentration of antithrombin III.

The test is performed by adding a known amount of factor Xa and antithrombin to the plasma of the patient. If heparin or LMWH is present in the patient plasma, it will bind to antithrombin and inhibit factor Xa. The amount of residual factor Xa is inversely proportional to the amount of heparin in the plasma. The amount of residual factor Xa is detected by adding a chromogenic substrate that resembles the natural substrate of factor Xa. Factor Xa cleaves the chromogenic substrate, releasing a coloured compound that can be detected by a spectrophotometer. Results are reported as anticoagulant concentration in anti-factor Xa units/ml, such that high anti-factor Xa values indicate high levels of anticoagulation. Deficiencies of antithrombin in the patient do not affect the assay, because excess antithrombin is provided in the reaction.

Specimen is collected in sodium citrate bottle (blue top) about four hours after subcutaneous injection of LMWH, otherwise, falsely low values may occur. Blood sample should be delivered to the processing bench immediately or else falsely low values may occur (because platelets release platelet factor 4 (PF4) which can neutralise heparin or LMWH). For the same reason, plasma must be separated from cells as soon as possible, ideally within one hour of specimen collection. Plasma can be stored for two hours at room temperature or on ice or can be frozen.

Laboratory is specifically notified as to which drug should be measured (heparin, LMWH). Limitations of this test include cost issues and less ready availability than the PTT for heparin monitoring.

Reference Range for Anti-factor Xa

Patients not on anticoagulants: 0 unit/ml.
Therapeutic range for anti factor Xa for a DVT patient differs according to the type of preparation used:

- Heparin 0.3-0.7 units/ml
- LMWH: 0.4-1.1 units/ml for twice daily subcutaneous dosing. For once daily subcutaneous LMWH dosing, the therapeutic range is less certain but is approximately 1-2 units/ml.
- Target range for deep vein thrombosis (DVT) prophylaxis (prevention): There is no defined target range for prophylaxis of deep vein thrombosis (DVT) because such anticoagulation is not usually monitored. When anti-Xa levels have been measured, mean values are usually <0.45 units/ml.

Therapeutic range :	UFH	:	0.3-0.7 U/L
	LMWH:		0.5-1.0 U/L
Prophylactic range:	UFH	:	0.1-0.29 U/L
	LMWH:		0.20-0.49 U/L

Causes of Sub Therapeutic Antifactor Xa Level

- Specimen drawn at incorrect time (collection times are four hours after injection of LMWH, six hours after injection of danaparoid)
- Specimen transportation time longer than two hours.
- Patient receiving prophylactic dose, therefore, therapeutic range is not applicable and anti-Xa level is actually appropriate for dose

Causes of Supra Therapeutic Antifactor Xa Level

- Renal failure (with LMWH or danaparoid) because of decreased renal clearance.
- Heparin contamination, if specimen was drawn from an IV heparinized line.

Automated Reticulocyte Count

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Reticulocyte count is an index of erythropoietic activity of bone marrow. Manual counting through supra vital stain is the most common procedure of reticulocyte counting however it is fraught with 20-25 per cent interobserver variation. Introduction of automated technologies have greatly increased the accuracy and precision of reticulocyte count with the coefficient of variation (CV) of 3-12.3 per cent.

The automated technologies are based on the principle of flowcytometry and determine the reticulocyte count after staining with fluorescent (thiazole orange, amamine-o, cyanene) or non fluorescent (oxazin 750, new-methylene blue) dyes. Briefly, residual RNA in reticulocyte is precipitated by the dye while haemoglobin from erythrocyte is cleared by acidic reagent leaving stained RNA. In each sample, 32,000 red cells are counted and assayed by volume conductivity and light scatter (VCS). The instrument separates reticulocytes from red and white blood cells as well as platelets. In addition, the immature reticulocyte fraction (IRF) can also be measured by automated instruments depending on the intensity of RNA content, whereby mature forms show less staining. IRF may be important from diagnostic point of view reflecting increased erythropoietic stimulation e.g. regeneration of erythropoietic activity in patients receiving bone marrow or stem cell transplantation.

Although the reagents used for automated reticulocyte counts seem expensive however when we consider the cost of labour in manual counting, the automated reticulocyte counting appears cost effective.

The automated counting is less laborious, more precise and accurate but it has certain limitations as howell jolly bodies, red cell fragments, nucleated red cells, siderotic inclusions; debris, large platelets and platelets clumps are potential sources of error for automated reticulocyte counting. Hb-H samples can also give erroneously low or high results depending on incubation time in the instruments which use new methylene blue dye.

Automated reticulocyte count like the one in manual count is useful in differentiating anaemia caused by marrow failure or haemolysis. Because of greater precision, the automated reticulocyte count can help in monitoring erythropoietin response in chronic renal failure and marrow recovery in aplastic anaemia or malignant disease after therapy. The immature reticulocyte fraction is another tool of automation with clinical significance. This may be normal in conditions like iron deficiency and anaemia of chronic disease, where reticulocyte response is poor. In contrast, in conditions like aplastic anaemia, acute myeloid leukaemia and myelodysplastic syndrome, the IRF may be increased in spite of low or normal reticulocyte count. Immature reticulocyte counts have also been found to predict the optimal time for stem cell harvesting in peripheral blood.

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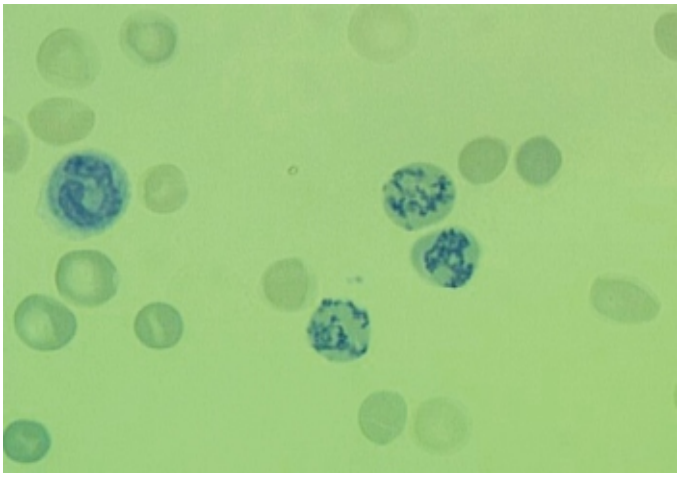

Figure1:
Reticulocyte count as stained by new methylene blue

Triple Screen Test

Dr Beena Umar
Resident, Histopathology

Triple test is a maternal blood screening test that looks for three specific substances; AFP, hCG, and Estriol.
AFP: *alpha-fetoprotein* is a protein that is produced by the foetus.
hCG : *human chorionic gonadotropin* is a hormone produced within the placenta
Estriol: *estriol* is an estrogen produced by both the foetus and the placenta
It is a non-invasive procedure done through a blood test with little to no known risk to the mother or developing baby.

How is the Triple Screen Test Performed?

The triple screen test involves drawing blood from the mother which takes few minutes. The blood sample is then sent to the laboratory for testing and the results come in few days.

When is the Triple Screen Test Performed?

The triple screen test is performed between the 15th and 20th week of pregnancy although results obtained in the 16th -18th week are said to be the most accurate.

All pregnant women should be offered for triple screen, but it is recommended for women who:

- have a family history of birth defects;
- are 35 years or older;
- used possible harmful medications or drugs during pregnancy;
- have diabetes and use insulin;
- had a viral infection during pregnancy;
- have been exposed to high levels of radiation.

What Does the Triple Screen Test Look For?

The triple screen is measuring high and low levels of AFP and abnormal levels of hCG and estriol. The results are combined with the mother's age, weight, ethnicity and gestation of pregnancy in order to assess probabilities of potential genetic disorders.

High levels of AFP may suggest that the developing baby has a neural tube defect such as spina bifida or anencephaly. However, the most common reason for elevated AFP levels is inaccurate dating of the pregnancy.

Low levels of AFP and abnormal levels of hCG and estriol may indicate that the developing baby has Trisomy 21(Down's syndrome), Trisomy 18 (Edwards Syndrome) or another type of chromosome abnormality.

What Do the Triple Test Results Mean?

The triple test signifies that the mother may be at a possible risk of carrying a baby with a genetic disorder. It is known to have a high percentage of false positive results. Abnormal test results warrant additional testing for making a diagnosis like a high resolution ultrasound and amniocentesis for determining karyotype. If the testing still maintains abnormal results, a more invasive procedure like amniocentesis may be performed.

2nd Biennial Conference for Chemical Pathologist: PSCP Collaborate with AKU

Reported by Dr Imran Siddiqui
Associate Professor, Chemical Pathology

Chief Guest
Lt. Gen (Retd,)
S. Azhar Ahmed

The two-day 2nd Biennial Conference of Pakistan Society of Chemical Pathologists (PSCP) opened at Liaquat National Hospital and PNS Shifa on March 9 and at Aga Khan University (AKU) on March 10, 2007. Organised by PSCP and hosted by AKU. The Conference also included two workshops. Invited speakers from Singapore, Islamabad and Lahore as well as from Aga Khan University Hospital (AKUH), presented their papers on this sub-specialty and findings from latest research. The Conference's inaugural session was well attended by professionals from all over Pakistan, and was opened by Lt. Gen (Retd) S. Azhar Ahmed, Vice Chancellor Baqai University, Karachi, who also

inaugurated a concurrent 'Scientific Poster Exhibition' and a display of pathology-related equipment exhibition.

Chief Guest Lt. Gen (Retd,), S. Azhar Ahmed Chancellor Baqai University Inaugurating the Scientific Exhibition

Dr Imran Siddiqui, Chair Organizing Committee

Following a recitation from the Quran, Dr Imran Siddiqui, Chairman Organising Committee, welcomed the guests and provided an overview of the Conference. Brigadier Farooq A. Khan, Deputy Commandant, Armed Forces Institute of Pathology and President PSCP recognised the efforts of PSCP and its contribution to promote education through such conferences, especially thanking the invited international and National speakers. The chief guest Lt. Gen (Retd) S. Azhar Ahmed, referred in his speech to the importance of linkages in healthcare, and highlighted the significance of the human factor-notwithstanding technological innovations.

Brig Farooq Ahmed Khan, President PSCP Addressing on Occasion

The key features of this Conference were two workshops namely Recent advancements in chemical pathology and role of PCR in chemical pathology. Seminar on infertility, plenary lecture and scientific session including poster presentations. Joint efforts from chemical pathologists of Ziauddin University, Liaquat National Hospital and PNS Shifa resulted in the success of this scientific meeting. Section of Chemical Pathology and Conference Secretariat of Aga Khan University led the proceedings. The contribution and support of Dean Medical College, Aga Khan University, Dr Mohammad Khurshid was immense.

Dr Shirnen Mansoor, Medical Office AKHU Receive Best Poster Presentation Award

The conference was designed to provide an opportunity to chemical pathologists, technicians and other members of Pakistan's medical community to expand and build upon their knowledge and understanding of the dynamic field, presently undergoing very rapid change with new technology. Available facilities in Pakistan are fast increasing and the organisers saw the necessity of expanding the pool of appropriately-educated chemical pathologists. It is important that not only chemical pathologists but all healthcare providers keep abreast of the latest trends and technologies. This conference provided a relevant and quality educational opportunity to the chemical pathologists of Pakistan and continued the AKU tradition of leadership and excellence in medical education. This conference also received coverage in the leading newspapers of Karachi.

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