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Expanding Horizons

PULSE

Technical Newsletter of SRL Limited

Dear Friends,

The power of our mind is infinite, but how we invoke and channelize it is very important. There are several instances during our work days when we harness it in ways that many others cannot. Such ability of harnessing our thoughts and capturing those into tangible presentations is what sets one individual apart from another. And such publication of ideas, observations and interpretations therein, enrich the knowledge of the community as a whole; sets the bar a little higher.

Pulse works like a melting pot of such presentations in Lab Medicine. It is an easy way of capturing the essence of scientific thoughts across the organization in 6 monthly intervals. It also works like a motivational tool, as many of you will agree, to work our minds that bit extra and churn out an article of merit.

In the *In Focus* section of this issue, we have Dr Amar Dasgupta and his team writing about Hb Fontainebleau, a rare alpha chain variant of hemoglobin with only a few cases reported from the Indian sub-continent. They tell us that Hb variant analysis of patients with Hb Fontainebleau reveals an abnormal peak in HPLC which is difficult to diagnose if one is not familiar with this entity.

In the *Medical Case Reports* section, we have cases ranging from Churg Strauss syndrome, nodular hidradenoma of scalp, neurocytoma, primary adenoid cystic carcinoma of lung to case discussion of intestinal spirochetosis in immunocompetent adults and the possibility of presence of human anti-mouse antibodies as an interference in immunoassays. Kindly take note of the *Brain Teaser* section as well.

I thank all the contributors who have authored the articles and everyone who have supported the authors in direct or indirect ways to contribute to *Pulse*.

Hope you will enjoy reading this 16th issue of *Pulse*.

Warm regards,

Dr. B. R. Das

Diagnostic Challenges Posed by a Rare Alpha Globin Chain Variant in a Pregnant Female and its Potential Effects in her Unborn Child

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Introduction

Hemoglobinopathies are a heterogeneous group of diseases that range clinically from being asymptomatic to causing severe hemolytic anemia. Structural hemoglobin (Hb) variants mainly arise from point mutations that result in single amino acid substitutions. Until date over 200 alpha chain variants have been identified [1]. Most of these are picked up during hemoglobinopathy screening programs. Here we report a rare alpha-globin gene variant, Hb Fontainebleau [alpha 21(B2) Ala →Pro], detected in the heterozygous condition in a 19 year-old primigravida as a part of her antenatal screening. Hb Fontainebleau was first described by Wajcman et al in 1989 in four members of a family of Italian descent [2]. This would be the fourth case of Hb Fontainebleau reported from the Indian sub-continent.

Case Report

A 19 year-old female born of non-consanguineous marriage in a Sikh family from Punjab underwent Hb variant analysis as a part of her routine antenatal screening. She had no previous history of anemia or blood transfusions. Laboratory investigation revealed hemoglobin 11.6 g/dL with red cell count 4.82x10^9/L, total leucocyte count 10.2x10^9/L with normal differential count and platelet count of 219x10^9/L. Red blood cell indices revealed a mildly reduced mean corpuscular volume (MCV) of 77.8 fL and mean corpuscular hemoglobin (MCH) of 24.2 pg with a normal mean corpuscular hemoglobin concentration (MCHC) 31.1 g/dL. Red cell distribution width (RDW) was 15.7%. Hb variant analysis was carried out on the Variant II Hb testing system (Bio Rad Laboratories Hercules, CA, USA) which revealed a small abnormal Hb peak (12.5 %) with retention time of 2.92 minutes, appearing as a hump following and close to the HbA peak [Figure1]. The other hemoglobins on HPLC were Hb A (83.6%), HbA2 (1.7%) and Hb F (0.3%).

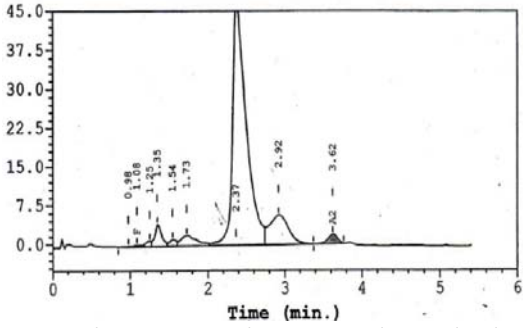

Figure 1: HPL chromatogram in the propositus showing the abnormal Hb peak (Hb Fontainebleau) at retention time 2.92 minutes.

HPLC analysis of the parental Hb was performed which revealed the presence of a similar (12.6%) abnormal hemoglobin peak with retention time of 2.93 minutes in the father. He had 2.5% of HbA2 and 0.2% of HbF. His hemoglobin was 16.1 g/dL with red cell count 4.84x10^9/L, MCV 90.8 fL, MCH 27.5 pg and MCHC 30.6 g/dL. Red cell distribution width (RDW) was 14.6%. HPLC analysis of Hb of the mother did not reveal any abnormality. Both the propositus and her father had low HbA2 levels.

Molecular characterization of the abnormal Hb in the father's blood sample by reverse DNA sequencing (Applied Biosystems, Foster City, USA) showed the presence of a heterozygous G>C substitution at codon 21 (alpha 2 globin gene) leading to substitution of alanine by proline at the beginning of the beta helix corresponding to Hb Fontainebleau [Figure2].

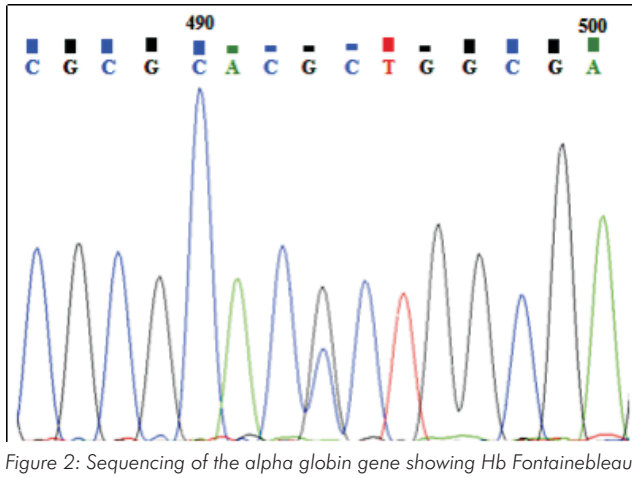

Figure 2: Sequencing of the alpha globin gene showing Hb Fontainebleau [a1-CD21GCTàCCT (Ala →Pro) in the father of the propositus.

The husband of the index case was also screened for hemoglobinopathies and showed an elevated HbA2 value of 5.7% along with a sharp abnormal hemoglobin peak 8.3% with a retention time of 4.67 minutes suggesting a double heterozygous state for HBQ India and Beta-thalassemia trait [Figure3].

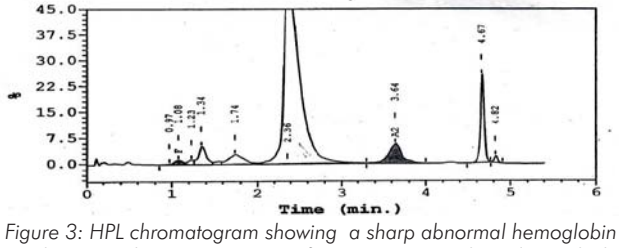

Figure 3: HPL chromatogram showing a sharp abnormal hemoglobin peak 8.3% with a retention time of 4.67 minutes and an elevated Hb A2 value of 5.7% in the father of the propositus.

Discussion

Studies involving the spectrum and frequency of alpha globin chain variants in India are very few in number[3]. The clinical importance of alpha chain variants lies in the possibility of them being co-inherited with other globin chain variants, especially beta globin chain variants, and the resultant alteration in the phenotype of otherwise clinically more severe forms of Hb variants, e.g. HbS. Hb Fontainebleau is a rare alpha chain variant in the Indian population which elutes out as an abnormal peak in HPLC of Hb and can pose considerable diagnostic difficulty to those unfamiliar with this entity.

Hb Fontainebleau was first described in the year 1989 in an Italian family and the new alpha chain variant derived its name from the place where the family resided. In this report published by Wajcman et al[2], the Hb variant was found to be associated with hereditary spherocytosis in three members of the family. The only member who did not have hereditary spherocytosis was clinically and hematologically normal, thereby highlighting the fact that Hb Fontainebleau per se does not cause any hematological abnormality. Subsequently, cases of Hb Fontainebleau were reported in Iraqis and Cypriots, and from United Kingdom and United Arab Emirates[4-9].

A total of three case reports of Hb Fontainebleau have been published previously from India. In the first report by Upadhaye et al.[6] a baby and her mother from Madhya Pradesh were detected to have Hb Fontainebleau during newborn screening for sickle cell disorders. Both were found to have Hb Fontainebleau in a compound heterozygous state with HbS and the baby had anemia at birth (Hb 11.4 g/dL). However, she had no cyanosis or icterus and didn't need transfusion. The second case reported from India[7] was a 35 year old lady who too was picked up during a routine antenatal screening program for thalassemia and had normal hematological indices. The third case[9], although evaluated for a moderately severe anemia and detected to have Hb Fontainebleau responded well to iron supplements with normalization of hemoglobin level and hence no deleterious effect of this Hb variant could be established. Further, his mother and both sisters were asymptomatic.

Our index patient and her father both did not show any clinical or hematological finding related to the presence of this rare alpha chain variant. The mildly reduced Hb value and microcytic indices in the propositus could have resulted from pregnancy and concomitant iron deficiency. Also we found that the HbA2 level in both the propositus and her father was low as has been previously reported in other cases.

Interestingly, the husband of the index patient is also a double heterozygote for beta thalassemia trait and HbQ India. This opens up the possibilities of multiple combinations of hemoglobinopathies in the unborn baby - she has only a 25 percent chance of not having any alpha chain variant. The child would otherwise have equal chance of acquiring the following possibilities [Figure4]

a. Double heterozygous for two alpha chain variants - both Hb Fontainebleau and Hb Q India
b. Hb Fontainebleau heterozygous state alone
c. Hb Q India heterozygous state alone

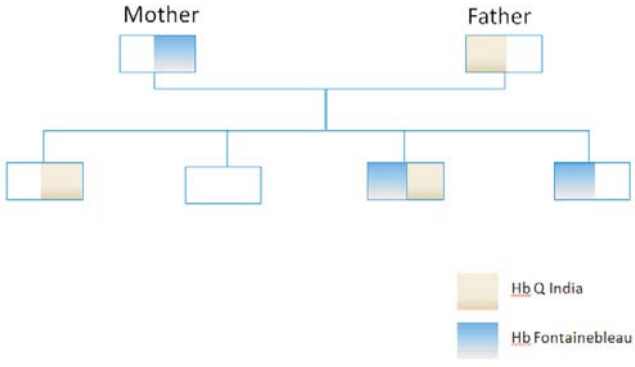

Figure 4: Possible genotypes of hemoglobinopathies in the children of the propositus and her husband.

But in this case the husband also has a beta thalassemia trait and there is a possibility of the child adiitionally acquiring the defective beta globin gene from the father with or without a concurrent inheritance of one or more alpha chain variants. The clincal presentation in such a situation would be complex and difficult to predict at this stage.

A recent study by Turner et al [8] reported 12 cases of Hb Fontainebleau in the United Arab Emirates including one that was homozygous, four with co-inherited deletional alpha thalassemia and one with a co-inherited non-deletional alpha thalassemia (alpha T Saudi). The latter case as well as the one homozygous Hb Fontainebleau had higher levels of Hb Fontainebleau as would be expected. However, only the case with a co-inherited non-deletional alpha thalassemia (alpha T Saudi) had mild anemia and a severely low MCV possibly due to a concomitant iron deficiency as evident from low serum iron level. The three of the four cases that had Hb Fontainebleau and a deletional alpha thalassemia had normal values of hemoglobin with mildly microcytic red blood cell indices.

Conclusion

Hb Fontainebleau is a rare alpha chain variant with only a few cases reported from the Indian sub-continent. Hb variant analysis of patients with Hb Fontainebleau reveals an abnormal peak in HPLC which is difficult to diagnosis if one is not familiar with this entity. From the published studies referred to above and from our own cases reported here, it appears that Hb Fontainebleau by itself does not cause any hematological abnormality. Therefore, a patient having anemia and Hb Fontainebleau should be investigated for other causes of anemia and be advised appropriately. However, the clinical importance of Hb Fontainebleau lies in the potential modifications it can bring about in the phenotype of severe forms of beta chain and alpha chain variants if co-inherited with the latter

variants. The unborn child of our index case is possibly one such example wherein various permutations and combinations of inheritance of multiple hemoglobinopathies including Hb Fontainebleau could potentially be observed as already discussed. Hence, it is pertinent to adopt a planned strategy for complete and extensive investigation of suspected cases of alpha and beta globin gene defects.

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Churg Strauss Syndrome

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Summary

We describe a case report of a 60 yr /F who presented with Hematemesis in the emergency ward subsequently admitted & to be investigated upon. The pt was allergic, had cough with wheeze and Chronic Sinusitis. She was on Anti asthmatic drugs since few years. There was H/O Joint pain with low grade fever. Her investigations revealed moderate to severe anemia, Eosinophilia with thrombocytopenia. X-ray chest showed bronchial thickening with hyperinflation.USG whole Abdomen was normal, upper GI endoscopy suggested of mild GI Bleed. A provisional diagnosis of churg strauss syndrome was made based on the clinical & laboratory findings. Further a bone marrow biospsy was done which was suggested Granulomatous inflammation with eosinophilia. Finally ANCA (Anti Neutrophil cytoplasmic Antibody) via Immunofluoresence was done and it showed p-ANCA (Perinuclear) positivity. Hence a Final Diagnosis of Churg Strauss syndrome was made based on ACR (American College of Rheumatology) criteria.

Background

Churg Strauss syndrome also known as EPGA (Eosinophilic Granulomatosis with Polyangitis) is a rare systemic vasculitis predominantly affecting small & medium sized blood vessels. It was first described by Dr. Jacob Churg & Dr. Lotte Strauss in 1951 as a syndrome consisting of Asthma, eosinophilia, fever and accompanying vasculitis of various organ systems. Churg & Strauss discovered the presence of granulomas as well as abundance of eosinophils which distinguished this disease from Polyarteritis nodosa and Wegener's Granulomatosis.

ACR (American College of Rheumatology) diagnostic Criteria is used to make a final diagnosis. 4 out of 6 below mentioned criteria are required to establish the diagnosis-

1. Asthma
2. Eosinophilia > 10%
3. Neuropathy
4. Pulmonary infiltrates
5. Paranasal sinusitis
6. Extravascular eosinophils on Biopsy

A Positive ANCA (Anti neutrophil cytoplasmic antibody) on Immunofluorescence/ ELISA also supports the diagnosis. It is positive in 40% cases. The usual pattern seen on Immunofluorescence is p-ANCA (Perinuclear).

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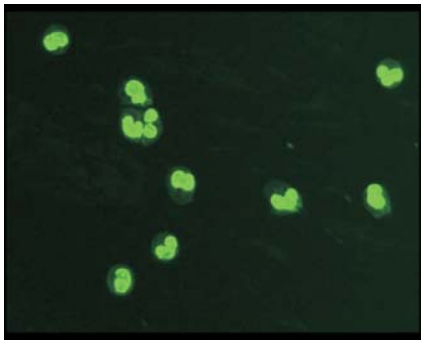

p-ANCA

Case Presentation

A 60 yr old female presenting in emergency with Hematemesis. She has H/O allergy, Asthmatic Bronchitis with sinusitis, low grade fever, joint pain. The pt. had severe anemia with eosinophilia & thrombocytopenia. LFT & KFT were normal. USG whole abdomen was normal. X-ray Chest showed bronchial thickening with hyperinflation. Upper GI endoscopy revealed mild GI bleed.

Investigations

CBC:
Hb – 5.5 gm %
TLC – 8000 / cumm
DLC - P 43 %, L 20 %, E 35 %, M 02 %
Platelet Count – 20,000 / cumm
Bone Marrow Biospsy – Hypercellular marrow with eosinophilia s/o Granulomatous Inflammation.
BIOCHEMICAL / SPECIAL INVESTIGATIONS –
LFT / KFT – Normal, Urine R/M - WNL
ANA – Negative, Rheumatoid Factor – Negative, Anti CCP - Negative
ANCA – Positive p-ANCA via Immunofluorescence

RADIOLOGICAL /OTHER INVESTIGATIONS:
X-ray Chest – Bronchial thickening with Hyperinflation
USG whole Abdomen – normal
Upper GI endoscopy – Mild GI bleed

Differential Diagnosis

- Hyper eosinophilic syndrome
- Asthma
- Bacterial / Eosinophilic Pneumonia
- Wegener's Granulomatosis
- Microscopic Polyangitis
- Polyarteritis Nodosa

Treatment and Outcome

The patient was put on Corticosteroids (Prednisolone) and Cyclophosphamide and was refered to higher centre for further treatment.

Discussion

Patients with Churg Strauss syndrome (EGPA) usually presents with Asthma, the next phase sees eosinophilia & the 3rd phase may be characterized by vasculitis which may also involve skin, lungs, kidney, nerves. As seen in this case a similar case published in JCR showed the patient had cough, breathlessness, asthmatic bronchitis with eosinophilia. The patient had a positive ANCA too. Another case published in case reports in Medicine showed an unusual presentation having severe pain in the left side of abdomen with h/o asthma & eosinophilia.

Take Home Message

1. Churg Strauss syndrome (EGPA) consists of Asthma, eosinophilia and accompanying vasculitis of various organs systems.
2. Granulomatous Inflammation with eosinphilia is characterstic which differentiates it from Wegner's Granulomatosis & Polyarteritis Nodosa.
3. American College of Rheumatology Criteria is used for the Diagnosis.
4. ANCA (Anti neutrophil Cytoplasmic Antibody) positivity is seen in approx. 40 % of the cases & its presence supports the diagnosis together with other signs & symptoms.

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Schwannoma Arising from Tongue: A Rare Case

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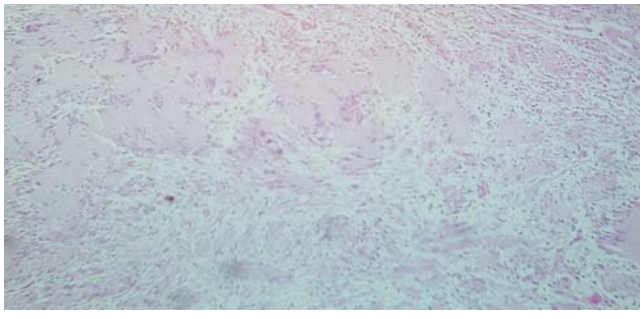
Summary
A 7 year-old male was referred to the Head and Neck Surgery department by his treating physician after he noticed a sessile nodule at the right lateral border of his tongue around 1 cm. The patient reported that the lesion had been there for around 2-3 months and had not grown significantly and denied any pain associated with the nodule, dysphagia, or change in voice. The remaining oral examination was unremarkable. No cervical lymph node enlargement was noted. The patient's routine haematological and urine examination were normal.

The nodule was excised and sent to our lab. On microscopy, a well-defined, circumscribed neural lesion was seen and it was diagnosed to be a benign schwannoma arising in the tongue.

Background
Schwannomas are slow growing benign tumors. Head and neck Schwannomas account for 25-40% of all cases [1,2]. Intra-oral schwannomas account for only 1% of all head and neck tumors [3]. Tongue schwannomas are rare tumors of the oral cavity. We present this case for the rarity of schwannoma in the tongue hence, they should be considered among the differential diagnoses when evaluating a patient presenting with a tongue mass, especially since it has favourable prognosis.

Case Presentation
- A 7 year-old male was referred to the Head and Neck Surgery department by his treating physician after he noticed a sessile nodule at the right lateral border of his tongue measuring around 1 cm.
- The patient reported that the nodule had been there for around 2-3months and had not grown significantly.
- The patient denied any pain associated with the mass, dysphagia, or change in voice.
- The remaining oral examination was unremarkable
- No cervical lymph node enlargement was noted.
- The patient's routine hematological and urine examination were normal.
- GROSS: The nodule was gray-tan in colour, measuring 0.7x0.4x0.2 cm.
- MICROSCOPY: The tumour consisted of alternating hypocellular and hypercellular areas made up of spindle shaped Schwannian cells. The hypercellular (Antoni A) areas showed cellular interlacing fascicles of spindled cells with focal nuclear palisading around eosinophilic areas characteristic of Verocay bodies. The hypocellular (Antoni B) areas showed myxoid change. Blood vessels with thick hyalinised walls and focal thrombus formation were seen amidst the tumour.
- IMMUNOHISTOCHEMISTRY: strong S100 positivity in the spindle tumour cells.



Schwanomma showing verocay bodies X100

Differential Diagnosis
- Schwannoma should be considered in the differential diagnosis of benign masses over tongue like lipoma, neurofibroma, hemangioma, lymphangioma, lingual thyroid, leiomyoma, and benign salivary gland tumors.
- Solitary neurofibroma should be excluded through histopathology to rule out chances of neurofibromatosis.

Treatment and Outcome
- Local excision or enucleation of these tumours is the treatment of choice [4].
- The non-encapsulated form requires a margin of normal tissue and careful separation from the involved nerve is also necessary to preserve normal function.
- Schwannomas are not radiosensitive and so radiotherapy has no role in the treatment [5].
- Recurrence is rare. Malignant transformation of a benign schwannoma is rare.

Take Home Message
Schwannoma in the tongue is a rare entity however they should be considered among the differential diagnoses when evaluating a patient presenting with a tongue mass.

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Nodular Hidradenoma of Scalp

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Summary
Nodular hidradenoma is a rare adnexal neoplasm of eccrine or apocrine origin. Clinically, it is a slow growing, well circumscribed, dermal lesion, has a slight predilection for the head, face, and upper extremities and show female predominance, with a mean age at presentation of 37.2 years [1].

A 32 years male presented with a scalp nodular swelling, for several months. On routine investigations, his fasting blood glucose levels were high and the clinical diagnosis of infected sebaceous cyst was made.

Lesion was excised and sent for histopathology. Microscopically it was lobulated (Figure 1) and composed of eosinophilic and clear cells with multiple ducts lined by cuboidal and apocrine cells (Figure 2 & 3). Occasional squamous morules were seen. Significant atypia and necrosis were not seen and mitosis were occasional.

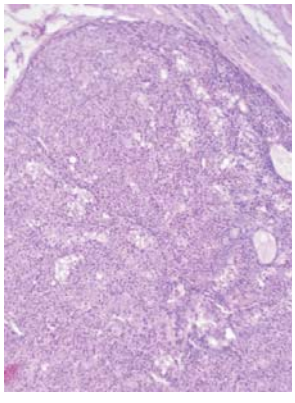

Figure 1: Well circumscribed tumor disposed in lobules (H&E, 100X)

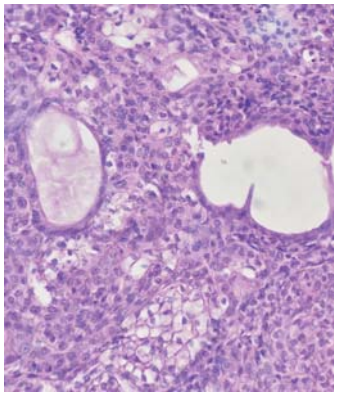

Figure 2: Tumor shows clear cells displaying clear cytoplasm and eccentric nuclei, eosinophilic cells with bland oval nuclei and ducts (H&E, 400X)

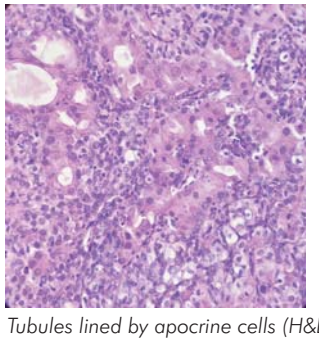

Figure 3: Tubules lined by apocrine cells (H&E, 400x)

The diagnosis of nodular hidradenoma was made with a note of caution to exclude other mimics via IHC and to exclude metastatic RCC. Also a wide excision with clear surgical margins was advised to prevent recurrence.

Background
A great majority of skin appendageal tumors are known to show clear cell change. Renal cell carcinoma is known for cutaneous metastasis to scalp and face. This case report is aimed at a comprehensive analysis of histological features of hidradenoma to differentiate from its mimickers and acknowledge that immunohistochemistry is sometimes essential for diagnosis.

Case Presentation
Patient presented with scalp swelling. No other significant past or family history.

Investigation
Routine investigations were unremarkable except elevated fasting glucose (137 mg/dl).

Differential Diagnosis
1. Metastatic renal cell carcinoma
2. Skin appendageal tumors of sebaceous / follicular / sweat glands origin
3. Glomus tumor
4. Clear cell melanoma

Treatment
Treatment consists of surgical excision with adequate margins and histological confirmation with margin status to avoid recurrence.

Outcome and Followup
Prognosis is good and malignant transformation of benign nodular hidradenoma has rarely been reported.

Discussion
Nodular (or clear cell or solid-cystic or acrospiroma) hidradenoma is a benign skin appendage tumor that shows vast array of histopathological features and may mimic other malignancies [1]. Histologically, nodular hidradenoma is well circumscribed, with a grenz zone separating it from epidermis. It is lobulated, may be solid cystic, with eosinophilc material in cysts. Solid areas show eosinophilic cells and clear cells. Focal apocrine and squamoid change can be seen.

Clear cell hidradenoma, the most common variant, contains predominantly clear cells containing glycogen and PAD positive material, but no lipid.

Poroid hidradenoma shows compact poroid cells with prominent ductal differentiation.

Mucinous cells (least common) are large cuboidal cells lining the tubules and may show evidence of apocrine differentiation [2].

Oncocytic, epidermoid, and pigmented variants of hidradenoma have also been reported [1,3].

Immunohistochemistry plays an important role in differentiation from other clear cell tumors of skin, metastatic RCC and glomus tumor. Hidradenoma shows positivity for PanCK, CK 5/6, CK7, CK 10, 34bE12, CEA, EMA and negative for CK20, vimentin, SMA, CD10, S100 protein [1].

The criteria for malignancy include poor circumscription, presence of nuclear atypia and mitotic activity, predominantly solid cell islands, infiltrative growth pattern, areas of necrosis and angio-lymphatic permeation. Nodular hidradenoma is labeled as atypical when there is no evidence of invasive features but it has a high mitotic rate or nuclear atypia [4,5].

Learning Points

- Differentiation of nodular/ clear cells hidradenoma from its mimics requires close attention to histology and IHC.
- High mitotic rate is not always predictive of malignancy.

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The Possibility of Presence of HAMA as an Interference in Immunoassay

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Summary

Circulating human antibodies reactive with animal proteins (anti-animal antibodies) are an often unrecognized and unsuspected source of interference in immunological assays, in particular two-site (sandwich) immunoassays. Although many human anti-animal antibodies may be detectable, the laboratory is mostly concerned with antibodies of sufficient titer and affinity to have an analytically significant effect. Anti-animal antibodies (IgG, IgA, IgM, IgE class, anti-isotype, and anti-idiotype specificity) arise as a result of iatrogenic and non iatrogenic causes and include human anti-mouse, -rabbit, -goat, -sheep, -cow, -pig, -rat, and -horse antibodies and antibodies with mixed specificity. Circulating antibodies can reach gram per liter concentrations and may persist for years. Prevalence estimates for anti-animal antibodies in the general population vary widely and range from <1% to 80%. Human anti-animal antibodies cause interferences in immunological assays and can affect the result of the assay. The most common human anti-animal antibody interference is HAMA, which causes both positive and negative interferences specially in two site mouse monoclonal antibody-based assays.This is a case report of such interference encountered during the testing procedure by ELISA.

The detailed study of the present case led to the conclusion that there was some interfering substance in the sample which led to the positive result in the existing platform. The probability of HAAA most probably HAMA antibodies, in the sample could be the reason of false positivity in this particular case.

Key words: HAAA's (Human anti animal antibodies), HAMA (Human anti-mouse antibodies), IMA's (immunoassays)

Introduction

Interference in immunoassays is a serious but underestimated problem. Interference can be defined as "the effect of a substance, present in the sample that alters the correct value of the result, usually expressed as concentration or activity, for an analyte". Immunoassays are analytically sensitive and measurements can frequently be performed without prior extraction. Specificity of an immunoassay does not only depend on the binding property of the antibody but also the composition of the antigen and its matrix is also important. Specificity can also be influenced by reagent composition and immunoassay

format. Substances that alter the measurable concentration of the analyte in the sample or alter antibody binding can potentially result in assay interference (Tate & Ward, 2004).

Interference can be analyte-dependent or analyte–independent and it may increase (positive Interference) or decrease (negative interference) the measured result. The common Interferences of hemolysis, icterus, lipemia, effects of anticoagulants and sample storage are independent of the analyte concentration. Analyte-dependent interferences in immunoassays are caused by interaction between components in the sample with one or more reagent antibodies. They include heterophil antibodies, human anti-animal antibodies, auto-analyte antibodies, rheumatoid factor and other proteins. Interferences may lead to falsely elevated or falsely depressed analyte concentration depending on the nature of the interfering antibody or the assay design (reagent limited versus reagent excess assays). The magnitude of the effect depends on the concentration of the interferant, but it is not necessarily directly proportional. It can also lead to discordant results between assay systems (Selby, 1999; Tate & Ward, 2004). Interference can have important clinical consequences and may lead to unnecessary clinical Investigation as well as inappropriate treatment with potentially unfavorable outcome for the patient (Ismail & Barth, 2001). It is important to recognize interference in immunoassays and put procedures in place to identify them wherever possible (Kricka, 2000).

Endogenous interfering substances can occur in both healthy and pathological patient samples. Sample properties are unique for each patient. Interference is caused by interaction with one or more steps in the immunoassay procedure and the analyte concentration or the antibody binding is influenced (Davies, 2005). Unsuspected binding protein(s) in the individual can interfere with the reaction between analyte and assay antibodies. In reagent excess assays, like the common two-site immunometric assay (IMA), there is an increased chance of a cross-reactant forming a bridge between the two antibodies. Conformational changes to antigens can be induced by antibodies which may alter the specificity of antibodies. For these reasons there may be a higher prevalence of unpredictable cross reaction in IMAs than in the single-site antigen-antibody reaction in reagent-limited assays (Boscato & Stuart, 1986). Exogenous antibodies given to a patient for therapy may also compete with the assay antibody for the analyte and disturb the antigen-antibody reaction resulting in immunoassay interference, e.g., administration of Fab fragments derived from anti-digoxin antibodies (Digibind) (Hursting et al., 1997).

Exogenous interferences are any interference caused by the introduction of external factors or conditions, in vivo or in vitro, not normally present in native, properly collected and stored samples. For example, hemolysis, lipemia, icterus, blood collection tube additives.

Background

Human anti-animal antibodies

Human anti-animal antibodies (HAAA) are high-affinity, specific polyclonal antibodies generated after contact with animal immunoglobulin. They show strong binding and are produced in a high titer. HAAAs can be of the IgG, IgA, IgM, or rarely, the IgE class (Kricka, 1999). They compete with the test antigen by cross-reacting with reagent antibody of the same species to produce a false signal. The most common HAAAs are human anti mouse antibodies (HAMA), but also antibodies to rat, rabbit, goat, sheep, cow, pig, horse may occur (Selby, 1999). HAMA is especially prevalent in serum of animal workers and in patients on mouse monoclonal antibody for therapy or imaging.

Interfering, endogenous antibodies should be called specific HAAAs when there is a history of medical treatment with animal immunoglobulin and immunoglobulin from the same species used in the immunoassay (Kaplan & Levinson, 1999). The nomenclature becomes confusing where the immunogen is not known and a heterophilic antibody is recognized in mouse or other animal-specific immunoassays.

HAMA interference has been reported for numerous analytes including cardiac markers assays (White & Tideman, 2002), thyroid function tests (Frost et al, 1998), drugs and tumour markers (Boerman et al., 1990). Two-site (sandwich) immunoassays are more prone to interference from antibodies to animal IgG in serum and may cross-react with reagent antibodies especially from the same species. HAMAs interfere by bridging between the immunoglobulin capture and the immunoglobulin detection antibodies resulting in false positive results. False negative results due to HAMA interference are also possible in two site assays, when the HAMA reacts with one of the antibodies preventing reaction with the analyte (Kricka, 1999). Methods that use only one mouse monoclonal in IMA assays are less prone to interference from HAMA.

Incidence of immunoassay interference

The prevalence of interference in modern immunoassays is low, but variable and dependent on the type of antibody interference. Heterophilic antibody and HAMA interference can vary from 0.05% to 6% depending upon the method of detection (Bjerner et al., 2002). Nonanalyte antibody binding substances have been detected in proximally 40% of serum samples using a modified immunometric assay, termed an “interference assay” and they cause 15% interference in non-blocked assays (Boscato & Stuart, 1986). Ward et al. identified 7 out of 21,000 samples from a hospital population with heterophilic interference and HAMA, the interference being as low as 0.03% in blocked IMAs. However, the addition of blocking reagent does not guarantee the complete elimination of interference.

Exogenous antibodies given to a patient for therapy may also compete with the assay antibody, for the analyte and disturb the antigen-antibody reaction, resulting in immunoassay interference. Administration of radioactive or fluorescent compounds, drugs, herbal medicines, nutritional supplements, sample storage and transport are all exogenous interferences that can adversely affect immunoassays (Selby, 1999).

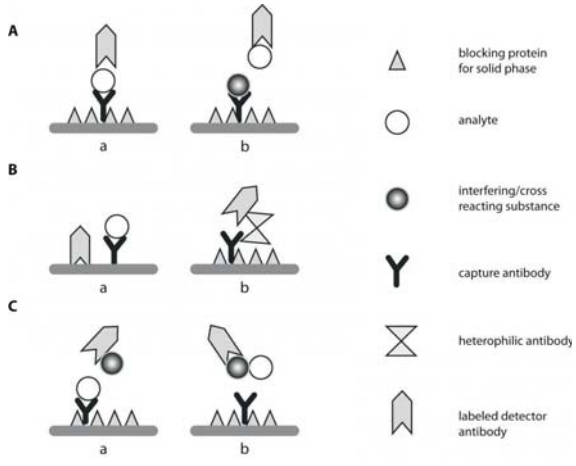

Fig. 1. Summarizes the possible interference mechanisms in IMAs (Immunoassays). Different interferences in immunometric immunoassays: Aa - assay without any interference; Ab - cross-reactivity of an interfering substance with capture antibody, resulting in false negative result; B - positive interference: Ba - unspecific binding of labelled detector antibody to a not blocked solid phase; Bb - "bridge" binding by heterophilic antibodies or HAMA, respectively; C - negative interference: Ca - change of sterical conformation after binding of interfering protein to Fc fragment of detector antibody Cb - masking of the epitope on analyte surface by a protein of the sample (Dodig, 2009).

Case Report

A 30 yr pregnant female with no earlier history of bad obstetrics' was tested for the entire torch panel as routine antenatal investigation along with other routine investigations. The sample was found to be positive for all the IgM and IgG for the entire Torch parameters including Toxoplasma, cytomegalovirus, rubella and HSV 1&2. Since this was a very rare case a recheck was done and the result was found to be consistent with the previous one.

A fresh sample was than demanded from the patient in order to rule out any preanalytical error. This sample was further rechecked for all the torch parameters(including IgM and IgG).The sample was also sent for a parallel inter lab comparison to the sister lab. The result of our sister Lab was also concordant. Since this was a very rare case a complete history of the patient was taken from the treating Gynecologist and a detailed discussion on the case was done with her. The history of the patient ruled out any autoimmune disease as well as rheumatoid arthritis. A parallel testing was also performed on different platform at labs other than SRL. The tests were found to be negative for all the parameters. A PCR testing was than suggested in order to confirm the status of the patient. The result of PCR was found to be negative for all the parameters for which the tests were positive in immunoassay.

The detailed study of the case led to the conclusion that there was some interfering substance in the sample which led to the positive result in the existing platform.

Diagnosis

Since all the torch parameters were found to be negative at two different platforms and PCR results were also negative an antenatal active infection was ruled out.

Discussion

The present immunoassay was based on the principle of IgM immunocapture. The Elisa plate wells are coated with Anti mouse Anti IgM to capture all IgM's and also the conjugating antibodies are mouse antibodies. The rare anti mouse antibodies are binding to plate and conjugate. So the sample will give positive reading for any parameter due to presence of HAMA in Immuno capture kits. This could probably the reason of positivity(IgM) for all the torch parameters in the above case.

Testing for interferences in samples suspected of interference

Immunoassay results on samples suspected of interference can be checked by different procedures. These include repeat analysis of the sample using a different immunoassay platform that, if possible, employs antibodies that are raised against different species normally gives agreement between methods. If HAMA interference is suspected, the alternate assays should not use monoclonal mouse antibodies because the assay may also be inaccurate. If a significantly different result is detected between methods there is a likelihood of interference. However, agreement between methods does not necessarily exclude interference nor does disagreement, if methods lack standardization and clinical decision limits differ (Tate & Wald, 2004). The false assumption that a result is correct because a majority of immunoassay methods give similar results was shown in the multicentric study by Marks in which nine of eleven LH and FSH methods were in agreement but gave falsely low results for a 72-year old postmenopausal woman who was positive for RF (Marks, 2002). Reanalysis using alternative technology such as liquid chromatography or tandem mass spectrometry should be considered if available.

Another procedure for detecting and identifying a suspected interfering antibody is the use of commercially available blocking antibodies (Emerson et al., 2003). Statistically discrepant results before and after incubation with blocking agent would be indicative of interference. A difference between initial and treated value of 3 to 5 standard deviation (SD) suggest possible heterophilic interference, >5 SD indicates definite heterophilic interference (Preissner et al., 2005). However, 20-30% of samples with interfering antibodies may yield similar results after treatment with the blocking antibodies (Ismael, 2009).

Another method is by making serial dilutions of the sample using manufacturer's diluent, provided that it contains non-immune globulin (Ismail, 2007). This could identify about 60% of samples with interference in which linearity and parallelism are lacking.

Using these three methods it is possible to identify interference in almost 90% of suspected samples

Conclusion

Interference in immunoassays from endogenous antibodies is still a major unresolved and underestimated analytical problem, which can have important clinical consequences. There is no single procedure that can rule out all interferences. It is important to recognize the potential for interference in immunoassay and to put procedures in place to identify them wherever possible. Most important is a consideration of the clinical picture. If there is any suspicion of discordance between the clinical and the laboratory data an attempt should be made to reconcile the difference. The detection of interference may require the use of another method, or measurement before and after treatment with additional blocking agent, or following dilution of the sample in non-immune serum. If testing is inconclusive and the interference cannot be identified, the analyte concentration should not be reported and laboratory report should indicate there is a discrepancy for that analyte due to some technical inaccuracy and suggest the test to be repeated using another sample.

Interference in immunoassay is one factor that contributes to the uncertainty of medical testing. Laboratories should be aware of the potential for interference in all immunoassays and how artefactual results may cause misinterpretation and a subsequent erroneous diagnosis and unwarranted treatment.

The recognition of such aberrant test results requires constant surveillance of both laboratory and clinician. Since these interferences are relatively uncommon, clinicians need to be aware of the fact and the mismatch of clinical and biological data. Interference can have important clinical consequences and may lead to unnecessary clinical investigation as well as inappropriate treatment with potentially unfavorable outcome for the patient (Ismail & Barth, 2001). It is important to recognize interference in immunoassays and put procedures in place to identify them wherever possible (Kricka, 2000).

Dialogue between the clinician and the clinical laboratory over unexpected immunoassay test results can avoid inappropriate clinical intervention based on abnormal test results.

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Renal Replacement Lipomatosis

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Summary
Renal replacement lipomatosis (RRL) is a rare and benign condition which occurs secondary to atrophy or destruction of renal parenchyma, with proliferation of excessive lipomatous tissue in renal sinus, renal hilum and peri-renal space. We report a case of 75 year old male patient presented with dull aching left flank pain for last 2 years. Intravenous pyelography revealed a non functioning kidney with a staghorn calculous in dilated left pelvicalyceal system. Left nephrectomy specimen we received. Grossly kidney size was small. On histopathological examination, renal cortical atrophy and extensive fatty infiltration of renal parenchyma was noted. We diagnosed the case as renal replacement lipomatosis (RRL).

Background
There are spectrum of changes associated with RLL, mildest form- renal sinus lipomatosis to almost total replacement lipomatosis at the other end of spectrum [1]. It is different from renal lipomas which are neoplastic, whereas this condition is thought to be a degenerative process. Therefore, the important aspect of this relatively uncommon entity is that it may be confused with renal neoplasms on radiology. It is associated with a poorly or non-functioning kidney and histologically there is gradual replacement of the renal parenchyma with mature adipose tissue leading to end stage renal disease. Renal replacement lipomatosis may be missed if not considered, so our aim is to spread more awareness to Urologists, Radiologists, and Pathologists of this relatively uncommon entity so that it can be recognised appropriately.

Case Presentation
We present a case of 75 year old male presenting with dull aching, non radiating pain in left flank for last 2 years. Investigations reveal mild high serum BUN and creatinine. USG revealed both kidneys are normal in size and shape. A large echogenic structure with posterior accoustic shadow measuring 3.71 cm is seen in the left pelvis. Pelvicalyceal system is dilated on left side. The impression was staghorn calculous with grade II hydronephrosis. Intravenous urography reveals a large staghorn calculous in the left kidney with non excretion of contrast and delayed nephrogenic effect. CT and MRI were not performed. Left nephrectomy specimen was received covered with perinephric fat measuring 10x6x4.8 cm. Capsule could not be stripped off easily. The kidney measured 6.4x4x2.5cm. On serial sectioning, yellowish fatty tissue was seen replacing normal renal parenchyma. Only rim of parenchyma was seen at renal cortex. Cortico-medullary ratio was not discernible. Staghorn calculous was seen occupying the renal pelvis. On microscopy, there was diffuse infiltration of renal parenchyma by fat with atrophied renal parenchyma (Fig1). The remaining parenchyma showed glomerulosclerosis and focal dense lymphocytic infiltrate into the interstitium. Tubules showed thyroidisation, and thickening of vessel wall was also seen. No granuloma or malignancy were seen (Fig 2).

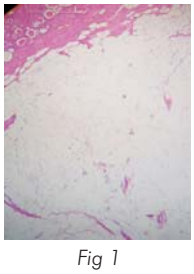

Fig 1

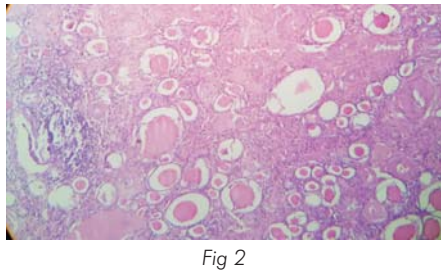

Fig 2

Based on gross and microscopic picture, we diagnosed the case as Renal replacement lipomatosis.

Discussion
Renal replacement lipomatosis can be defined as the proliferation of fibrofatty tissue which subsequently replaces the destroyed renal parenchyma. It may be rarely idiopathic or may be secondary to destruction of the renal parenchyma, mostly due to a calculus or due to infection like Tuberculosis.

RRL was first described by Kurtzmann in 1931 as reported by Peacock and Balle [2]. There is spectrum of changes of fatty infiltration ranging from mild lipomatosis in the renal sinus with underlying normal parenchyma (renal sinus lipomatosis), occurs in sixth to seventh decade, associated with old age, obesity, atherosclerosis, or exogenous steroids and it may progress to near complete replacement (renal replacement lipomatosis). There is no specific clinical feature to diagnose this condition. Patients usually present with complaints of recurrent flank pain, fever, weight loss and mass per abdomen.

The differential diagnosis include malakoplakia, xanthogranulomatous pyelonephritis, or lipid containing tumors like lipoma, liposarcoma or angiomyolipoma [4]. In RRL, the renal parenchyma is atrophied and may undergo hydronephrosis associated with destruction of parenchyma. This feature helps to distinguish it from tumors of the kidney where the renal parenchyma is not atrophied.

This case is reported to remind this entity once again as Replacement renal lipomatosis is an uncommon entity, and a high index of suspicion is necessary to achieve an accurate diagnosis.

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Intestinal Spirochetosis in Immunocompetent Adults: 3 Case Reports

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Summary

Intestinal spirochetosis can be found in immunocompetent and in immunocompromised patients. They colonize the surface of the colonic mucosa. The colonization may be asymptomatic or lead to symptoms of diarrhea and rectal bleeding. Diagnosis is with the help of a colonic biopsy wherein the diagnostic histopathological feature of a blue false brush border is seen. Special stains like silver stains can help in the confirmation of the diagnosis. Treatment with a course of metronidazole usually helps in resolution of the symptoms. We present three cases of intestinal spirochetosis which were diagnosed on a biopsy and confirmed with the help of silver stains. All three patients presented with diarrhea or rectal bleeding and were treated with metronidazole. All the patients obtained symptomatic relief with the treatment.

Background

Spirochetes are pathogenic in animals, but their significance in humans has not yet been established. Spirochetes have been associated with rectal bleeding and diarrhea. Spirochetes have a distinct histological picture and are easily recognizable on microscopy as a false blue brush border. Intestinal spirochetosis can be diagnosed easily with multiple biopsies, and should be suspected in a patient with persistent diarrhea or rectal bleeding.

Case Presentation

We present 3 cases of intestinal spirochetosis.

Case 1: A 19 year old male presented to the OPD with history of diarrhea. Clinically he was suspected to have Ulcerative Colitis. Colonoscopy revealed grade 3 colitis in the rectum. Multiple biopsies were taken from the right and left colon and rectosigmoid region.

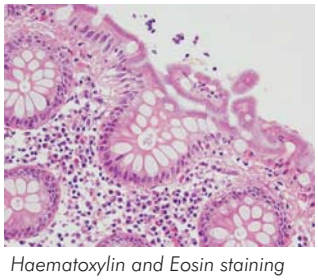
Case 2: A 22 year old male presented to the OPD with a history of rectal bleeding. Colonoscopy revealed proctitis with rectosigmoid ulceration. He too was suspected to have Ulcerative Colitis. Multiple biopsies were taken from the left colon and rectosigmoid area.

Case 3: A 60 year old male presented with history of weight loss and diarrhea. Clinically the differential diagnoses given were Coeliac disease or Microscopic colitis since the upper gastrointestinal endoscopy revealed gastritis and colonoscopy findings were normal.

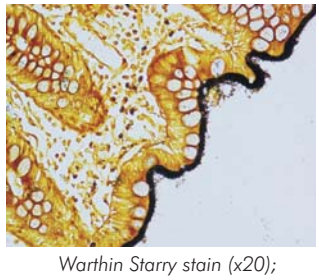
All 3 patients were immunocompetent.

Investigations

The colonic biopsies in all 3 patients revealed a haematoxyphilic brush border on the surface epithelium on H&E staining. This was characteristic of Spirochetosis. PAS stain positivity was seen and Warthin Starry silver stain on the sections easily demonstrated the spirochetes. Additional findings in the colonic biopsies included focal active colitis in the 19 year old, a mild lymphoplasmacytic infiltrate in the lamina propria in the 22 year old and lymphocytic colitis in the 60 year old patient.



Haematoxylin and Eosin staining (x 40); colonic mucosa with a haematoxyphillic brush border



Warthin Starry stain (x20); spirochetes along the luminal surface appear black with silver stain

Differential Diagnosis

The microscopic picture of spirochetosis is very characteristic and diagnostic for the particular organism.

Treatment

All 3 patients were treated with a course of metronidazole and showed symptomatic relief.

Outcome and Followup

The patients responded to the treatment and follow up was uneventful.

Discussion

First recognized in humans by van Leeuwenhoek in his own diarrheal stool in the 17th century (named as animalcules), intestinal spirochetes in humans are still poorly understood in their biology, origin, and state as commensals or pathogens in the human large intestine [1]. Spirochetes are currently divided into three phylogenetic groups: Spirochaetaceae including Borrelia, Spirochaeta, Spironema and Treponema; Leptospiraceae including Leptonema and Leptospira; and finally Brachyspiraceae containing the intestinal spirochetes of Brachyspira (Serpulina). Brachyspira aalborgi (B. aalborgi) and Brachyspira pilosicoli (B. pilosicoli), the two members of Brachyspiraceae family, are both described in humans and are considered as cause of human intestinal spirochetosis [2].

The prevalence of spirochetosis in human varies from 2.5 to16% in western countries. The prevalence in homosexual and immune-compromised patients, based on stool culture and biopsy finding was as high as 50%. In human, the pathological and clinical significance of these organisms is far less clear and controversial although there have been reported cases associated with rectal bleeding and diarrhea [3]. Spirochaetes are difficult to grow in culture media and are not detected by routine examination of stool, so diagnosis usually requires a biopsy specimen of the colon. Typical histological findings on the biopsy specimen including a band-like growth of spirochetes adherent to the colonic luminal surface, giving an accentuated brush-border appearance. Special stains including Giemsa, periodic acid-Schiff and silver stains are used to visualize the organisms, although most cases can be identified readily on sections stained with haematoxylin and eosin [4,5].

Treatment strategies have been proposed for intestinal spirochetosis eradication, including macrolids and clindamycin, but metronidazole seems to be the drug of choice, with a dose regimen of 500mg 3 times a day for 10 days in adults and 15mg per kg bodyweight 3 times per day for 5 days in children [2].

The significance of spirochetosis in humans is debatable. Takuchi et al and Gear and Dobbins considered the presence of spirochetosis insignificant as they did not correlate with the symptoms or the presence of mucosal inflammation. However, Gad et al and Douglas and Crucioli reported cases of diarrhea and rectal bleeding which improved after treatment with metronidazole.[6] In the 3 cases reported by us, 2 patients presented with diarrhea and one patient presented with rectal bleeding. All the three patients obtained symptomatic relief with a course of metronidazole.

In conclusion, spirochetosis should be considered as a possible diagnosis in patients presenting with diarrhea and rectal bleeding. A high degree of suspicion is required to identify the typical histopathological picture of a false brush border, a finding that can easily be missed.

Learning Points

- Intestinal spirochetosis can present with diarrhea or rectal bleeding
- Histopathological features include a haematoxyphilic false brush border.
- Silver stains like Warthin Starry help in the confirmation of the diagnosis.
- Treatment is simple and a course of metronidazole provides symptomatic relief.

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ETP ALL or Mixed Phenotype Acute Leukemia: Diagnostic Dilemma in Acute Leukemia with Simultaneous Expression of Thymic and Myeloid Markers

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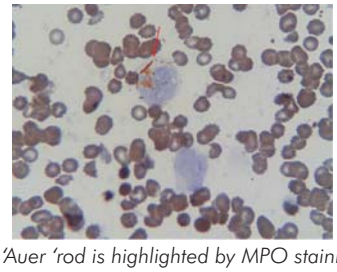
Introduction

Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is most immature subtype of thymic (T) precursors cell ALL, capable of expressing one or more stem cell markers and /or myeloid markers (1). Among T-cell lineage markers, ETP-ALL blasts are negative for CD1a, CD8 and sCD3. CD5 is weak (<75% cells positivity) or negative. This entity is not adequately described in 2008 WHO classification of hematolymphoid neoplasms. ETP-ALL has a poor treatment outcome and high relapse rate. Cytogenetic findings are not disease specific but may have some myeloid associated mutations. On the other hand, the T/Myeloid subtype of mixed-phenotypic acute leukemia (MPAL), as defined by the WHO 2008 classification, can resembles ETP–ALL. Flow cytometric immunophenotyping is essential for the diagnosis of MPAL. For assigning T-lineage to the blast cells, either cCD3 or sCD3 should be positive and for myeloid lineage either Myeloperoxidase (MPO) or monocytic markers should be expressed by these cells (2). Here, we report the case of a 35-year old female whose blast cells had immunophenotypic features favouring the diagnosis of ETP-ALL but cytochemical findings showed MPO positivity in these cells along with the presence of 'Auer' rods thereby questioning their ETP phenotype and suggesting the diagnosis of MPAL.

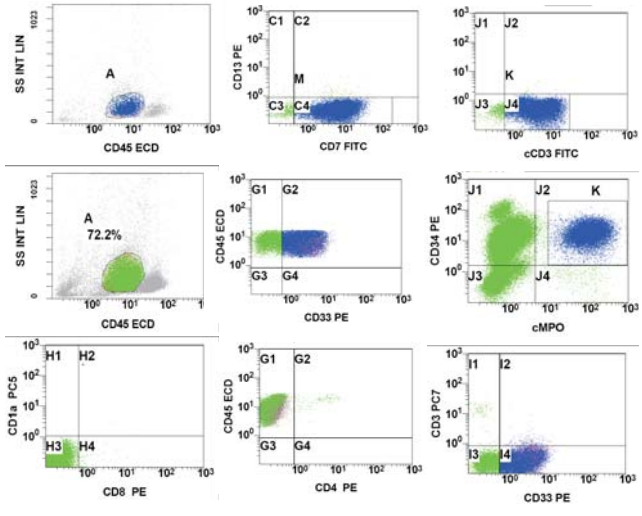
Case Presentation

A 35-year female presented with fever, weakness and petechial hemorrhage. Laboratory investigations showed leucopenia (820/microL), low hemoglobin (4.8g/dL) and low platelet count (15000/microL). Morphological examination of bone marrow revealed ~75% blast cells with coarse cytoplasmic granulation in a number of these cells. Some of the blasts also showed slender 'Auer rods' (Figure 1) that was more evident in MPO stained bone marrow smears.

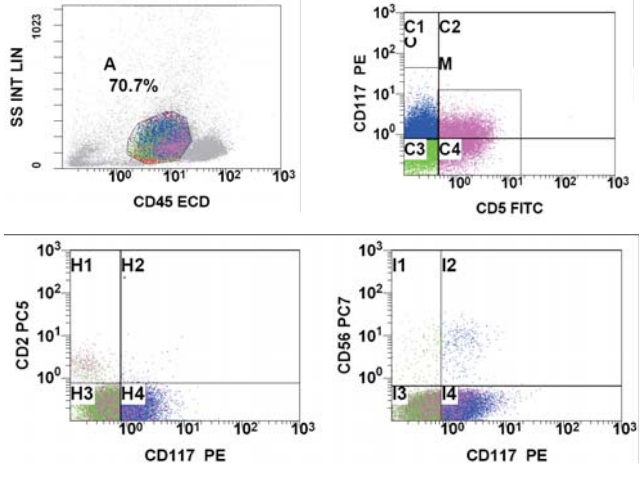


Blast with 'Auer 'rod is highlighted by MPO staining (arrow)

In Flow Cytometric immunophenotyping the gated population of blast cells in CD45/SSC dot plot expressed immaturity markers [CD34 and CD117], myeloid marker [CD33] and T lymphoid markers [cytoplasmic CD3, CD7 and CD5 (dim and partial)]. Other T-lymphoid markers such as CD1a, CD4, CD8 and surface CD3 were negative in blast cells thereby suggesting their early T-precursor phenotype. Interestingly, cytoplasmic MPO was expressed only in a subpopulation (9%) of the gated CD34 positive blast population, corresponding to the low percentage (6%) MPO positivity in blast cells in the bone marrow smears. Based on cytochemical MPO positivity, the presence of 'Auer' rods and the above immunophenotypic features of the blast cells the diagnosis of MPAL – T/Myeloid was preferred to ETP-ALL.



Gated blasts population expressing CD33, CD7, cCD3 and MPO positivity in 9% of blasts cells (K window); Blasts are negative for CD1a, sCD3, CD4 and CD8



Gated blasts show weak CD5 and CD117 positivity and negative for CD2 and CD56

Discussion

In flow cytometric immunophenotyping of acute leukemia, positivity for a surface marker is based on expression of the marker in more than or equal to 20% of blast cells and for the cytoplasmic markers the cutoff of 10% or more has been suggested in some studies (3,4). However, there is a lack of consensus on these cutoffs, especially for the cytoplasmic markers. According to the 2008 WHO guidelines, MPO is a myeloid lineage specific marker but the guidelines did not define the cutoff for percentage positivity for MPO by flow cytometry. On the other hand, the cutoff of 3% has been described for cytochemical MPO positivity in morphological analysis of bone marrow smears. Furthermore, the outcome and interpretation of immunophenotyping data can be affected by the type of sample, aging of the sample, hemodilution of the bone marrow sample, the presence and percentage of normal myeloblasts, reagents and fluorochromes used, method and techniques followed etc. One or more of these variables can lead to false positive or negative results. In our experience cytochemical MPO staining in conjunction with flow cytometry data has been found more useful in sorting out the abovementioned issues than either modality of diagnosis alone; but in some cases the dilemma could persist in spite of applying both. This is well demonstrated in the case under discussion in that the presence of MPO positive 'Auer rods' in addition to ~6 % MPO positive blast cells in bone marrow smears and ~9% MPO positivity in flow cytometry confirmed the concomitant 'myeloid' phenotype and the neoplastic nature of a subset of blast cells, in addition to their early T-precursor phenotype, i.e. T/Myeloid MPAL phenotype.

The blast cell phenotype of CD33, CD117, CD34, cCD3, CD7 positivity, dim CD5 expression and CD4, CD8, CD1a and sCD3 negativity in the absence of other B cell markers favours the diagnosis of ETP-ALL in our case (Figure 2 and 3). This is the most immature subtype of T-ALL associated with myeloid antigen expression. Distinguishing these cases from true MPAL is difficult due to similar immunophenotype of blast cells in the two conditions complicated by nebulous diagnostic criteria as outlined above, but this distinction is important in view of different therapeutic approaches in the two conditions(5). All of the above however, also point to the fact that in ontogeny, cells of the myeloid and the T-lineage share a common origin at the stem cell level and leukemic blast cells expressing both T-lymphoid and myeloid phenotypes represent neoplastic cells at an early stage of maturation and differentiation in the common T/Myeloid lineage. This possibility is also supported by other cellular and molecular evidences; both old and new (6).

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Renal Hypodysplasia with Mesonephric Duct Hyperplasia Resembling Epididymis

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Summary
Embryologically, the mesonephric ducts also known as Wolffian ducts develop into epididymis, Vas deferens and seminal vesicles under the influence of testosterone in males. In females the structures usually regress completely. Sometimes the mesonephric duct remnants can persist in the genitourinary system and pose a diagnostic challenge both clinically and histologically [1]. Mesonephric duct remnants have been reported in the spermatic cord, vas deferens, urethra, prostate and renal pelvis. In females, the mesonephric ducts can present as Skene's glands as well as Gartner's duct or cyst. They may be asymptomatic or present with symphoms like vaginal bleeding or cervical mass lesion [2-4]. Embryologically the kidneys develop from the metanephric blastema and the ureteric bud which itself is an outgrowth of the caudal mesonephric duct. Abnormalities of metanephric blastema or ureteric bud differentiation give rise to congenital malformations of the kidney resulting in aplasia, hypoplasia or dysplasia. Presence of mesonephric duct remnants, their hyperplasia in a structurally abnormal kidney is rare. We present here a case of unilateral hypodysplastic kidney with mesonephric ductal hyperplasia in a twelve year old male child.

Case Presentation
A 12 years old male presented to the nephrologist with recurrent UTI. A plain and contrast CT study of the KUB region revealed a normal right kidney and a smaller left kidney. Left pelvicalyceal system and left ureter were moderately dilated and renal parenchyma was thinned out. No calculus was seen. The CT report stated small left kidney with left moderate hydronephro-ureterosis. Subsequently the patient underwent nephrectomy of the left kidney and the specimen was received in the Laboratory.

Gross findings: The kidney measured 6 x 2 x 3 cm, appeared shrunken grossly. It weighed only 70 grams. Ureter attached to the specimen measured 4.7 cm in length and was dilated. Cut section of the kidney was grey white with fibrous areas.

Microscopic findings: The renal parenchyma was markedly thin and hypoplastic. The renal pelvis was dilated. The cortico-medullary differentiation was lost and there was lobar disorganisation. The parenchyma showed hyperplastic aberrant ductular structures [Fig 1]. The ducts were lined by columnar ciliated epithelium and resembled the epididymis [Fig 2]. The nuclei were uniform and did not show any atypia. An acinar or tubular pattern of mesonephric ducts lined by cuboidal epithelium was also identified.

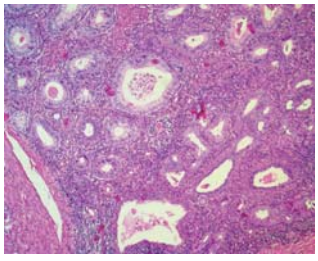

Fig 1 Showing mesonephric duct remnant hyperplasiaX100 (Large)

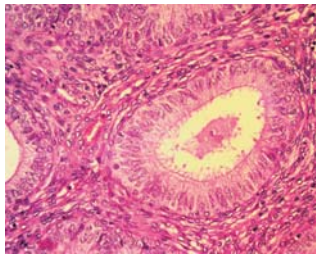

Fig 2 showing epididymis like ductal structures lined by brush border epithelium x 400 (Large)

The surrounding stroma showed dense chronic inflammatory cell infiltrate. Some of the tubules showed thyroidisation [Fig 3]. The number of glomeruli was markedly reduced. The ureter was dilated.

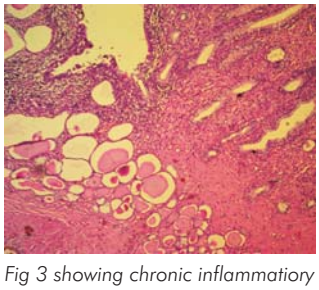

Fig 3 showing chronic inflammatiory cell infiltrate & thyroidisation of tubulesx100 (Large)

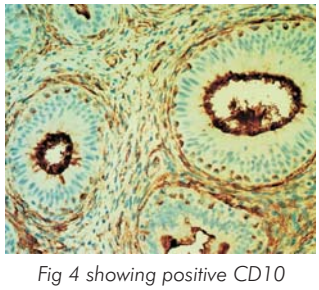

Fig 4 showing positive CD10 staining of brush border epithelium of epididymis like structures x 100 (Large)

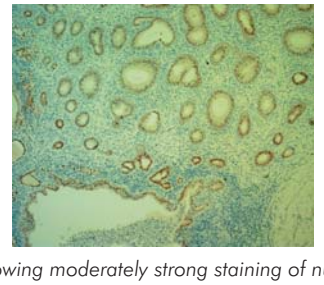

Fig 5 showing moderately strong staining of nuclei with PAX 2x100 (Large)

Immunohistochemistry studies for CD 10 and PAX 2 were performed. The CD 10 stain was positive in the brush broder of lining epithelial cells of the ducts [Fig 4]. PAX-2 showed moderately strong nuclear staining of the aberrant ducts [Fig 5].

These immunohistochemical stains supported their mesonephric origin and in conjunction with the histomorphology of H & E stained sections a diagnosis of renal hypo-dysplasia with mesonephric duct hyperplasia with mixed epididymis and acinar or tubular morphology was rendered.

Discussion

Congenital anomalies of kidney can be broadly classified as agenesis, aplasia, hypoplasia. They can be associated with family history commonly [6]. In our case however we could not trace the family history.

Embryologically, the mesonephric ducts develop into the urinary and male reproductive system giving rise to the ureter, renal pelvis, collecting tubules, ducts deferens, ejaculatory ducts and seminal vesicle, under the influence of testosterone. Most of the embryonic mesonephric ducts are eventually replaced by metanephric ducts to form the permanent kidney. Mesonephric ducts remnants that have been reported in renal pelvis, spermatic cord, vas deferens urethra, prostate and prostatic urethra have been in the form of small acinar or tubules lined by cuboidal to low columnar epithelium [1]. Epididymis like mesonephric remnant have recently been reported in the prostate and kidney [1-2]. Similar to the case reported by Xiao G Q et al, our case also showed epididymis like mesonephric remnant proliferation along with acini-tubular patterns associated with hypodysplastic kidney.

Incidence of unilateral kidney has been reported as 1 in 500 autopsies. A kidney can be small because of congenital hypoplasia or pyelonephritic shrinkage or both. Simple renal hypoplasia which means small kidney with reduced nephrons is distinct from renal dysplasia which shows parenchymal malformation. In renal hypodysplasia, small kidney (reduced number of nephrons) are further associated with dysplastic features. Concurrent hypoplasia and dysplasia are more common than hypoplasia alone.

When renal malformations are associated with lower urinary tract abnormalities, they constitute a spectrum of disorders known as congenital anomalies of kidney and urinary tract (CAKUT). CAKUT occurs 1 in 500 live births and account for 40 - 50 % of chronic renal disease in children [8-10].

In our patient, the kidney showed features of hypodysplasia as well as ipsilateral ureteric abnormalities. Congenital anomalies of kidney can be caused by genetic or environmental factors. When unilateral, the anomaly may go unnoticed until adulthood when symptoms appear. In our case, the patient was diagnosed at the age of 12 when he was suffering from repeated urinary tract infections and subsequently underwent imaging investigations followed by nephrectomy.

Differential diagnosis

Intrarenal parenchymal mesonephric recurrent hyperplasia should be differentiated from neoplastic nephron and Wilm's tumour. They can be diagnosed based on presence or absence of mass lesion, their histomorphology and aided by immunohistochemical studies.

Management

Treatment depends upon the degree of chronic renal disease and presence of associated conditions that may worsen renal damage. A conservative approach is preferred if the contralateral kidney is normal [8].

This case is interesting because it is a rare congenital abnormality. It is important to distinguish it from neoplastic processes as discussed under differential diagnosis. Genetic studies, prenatalUSG studies and family screening has a role in early diagnosis and better management and prognosis of the disease.

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lymph nodes by direct invasion. Tumors also spread by perineural invasion and the hematogenous route. Immunoperoxidase stains show that the neoplastic cells have a variable ductal and myoepithelial phenotype, the cells expressing cytokeratin and also vimentin, smooth muscle actin, calponin, S-100 protein, p63, and GFAP. The surrounding matrix recapitulates a basement membrane like material in that it stains positive with antibodies directed at Type IV collagen, laminin, and heparin sulfate. Primary treatment is surgery with supplemental radiation, especially by linear accelerator. Poor prognosis is related to stage of the tumour at the time of diagnosis, the presence of positive margins, and a solid cellular growth pattern.

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Neurocytoma - A Rare CNS Tumor

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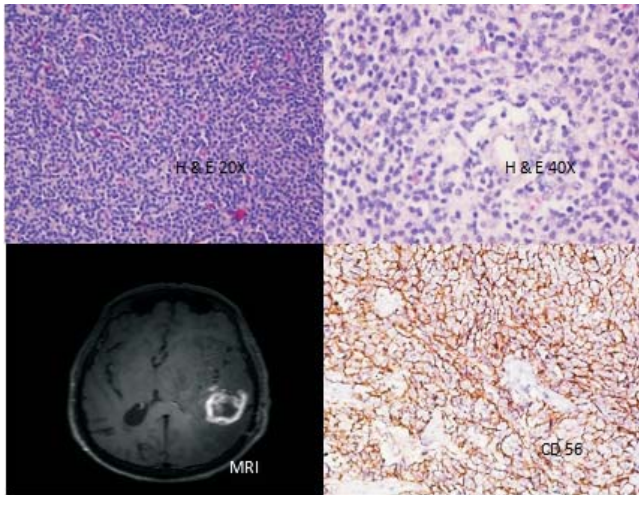
Summary
Neurocytomas are rare CNS tumours with an incidence of less than 1% of all CNS tumours. Central neurocytomas (CN) and rarer variant the extra ventricular neurocytoma (EVN) are known usually both types are benign recently atypical features have been described associated with aggressive behavior of tumor. We describe a case of classic Central neurocytoma and a case of extra ventricular neurocytoma with atypical features.

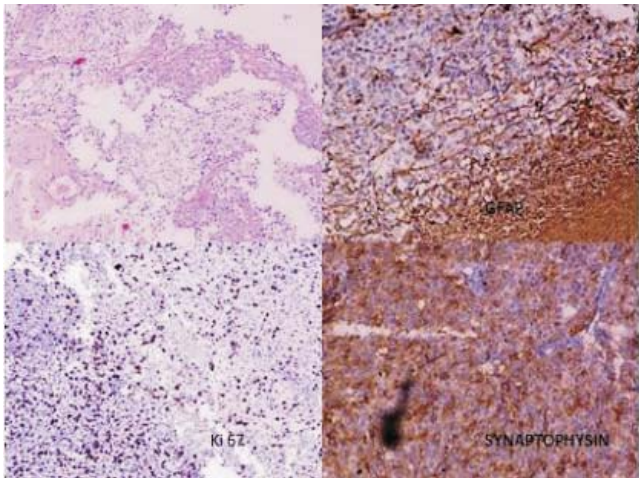
Background
CN usually involving the lateral ventricles in young adults and display characteristic neuroimaging and histomorphologic findings. EVN have described in cerebrum, cerebellum and spinal cord. Both variants are usually benign and when adequately resected are associated with good prognosis. Recently certain atypical features have been described which are associated with aggressive behavior of tumor and increased incidence of local recurrence. These features include >2% of Ki67 labeling index, presence of mitosis, necrosis and microvascular proliferation. Such cases may require adjuvant therapy.

Case Presentation
Case 1:
A 33 year old man presented with headache and weakness of right lower limb for 15 days. Brain MRI revealed a left lateral ventricular mass. This on histopathology showed tumor composed of round cells with arborizing blood vessels. Cells do not show significant pleomorphism or mitotic activity. No necrosis and microvascular proliferation is noted. Small areas show neuropil. IHC shows tumor cells do not express Glial fibrillary basic protein (GFAP) or epithelial membrane antigen (EMA). Tumor cells express Synaptophysin and CD56. Ki 67 labeling index is 1.5%. This case was Central neurocytoma WHO grade II.



Neurocytoma (20X and 40 X), tumor composed of round cells with arborizing blood vessels. Cells do not show significant pleomorphism or mitotic activity and tumor cells expressing CD56

Case 2:
65 year old man presented with aphasia and right hemiparesis for one year. Neuroradiology showed a tumor in left parieto-temporal region with radiological features suggestive of a high grade glioma. Histomorphology shows tumor composed of round cells with thin vasculature with areas of hypercellularity with brisk mitotic activity. Focal necrosis and microvascular proliferation also noted. IHC shows tumor cells do not express GFAP or EMA. Tumor cells express Synaptophysin and CD56 with Ki67 labeling index of 30%. P53 is also over expressed. This case was diagnosed as atypical neurocytoma.



EVN - Tumor composed of round cells with microvascular proliferation, Tumor cells do not express GFAP but Synaptophysin and Ki 67 is 30%

Differential Diagnosis

Purely on histomorphology this tumour can be mistaken for oligodendroglioma and for intraventricular tumour ependymoma is a close differential. For differential diagnosis IHC is required. The ultrastructure studies and molecular tests also help in confirming the diagnosis.

Discussion

Neurocytomas express Synaptophysin and other neuronal markers like CD56, neu-N etc which are characteristically negative in oligodendroglioma. In contrast oligodendrogliomas show deletion of 1p/19q not seen in neurocytomas. Both types of neurocytomas when resected adequately have a good outcome. When adequate surgery is not possible or when these tumours have atypical features like Ki67 labeling index > 2%, high mitotic activity, necrosis or microvascular proliferation, the tumours are likely to have a more aggressive behavior and likely to recur. Rades ét al 2004 have documented that complete resection in atypical EVN offers better local control and survival rate. Survival is improved with post surgery radiotherapy and recently adjuvant chemotherapy has also shown improved survival.

Learning Points

1. Central neurocytoma is intraventricular tumor of lateral ventricles in young adults
2. Use of appropriate IHC helps in achieving accurate diagnosis
3. EVN is also benign tumor and often difficult to differentiate from oligodendroglioma and use of appropriate IHC is required to come to accurate diagnosis
4. Recently described atypical features are associated with poorer outcome and should be recognized and reported.

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Make a Diagnosis

The stool sample came for identification of parasite from Tanda, Himachal Pradesh. What is the diagnosis?

Contributed by
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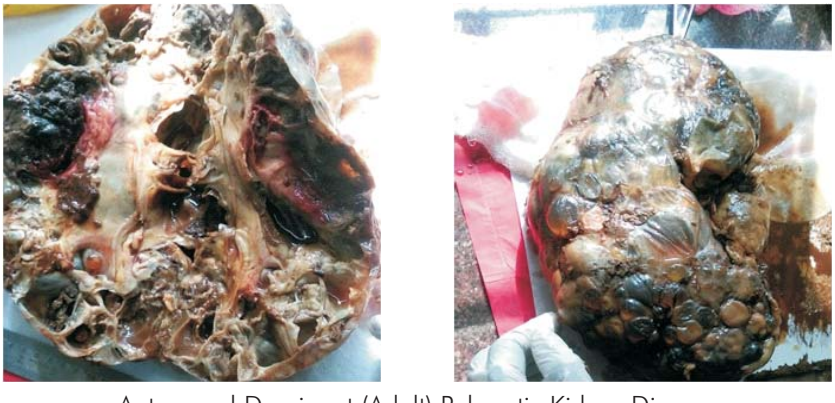
Answer for Picture Quiz (Dec 2016)



Autosomal Dominant (Adult) Polycystic Kidney Disease

SRL Activities

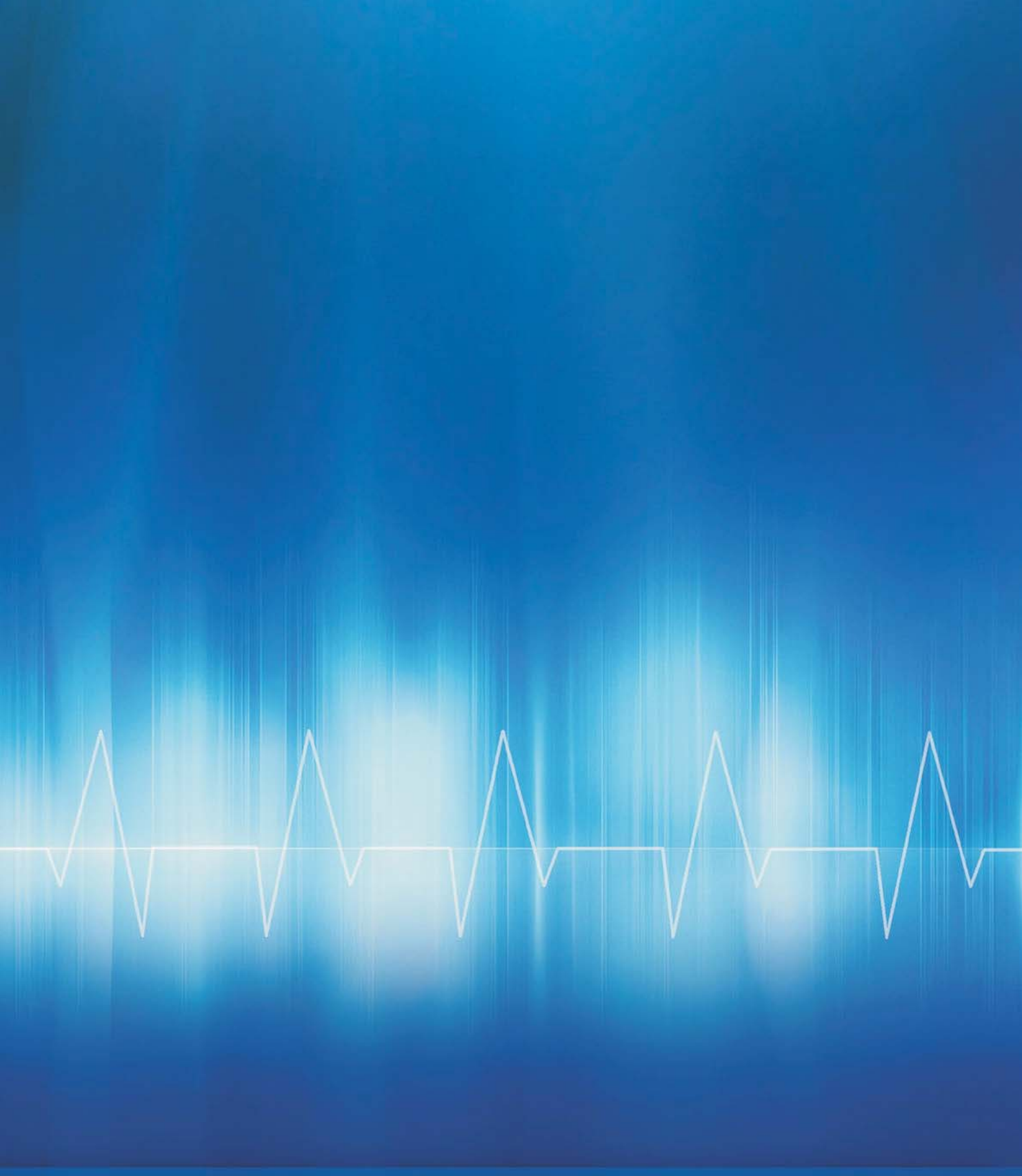
Recent Tests Released

Test	Specialty	Significance
Next Generation Sequencing based Non-invasive prenatal testing (NIPT)	Gynecology	NIPT offers rapid and sensitive means for screening of chromosomal aneuploidies in fetus. It involves sequencing of cell-free DNA in maternal plasma and is a highly effective screening test for Down's syndrome with sensitivity of around 99% and false positive rates of < 0.1% in both high risk and general populations. Screening of other chromosomal aneuploidies such as chromosome 13 and 18 trisomies and sex chromosome aneuploidies is also possible; however current performance of NIPT for these aneuploidies is limited. In India SRL was the first Lab to offer NIPT in the year 2013.
Microsatellite Instability (MSI) – PCR-Multiplex Fragment Analysis MLH1 and MSH2 – Whole Gene Sequencing	Oncology	The two most common genetic conditions associated with a significantly increased risk of colorectal carcinoma (CRC) are familial adenomatous polyposis (FAP) and Lynch syndrome (hereditary non polyposis colonic cancer, HNPCC). In FAP the risk of CRC is up to 100% and in Lynch syndrome the risk is up to 80%. While FAP is majorly associated with mutations in APC gene, over 200 different mutations in Mismatch Repair (MMR) genes: MSH2, MLH1, PMS2, MSH6, MLH3, MSH3, PMS1 are associated with HNPCC. Up to 90% of HNPCC cases are caused by germline (inherited) mutations in the MLH1 (40-50%) or MSH2 (30-40%) genes. Though whole gene mutation testing is most ideal for identifying heritable mutations in HNPCC cases, due to high cost of detecting mutations in multiple genes, pre-screening is done by other two methods: microsatellite instability (MSI) testing by PCR and by immunohistochemical (IHC) analysis.

Recent Publications

1. Bhandari P, Ahmad F, Das BR. (2017) Molecular profiling of gene copy number abnormalities in key regulatory genes in high-risk B-lineage acute lymphoblastic leukemia: frequency and their association with clinicopathological findings in Indian patients. Med Oncol. 2017 May; 34(5):92.

2. Bhandari P, Ahmad F, Mandava S, Das BR. (2016) Association of Genetic Variants in ARID5B, IKZF1 and CEBPE with Risk of Childhood de novo B-Lineage Acute Lymphoblastic Leukemia in India. Asian Pac J Cancer Prev. 2016; 17(8):3989-95.



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