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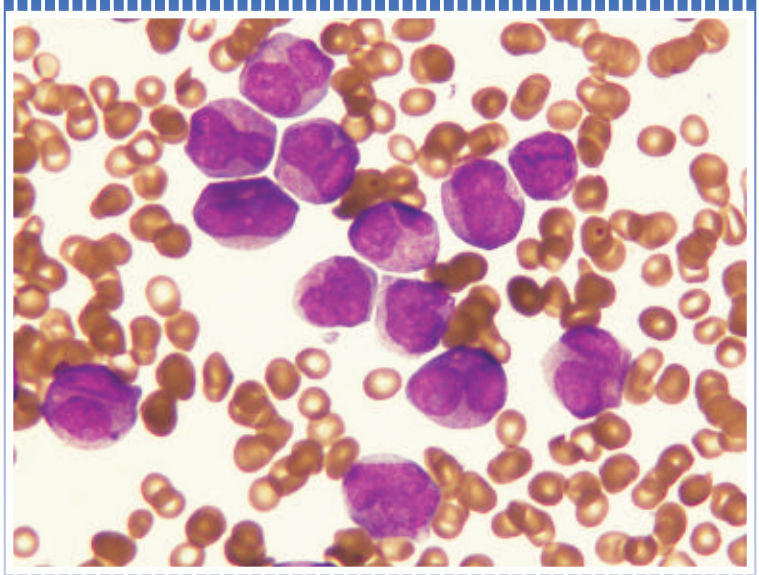
LABRAD : Vol 33, Issue 2 - May 2008

Aga Khan University Hospital, Karachi

Newsletter of Departments of Pathology and Microbiology, and Radiology

LABRAD

May 2008 Vol. 33, Issue 2



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Labrad

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

May 2008,
Volume 33, Issue 2

Labrad Administration Office
Mr Kokab Mirza
Clinical Laboratories
Department of Pathology and Microbiology
Aga Khan University Hospital
Stadium Road, P.O.Box 3500
Karachi 74800, Pakistan
Tel: 486-1551
Fax: (92 21) 493-4294,
493-2095

Website:
http://www.aku.edu/akuh/hs/cs/pathology.shtml

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Tartrate Resistant Acid Phosphatase (TRAP): An Important Diagnostic Tool for Hairy Cell Leukaemia

Mashhooda Rasool Hashmi, Haematology

Hairy cell leukaemia (HCL) is a low grade B-cell lymphoproliferative disorder with distinctive morphological, cytochemical and immunological characteristics. HCL, with respect to cytological characteristics and size, is a disease of monotonous cells known as hairy cells (Figure 1).

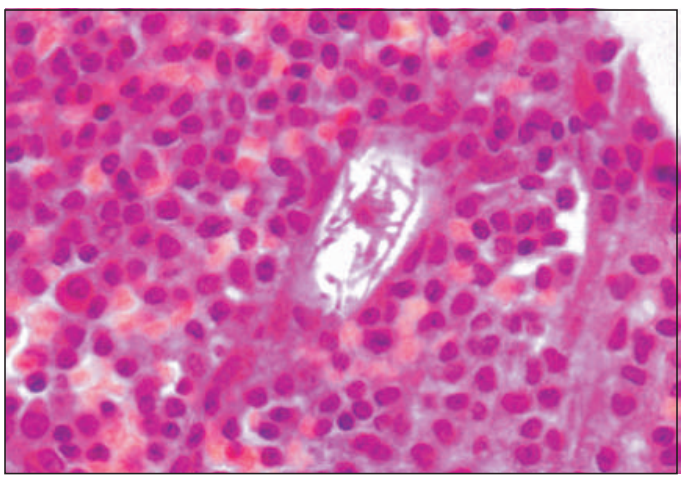

Figure 1: Bone trephine H & E sections showing infiltration with monotonous cells

The hairy cell is 1.5 to 2 times the size of a mature lymphocyte and the nucleus occupies one half to two third of the cell's area (Figure 2). Although there may be a moderate degree of cell size variation between patients, an individual patient usually displays a remarkably homogenous population of hairy cells.

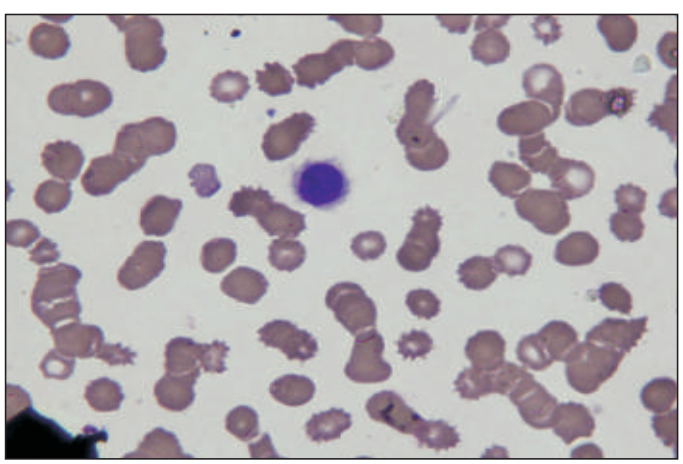

Figure 2: Hairy cells in peripheral blood smear stained with Leishman's stain

Correct and accurate diagnosis of this disease depends upon the recognition of hairy cells in blood, bone marrow and spleen. Suitable preparations include smears of the peripheral blood or bone marrow and touch imprints of biopsy specimens.

A critical cytochemical feature of the hairy cell is the expression of isoenzyme 5 of acid phosphatase that is uniquely resistant to treatment with tartaric acid. This characteristic was utilised in 1970s for the development of cytochemical stains that permitted the identification of tartrate resistant acid phosphatase (TRAP) activity of cells in cytological preparations (Figure 3).

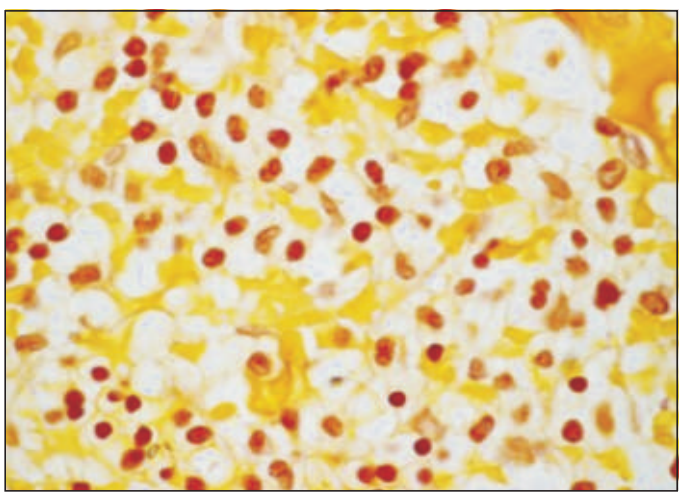

Figure 3: TRAP activity in bone trephine section

Acid phosphatase stains are particularly useful when fresh tissue is not available for phenotypic analysis or in the evaluation of borderline processes with ambiguous morphological or immunophenotypic features.

Acid phosphatase stains are technically challenging. Staining for acid phosphatase activity without tartrate treatment should always be performed to ensure that there is appropriate staining of normal cells, particularly monocytes and lymphocytes. A negative TRAP stain does not exclude the possibility of false negative results, as positive control slides are available rarely because of the unstable TRAP activity in air-dried

preparations. A second critical consideration is the definition of positive TRAP stain. Often, hairy cells are heterogeneous in the expression of TRAP with many negative cells. Interpretation of stain as positive is based on the intensity of the reaction in individual cells, rather than the number of positive cells. Only a few brightly stained positive mononuclear cells with cytological features of HCL are required for a positive study (Figure 4).

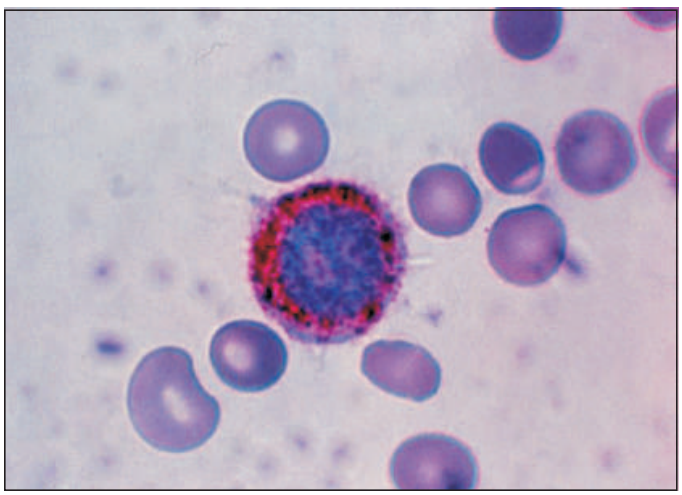

Figure 4: Acid phosphatase indicated by red granular precipitate

In B-cell disorders, acid phosphatase activity is often weak or negative with the exception of HCL, the cells of which show a strong positive activity. This, unlike other lymphoid cells, is however resistant to treatment by tartrate in majority of the cells.

The haematology section of the Clinical Laboratories is performing this test on Fridays with reporting on the next working day. The specimen type includes blood, bone marrow and bone trephine sections. The results are interpreted as positive or negative depending upon the presence and intensity of reaction.

References

1. Sharpe RW, Bethel KJ. Hairy cell leukemia: Diagnostic Pathology. Hematol Oncol Clin N Am 2006; 20: 1023-1049
2. Yam LT, Li CY, Lam KW. Tartrate resistant acid phosphatase isoenzyme in the reticulum cells of leukemic reticuloendotheliosis. N Eng J Med 1971; 284: 357-60

Heterotopic Pregnancy: A Case Report

Dr Imrana Masroor, Dr Fatima Mubarak,
Radiology

Introduction

Heterotopic pregnancy refers to the simultaneous coexistence of an intrauterine and an extrauterine pregnancy. It is a potentially fatal condition, rarely occurring in natural conception cycles. The incidence has risen from 1 in 30,000 births, to an approximate range of 1 in 4,000 to 1 in 8,000 pregnancies. This increased incidence appears to be related to the presence of partially damaged fallopian tubes (a causative factor for many ectopic pregnancies), often owing to partially treated pelvic inflammatory disease and the use of assisted reproductive techniques (1). The incidence of heterotopic pregnancy increases to 1 in 100 following ovarian hyper stimulation and in vitro fertilisation (2,3).

A case of heterotopic pregnancy is presented and the incidence, risk factors, diagnostic and management modalities available are reviewed.

Case Report

A 30-year-old female was self-referred to emergency department, with positive ßhCG levels and an ultrasound report from outside. Patient was married for 13 years and was gravida 4 and Para 3, she had menarche at 13 years, and the menses were regular. Pelvic examination showed no bleeding per vagina and os was closed. According to the patient on 13/01/06 patient's periods were overdue, so ßhCG was done by self referral, which showed a level of 9991 mUL/ml corresponding to a 5 plus week pregnancy. Then patient decided to have an ultrasound examination for confirmation of pregnancy, it was done on 18/01/06 outside AKUH and described a single well defined gestational sac in right adnexa with tubal ring sign positive (Figure 1), showing a foetal pole with positive cardiac activity. Both ovaries were normal, there was no free fluid in pelvis. Minimal fluid was seen in the uterine cavity with decidual reaction, however no foetus was seen. A diagnosis of unruptured right tubal

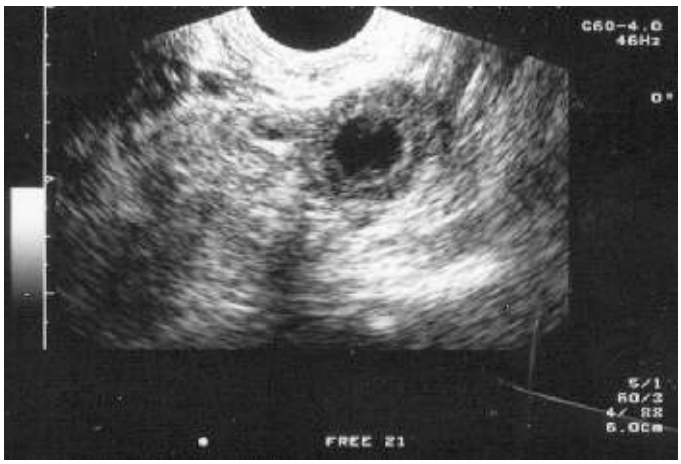

Figure 1: Trans vaginal ultrasound image showing a positive tubal ring sign in right adnexa (arrow head).

ectopic pregnancy was made. Ultrasound was not repeated when she presented to AKUH. On 19/01/06 patient underwent laparoscopic removal of right tubal ectopic pregnancy. The laparoscopic finding were right ampullary ectopic, an endometriotic lesion was, noted posterior to the broad ligament. Right linear salpingectomy was performed, trophoblastic tissue density was adherent to tube. The distal 1.5 cm of right tube was resected leaving behind 2.5 cm of the right tube.

At the end of the procedure, a dye test showed spillage from right bisected tube with no spillage from left tube despite repeated attempts. At that time it was planned to perform hysterosalpingogram after 6 weeks and if required tuboplasty would be done.

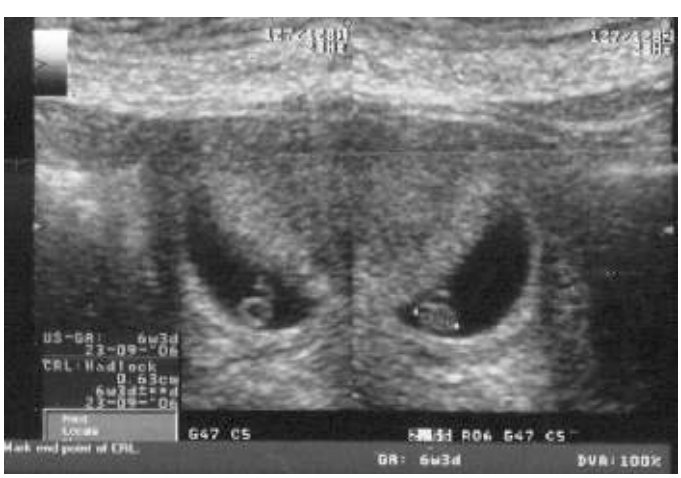

Figure 2: Transvaginal ultrasound showing a well defined gestational sac with foetal pole (arrow head) corresponding to 6 weeks of gestation. Cardiac activity was positive (Rt image).

Histopathology report revealed haemorrhage in tubal mucosa and underlying wall. Chorionic villi and degenerated decidua noted no evidence of molar pregnancy. Ectopic tubal pregnancy was confirmed. The recovery was uneventful and she was discharged with advice to repeat the ßhCG levels and to come for follow up in clinic. On 26/01/06 the ßhCG level was repeated and it showed rising level of 29613 mIU/ml due to which ultrasound pelvis was advised. The ultrasound pelvis performed on 27/01/06 showed a single live intrauterine pregnancy corresponding to 6 weeks of gestation with positive cardiac activity (Figure 2). Both ovaries were normal and there was no free fluid in pelvis. The patient was counseled regarding heterotopic pregnancy, on 28/01/06, and was discharged and advised follow up after two weeks. On third day of discharge, she presented to emergency department with mild lower abdominal pain. On examination, there was no bleeding per vaginum and os was closed. Ultrasound pelvis was repeated on the same day redemonstrating the findings of the previous ultrasound. Patient was admitted for observation and discharged the next day with advice to come for follow up in the clinic after six weeks. On 16/03/06, she had her follow up and an ultrasound examination, that showed a normal 12 weeks 5 days live foetus, corresponding to the previous ultrasound.

Discussion

Combined intrauterine and extrauterine pregnancy though rare, is increasing in incidence. Its incidence is even much higher in women undergoing in vitro fertilisation, in which more than five embryos are transferred in utero and is estimated to be 1 in 50 pregnancies. Though heterotopic pregnancy is an exceedingly rare condition, but over a thousand cases have been reported in the literature since the first description of heterotopic pregnancy in 1708 (2, 4).

The established risk factors are the same as for ectopic pregnancy which include salpingitis, use of IUCD (Intrauterine contraceptive device), progesterone only contraceptives, previous tubal

surgery and especially chronic pelvic inflammatory diseases (5).

Spontaneous heterotopic pregnancy is a rare occurrence with an estimated frequency of <1 per 30,000 pregnancies (6).

In this case, there was no history of above mentioned risk factors, however on laparoscopy an endometriotic deposit was seen adjacent to broad ligament, which may have caused pelvic adhesion leading to development of heterotopic pregnancy.

Ultrasound has a definitive role in diagnosis of heterotopic pregnancy but in this case, the gestational age of two foetuses was different by approximately four weeks. On first ultrasound, the ectopic was diagnosed and the intrauterine changes were reported as decidual reaction with minimal fluid in the endometrial cavity. True gestational sac shows a double decidual sac sign, which is an echogenic rim with an intervening hypoechoic line surrounding the sac, representing the decidua capsularis in close proximity with decidua parietalis. This sign was not seen on first ultrasound examination. Ultrasound can still miss half of heterotopic pregnancies.

There is little agreement regarding surgical management of heterotopic pregnancy. If the extrauterine gestational sac is intact with no intraperitonal haemorrhage, local low dose injection of methotrexate has promising results without affecting the co-existent intrauterine pregnancy. The conventional treatment of laparoscopy or lapratomy depends upon the haemodynamic status of the patient. In this case, the diagnosis was confirmed by history, examination, laboratory tests, and ultrasound findings. As the patient was stable with unruptured ectopic pregnancy, laparoscopic removal was done.

Diagnosing a heterotopic pregnancy is not easy, earlier diagnosis allows for less invasive procedures and potentially eliminates the need for blood products.

References

1. William DM, Alfred BK, Barbara SH. Ultrasound Requisites, Second ed,2004 Missori:Mosby, 359.
2. Dumesic DA, Damario MA, Session DR. Interstitial Heterotopic pregnancy in a women conceiving by fertilization after bilateral salpingectomy. Mayo Clin Proc 2001; 76:90-2.
3. Fonseca M, Potwar S, Badhwar V. Heterotopic pregnancy: a case report. Obstet. Gynecol 2001; 51: 169.
4. Bukhari AS, Bukhari S. Heterotopic Pregnancy: a case report of 2 cases in women with out risk factors. J Ayub Med Coll 2001; 13: 45-8.
5. Shahina Z, Munawer H, Haleema Y, Khurshid JN. Heterotopic pregnancy- outcome management. JCPSP 2004; 14: 494-6.
6. Duce MN, Ozer C, Egilmez H, Apaydin FD, Yildiz A, Kara E. Heterotopic pregnancy: Case report. Abdom Imaging 2002; 27: 677-9.

Biochemical Markers of Excessive Alcohol Use

Dr Lena Jafri, Chemical Pathology

Diagnosis of alcohol abuse can be difficult because many patients conceal this information. The blood tests traditionally used as markers of recent drinking are the liver enzymes, gamma glutamyltranserase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and the mean volume of the red blood cells (mean corpuscular volume, MCV).

Gamma Glutamyl Transferase (GGT)

GGT is one of the longest established biochemical tests for excessive alcohol consumption. It often provides the first indication of a patient's increased alcohol consumption. Clinically, it has been used as a measure of liver function or damage, but it is also found in the kidney, brain, spleen, pancreas and heart. This is one reason why increases in GGT are not specific for excessive alcohol consumption. Hepatic GGT levels increase in response to exposure to a variety of drugs and to alcohol. This may be mediated via oxidative stress, with resultant reductions in glutathione levels. The metabolism of

alcohol, for example, is known to result in free radical formation (1). Normally, small amounts of GGT are released from the cell membrane into the circulation. In people with repeated excessive alcohol consumption, there may be increased release of GGT from the cell membrane. In cases with inflammation and liver cell damage, there may also be cell necrosis with release of the enzyme. Serum levels of GGT rise in response to alcohol consumption to a variable extent. The response varies between individuals and within individuals according to the phase in their drinking history. GGT does not respond to a single dose of alcohol unless the person has previously been an excessive drinker. GGT levels respond to even low levels of habitual drinking (2), but generally sustained excessive drinking is needed to raise a significant proportion of drinkers' levels above laboratory reference ranges.

The Aminotransferases (Aspartate Amino Transferase and Alanine Amino Transferase)

The serum aminotransferases (formerly called transaminases) are sensitive indicators of liver cell injury. The most commonly measured are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The serum ALT and AST concentrations are normally less than 30 to 40 IU/L The location of the aminotransferases within cells is variable. ALT is found exclusively in the cytosol, while AST occurs in the cytosol and mitochondria. While present in the greatest concentration in the liver, AST is also present in heart, muscle, kidney, brain, pancreas, lung, leucocytes and erythrocytes. Because of this, it has limited specificity for alcohol use. Because ALT is found predominantly in the liver, it is affected less by non-hepatic insults. Like GGT, aminotransferases are not increased by a single episode of excessive drinking. The aminotransferases are less sensitive than GGT in detection of excessive alcohol consumption. Like GGT, the aminotransferases act not only as markers of alcohol consumption but also as indicators of hepatic damage from alcohol. The pattern and height of aminotransferase elevation assists in initial assessment of the nature of alcohol induced liver damage and in differential diagnosis. GGT levels are typically higher than aminotransferase levels in alcohol induced liver damage. When aminotransferases are elevated, if the AST: ALT ratio is greater than 2.0, 90% of cases are due to alcohol. In contrast, in viral hepatitis, the ALT is typically higher than the AST, and indeed there may be an isolated increase in ALT.

Mean Corpuscular Volume (MCV)

In alcohol excess, the majority of cases of macrocytosis occur in the presence of normal folate levels (3) and without anaemia, and do not respond to folate treatment. The cause of macrocytosis is complex. Ethanol appears to have a direct marrow toxic effect, causing reduced marrow cellularity and vacuolization of red cell precursors, similar to that seen in choramphenicol toxicity. MCV is a test that is performed so commonly that there is opportunity to use it in opportunistic case finding.

Conclusions

While the traditional markers of alcohol use, GGT, AST, ALT and MCV have limited sensitivity and specificity, they remain useful adjuncts in the assessment and management of excessive drinkers. A two fold elevation of the GGT in patients with AST to ALT ratio greater than 2:1 strongly suggests alcohol abuse. Their levels may indicate complications of drinking or concurrent conditions that may be affected by drinking.

References

1. Bailey, S. M. & Cunningham, C. C. (2002) Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. Free Radical Biology and Medicine
2. Sillanaukee, P., Massot, N., Jousilahti, P., Vartiainen, E., Sundvall, J., Olsson, U., Poikolainen, K., Ponnio, M., Allen,J. P. & Alho, H. (2000) Dose–response of laboratory markers to alcohol consumption in a general population. American Journal of Epidemiology
3. Maruyama, S., Hirayama, C., Yamamoto, S., Koda, M., Udagawa, A., Kadowaki, Y., Inoue, M., Sagayama, A. & Umeki, K. (2001) Red blood cell status in alcoholic and non-alcoholic liver disease. Journal of Laboratory and Clinical Medicine, 138, 332–337.

Osmotic Fragility

Dr Natasha Ali, Haematology

Osmotic fragility is the most commonly used test in the diagnosis of hereditary spherocytosis. The test may also be used in screening for thalassaemia.

Principle

Osmotic fragility measures the red cell resistance to haemolysis by osmotic stress, depending primarily on the volume of the cell, the surface area and the cell membrane function. Red cells that are spherocytic, for whatever cause, take up less water in hypotonic solution before rupturing than normal red cells.

Method

No special preparation is needed for the test; however history of anaemia and jaundice and significant family history along with evidence of splenomegaly is important and helps in the diagnosis. A 5 ml venous blood sample in heparin tube is required along with 2 to 3 ml of blood in EDTA tube for review of peripheral film and blood counts. Red cells are incubated in varying concentrations of hypotonic solution of sodium chloride NaCl (0.95%, 0.70%, 0.60%, 0.50%, 0.45%, 0.40%, 0.2%, and 0%). As the concentration of NaCl decreases, cells take up water to produce osmotic equilibrium. Absorbance of each tube is read at 546nm with distilled water as blank to check the degree of haemolysis. The test is repeated after overnight incubation of blood sample at 37°C to increase the sensitivity of the test. Normal control sample is run simultaneously (Figure 1).

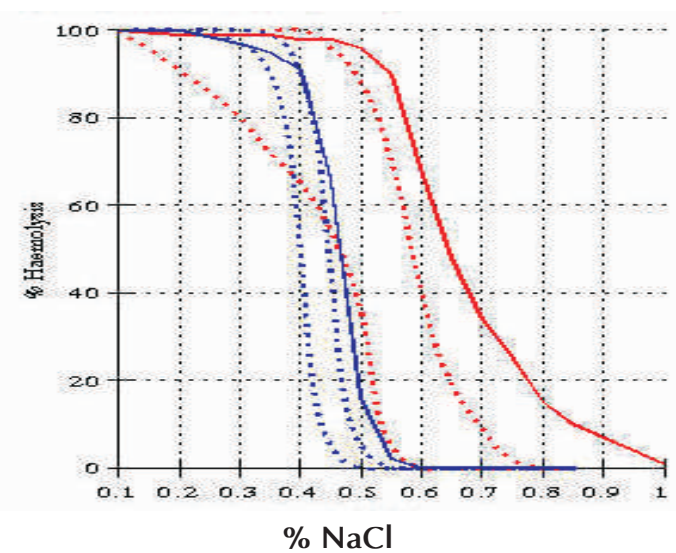

Figure 1: Osmotic fragility curves before and after incubation

Blue line indicates patient before incubation and red line after incubation. Dotted line indicates normal curves before (blue) and after (red) incubation

Interpretation

The ability of the normal red cell to withstand hypotonicity results from its biconcave shape which allows the cell to increase its volume by about 70% before the surface membrane is stretched; once this limit is reached, lysis occurs. Normal cells begin to lyse at NaCl concentrations of approximately 0.50%. Because of their increased volume to surface area ratio, spherocytes cannot expand as much as normal discoid cells; they are therefore particularly susceptible to osmotic lysis. Lysis is usually complete by 0.4-0.5%, starting at higher NaCl concentrations.

A shift to the right shows increased osmotic fragility (e.g. hereditary spherocytosis, autoimmune haemolytic anaemia, G6PD deficiency). A shift to the left shows decreased osmotic fragility (e.g. iron deficiency anaemia, thalassaemia minor). This indicates the presence of unusually flattened red cells in which the volume to surface area ratio is decreased. The osmotic fragility of red cells after incubation for 24 hours at 37°C is also a reflection of their volume to surface area ratio.

Results

Osmotic fragility is expressed in terms of the concentration of saline causing 50% lysis i.e. median corpuscular fragility (MCF). In health, MCF is 4.0 to 4.45 g/l at room temperature and 4.65 to 5.9 g/l after incubation.

The test is performed daily at Aga Khan University Hospital's Clinical Laboratory and reported after two days.

Pattern of Drug Abuse in Patients Presenting at a Referral Lab

Drs Farhan Dar, Aysha Habib Khan,
Farooq Ghani, Imran Siddiqui,
Chemical Pathology

Introduction

Drug abuse exists in our country, however very few users ever get tested or seek medical intervention. Drug addiction is compulsive use of a substance, despite its negative effects, which might lead to serious medical and emotional complications. Use of illicit drugs or the abuse of prescription or over-the-counter drugs may lead to addiction.

Objective

The objective of this study was to identify the pattern of drug abuse in a population presenting at a referral laboratory.

Methods

A retrospective analysis was done for drug abuse testing at AKUH's Clinical Laboratory from July 2006 to July 2007. The common drugs screened at AKUH Clinical Laboratory include alcohol, amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates.

Among the drugs, testing for amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates were performed in random urine. Analysis of barbiturates, benzodiazepines and cocaine was performed by enzyme immunoassay on Beckman Synchron Cx 7 while amphetamine, cannabinoids and opiates are tested by fluorescent polarisation immunoassay on Axysm. Testing for alcohol was performed in plasma by an enzymatic method on Synchron Cx 7.

Results

A total of 64 patients were screened from July 2006 to July 2007. Of these, seven (11%) were females and 57 (89%) were males. Mean age of the patients was 31 years (age range: 15 to 66 years). The most commonly used drug was benzodiazepines: 16 cases (25%) followed by cannabinoids: 5 cases (7.8%) and alcohol: 2 cases (3.3%). The least positive drugs were found to be barbiturates, cocaine and opiates: 1 case each (1.6%). Amphetamine was not detected in any of these cases. Patients aged between 20 to 29 years were more involved in usage of abusive drugs.

The mean age of the females tested for drug of abuse was 25 years (age range 17 to 39 years). Among seven females, 2 (28.6%) were positive for benzodiazepines only, while all other drugs tested were negative.

Conclusion

Most common drug of abuse in our cases is benzodiazepine followed by cannabinoids. Males are more involved in the usage of abusive drugs than females. Women are less likely than men to use illicit drugs and to develop drug-related problems.

Achondrogenesis

Drs Ruqaiya Shahid, Asim Qureshi, *M Yousaf,
Histopathology and *Radiology

Introduction

Achondrogenesis is a group of disorders that affect cartilage and bone development. There are at least two types of achondrogenesis, characterised by small body, short limbs and skeletal abnormalities. Affected infants are born prematurely, are stillborn or die shortly after birth from respiratory failure.

Case Report

A 16-week foetus was received in the laboratory. Gross examination of the foetus revealed short upper and lower limbs, disproportionately large head, soft skull, convex fascies, long philtrum, small nose, retrognathia and low set ears. Genitalia were not fully developed. No abnormalities were seen in the cardiovascular, gastrointestinal, renal and endocrine systems. However, sections from the lungs revealed hypoplastic lung tissue and sections from femoral shaft showed immature cartilage (Figure 1).

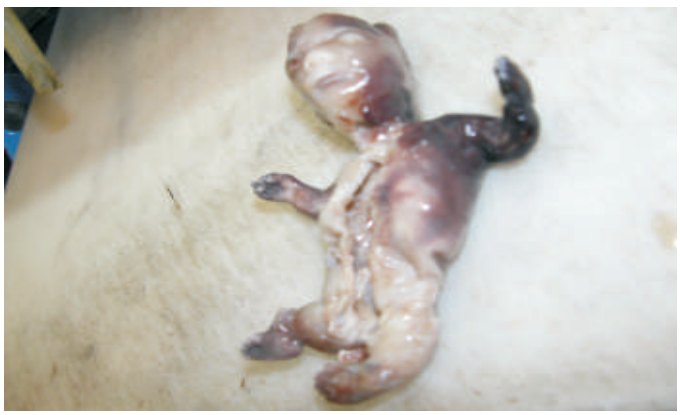

Figure 1: Foetus with achondrogenesis showing short lower limbs and soft bosselated head

Radiologic findings included very short tubular bones with metaphyseal expansion and cupping. Iliac wings were small with unossified pubis and ischium and short ribs. No ossification of the vertebrae and sacrum was found with relatively normal ossification of calvarium (Figure 2).

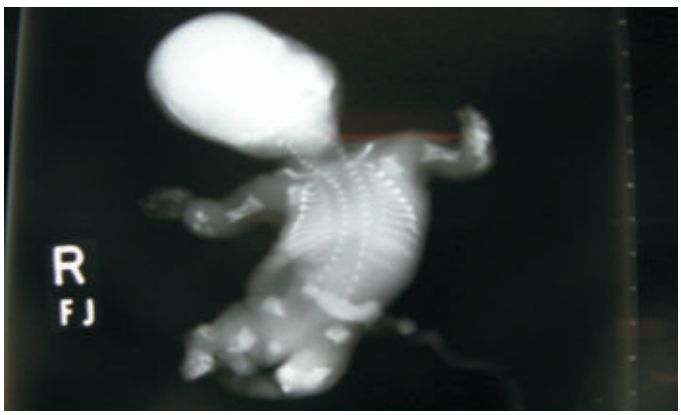

Figure 2: X-ray of the foetus showing short tubular bones of lower limbs and ossified pubis and ischium

Discussion

Achondrogenesis type Ib is characterised by extremely short limbs, a narrow chest and a prominent rounded abdomen. The fingers and toes are short and feet may be rotated inwards. Affected infants usually have an umbilical hernia. Achondrogenesis type Ib results from mutation of SLC 26 A2 gene that is essential for the development of cartilage and its conversion to bone. It is inherited as an autosomal recessive disorder. Most of the parents of an individual with an autosomal recessive disorder are the carrier of one copy of an altered gene but do not show signs and symptoms of the disorder.

Achondrogenesis type II is one of several disorders caused by mutations in COL 2 A1 gene which results in amino acid substitution, interrupting the normal formation of stable triple helical type 11 collagen molecules. There is complete absence of type 2 collagen in the cartilage which has a gelatinous composition. Type 2 achondrogenesis is considered an autosomal dominant disorder, almost always caused by new mutations in COL 2A1 gene and typically occurs in patients with no history of the disorder in the family. The affected individuals do not pass the disease to the next generation because they do not live long enough.

Conclusion

Clinical, radiological, gross and microscopic findings were consistent with achondrogenesis. Due to lack of genetic studies at our center, exact gene type could not be confirmed.

References

1. ACOL 2A1 mutation in Achondrogenesis type 2 results in the replacement of type 2 collagen by types 1 and 3 collagen in cartilage. Am Soc Biochem Mol Biol 1995; 270:1747-53

Molecular Diagnosis of Human Papilloma Virus

Sadia Tabassum and Maheen Hassan,
Molecular Pathology

Introduction

Human papilloma virus (HPV) belongs to the family papoviridae containing double stranded DNA. HPV are common viruses that can cause warts. There are more than 100 types of HPV. Most are harmless but about 30 types can cause severe type of infection which can lead to cancer. HPV is classified into low risk and high risk groups. Low risk HPV can cause warts and high risk HPV can lead to cancers of genital organs in males and females. HPV has several subtypes; for example, subtypes 6 and 11 are predominately found in benign warty growth lesions (low grade). Subtypes 16, 18, 31, 33 and 35 are seen in high grade lesions (Table 1).

Table 1: HPV Types and Associated Diseases

HPV Types	Associated Disease
2, 7	Common warts
1, 2, 4	Plantar warts
3, 10	Flat cutaneous warts
6, 11, 42, 43, 44, 55	Anogenital warts
16, 18, 31, 33, 35, 39, 45, 51	Genital malignancies
13, 32	Focal epithelial hyperplasia (oral)
6, 7, 11, 16, 32	Oral papillomas

Molecular Diagnosis of HPV

HPV qualitative assay is based on the isolation of viral DNA from cervical swab specimen, amplification and detection of E1-E2 regions of HPV genome using real time PCR (Figure 1).

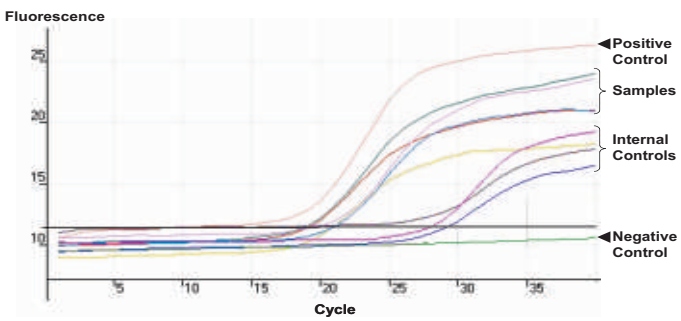

Figure 1: Real time PCR assay: fluorescence raw data

Genotyping of High Risk HPV

HPV PCR assay that is used in clinical laboratory easily detects the HPV (high risk) subtypes (16, 18, 33, 35, 39, 45, 52, 56, 58, 59, and 66) in the urogenital swab biopsies. HPV high risk typing test is based on the DNA extraction of HPV, multiplex amplification of DNA using HPV specific primers and detection of amplified products on agarose gel (Figure 2).

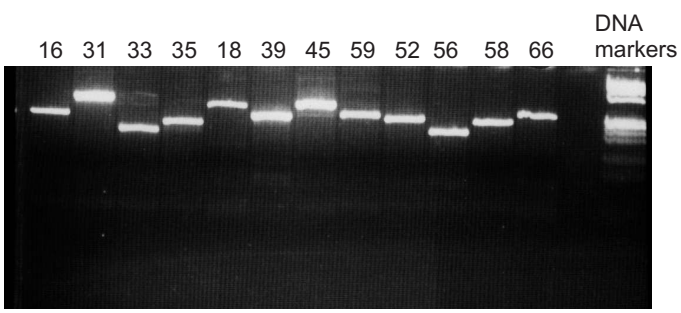

Figure 2: A) DNA samples of various HPV subtypes were amplified and electrophoresed on an agarose gel, stained with ethidium bromide and photographed. The number on each lane represents a specific HPV subtype.

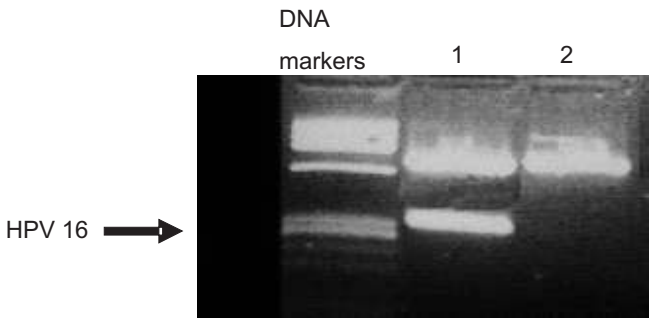

Figure 2:#B) Amplified products obtained from HPV positive and negative patient samples. The HPV type 16 is identified in the patient sample. Lane 1: positive sample; Lane 2: negative sample

Conclusion

Molecular testing can detect the HPV DNA and its high risk subtypes. The test is performed AKUH's Clinical Laboratory on cervical swab specimens.

Diagnosis of *Clostridium Difficile* Associated Diarrhoea

Dr Kauser Jabeen, Microbiology

Clostridium difficile is a spore-forming, gram-positive anaerobic rod that is responsible for 15-25% of all episodes of antibiotic associated diarrhoea due to production of two exotoxins, A and B. The disease spectrum ranges from pseudomembranous colitis to toxic megacolon, perforations of the colon, sepsis and rarely death. It is mainly a health care associated infection occurring with increased frequency in patients with antibiotic exposure, gastrointestinal surgery/ manipulation, prolonged stay in hospital, a serious underlying illness, immunocompromised conditions and advanced age. The main clinical symptoms include watery diarrhoea, fever, loss of appetite, nausea and abdominal pain/tenderness.

C. difficile is excreted in faeces and any surface, device or material (e.g., commodes, bathing tubs and electronic rectal thermometers) that becomes contaminated with faeces may serve as a reservoir for the C. difficile spores. C. difficile spores are transferred to patients mainly via the hands of health care personnel who have touched a contaminated surface or item.

It is important to differentiate between C. difficile colonisation and C. difficile associated disease. In colonisation the patients exhibit no clinical symptoms; however stool is positive for C. difficile organism and/or its toxin. Whereas diseased patient has clinical symptoms with stool positive for the C. difficile organism and/or its toxin.

Laboratory Diagnosis

Stool Culture for *C. difficile:* This is the most sensitive test available, but does not differentiate between the toxigenic and non-toxigenic strains. Stool cultures for C. difficile also are labour intensive and require the appropriate culture environment to grow anaerobic microorganisms therefore most commercial laboratories do not routinely perform cultures for C. difficile.

Antigen Detection for *C. difficile:* These are rapid tests (<1 hour) that detect the presence of C. difficile antigen by latex agglutination or immunochromatography. Antigen detection should be done with toxin testing to verify diagnosis. This test is available at AKUH's Clinical Laboratory.

Toxin Testing for *C. difficile:* This is the most widely used test for the diagnosis of C. difficile associated diarrhoea. This could be detected by either enzyme immunoassay (EIA) that detects toxin A, toxin B or both A and B or tissue culture cytotoxicity assay.

PCR for Detection of Toxin Gene: This is currently only a research tool and is not commercially available.

Management

Discontinuation of the offending antibiotic with fluid replacement is important. The infection can usually be treated with metronidazole or vancomycin (administered orally). After treatment, repeat C. difficile testing is not recommended if the patients' symptoms have resolved, as patients may remain colonised. Asymptomatic colonisation should not be treated.

Prevention

Disease is prevented by judicious use of antibiotics, contact precaution for patients with known or suspected C. difficile-associated disease, hand hygiene using either an alcohol-based hand rub or soap and water and proper use of disinfectant and environmental cleaning.

Pakistan Association of Pathologists – Meeting Report

Dr Natasha Ali, Haematology

The Pakistan Association of Pathologists conducted its 31st annual conference at Khyber Medical College, Peshawar from November 29 to December 1, 2007. The pre-conference programme mainly consisted of workshops in all fields of pathology facilitated by Professor Farooq Ahmad Khan, Dr Nuzhat Mushahid, Dr Raheel Qamar, Dr Nizamuddin and included updated knowledge of safe blood transfusion practices, role of molecular diagnostic techniques in the diagnosis of infectious diseases and proficiency testing and laboratory accreditation.

The conference was inaugurated on November 30, followed by the Raazi Lecture delivered, by Professor Farooq Ahmad Khan on `Metabolic Disorders'. The second half of the day included oral and poster presentations, which were chosen by peer reviewers from abstracts submitted prior to the meeting. These presentations contained the latest and exciting developments in scientific research. Four parallel symposia took place simultaneously. Presentations on various subtypes and antimicrobial resistance of shigella, platelet aggregometry, focus on leishmaniasis and G6PD deficiency comprised

Dr Ghulam Nabi Kakepoto chaired a hematology session at PAP conference 2007

of cutting-edge inferences. The second day of the conference started off with state of the art lecture delivered by Professor Waheed uz Zaman Tariq on `Challenges in the Diagnosis of Viral Diseases'. After the lecture, there were oral presentations in histopathology, haematology, chemical pathology and microbiology and some of the topics included were ultrasound guided core needle biopsy, focus of leishmaniasis in Abbottabad, automation of laboratory and chronic granulomatous disease, to name a few. Chairmen (for both days) were Professors Manzoor Ahmad, Muhmmad Saleem, Sajid Mushtaq and Muhammad Tariq.

On the eve of November 30, a gala dinner was held followed by a cultural programme.

The conference ended on December 1. These kinds of conferences offer opportunities to update and increase knowledg of everyday practice. Pathology is a dynamic field and it is important for the medical community to keep up with the changing trends, which this conference has helped in solidifying.

Residents observed scientific posters with keen interest.

The conference provided relevant and convenient quality learning opportunities to pathologists in Pakistan and continued the tradition of excellent medical education.

AKU delegates attending PAP Conference 2007 at Peshawar

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THE AGA KHAN UNIVERSITY

آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

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