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MARCH 2023

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NABL

Editorial board

From the Editors' Desk

Dear Reader,

The frost clears & makes way to spring. The scenery brightens; the birds chirp & the festival of colour brings new cheer!

We are rejuvenated after celebrating our 'Founder's day', drawing inspiration from our chairman, Dr.Pratap.C.Reddy, who revolutionized healthcare in India & continues to inspire a multitude of healthcare professionals.

We also take this opportunity to thank all our patrons for the immense support we have garnered over the years. We will continue to encouragecontributors to write on their experiences& share their expertise.

In this third edition of the year we showcase two conditions with genetic etiology.Ochronosis & alkaptonuria have been described in medical literature for long, however the Histopathological lesions encountered in this condition may are interesting & are hence reviewed. We share a case reports of Dandy Walker malformation presenting with a numerical chromosomal anomaly. Both our case reports the feature in the March issue have a genetic link, these need to be identified. Such disorders play an important role in patient management.

We have included 2 articles which delve into the subtle aspects of lab medicine. The first article sheds light on the various lesions encountered in histopathology & how terminology in histopathology practice is profound as well as sophisticated. The second article reviews LC- MS, a technology which is gradually replacing traditional immunoassays in referral laboratories.With LC- MS technology largely being adopted for new born screening & for hormone studies traditional immunoassays may be supplanted by the same. In another first time inclusionwe have included an interesting slide from histopathology in a concise format for easy revision. We expect our creative tendencies to gravitate towards biochemistry ballads, serology sonnets & musings from microbiology in the forthcoming issues.

We thank the contributors for taking time to put pen to paper & covering a gamut of topics with aplomb. AD express has gained considerable impetus by the way of your contributions & we welcome more from you all.

Also humbly request you to share your feedback on 'AD express' & we assure you that feedback from you will make each next issue ever more interesting !

Wishing you all a happy Holi & great year ahead!

Best regards,

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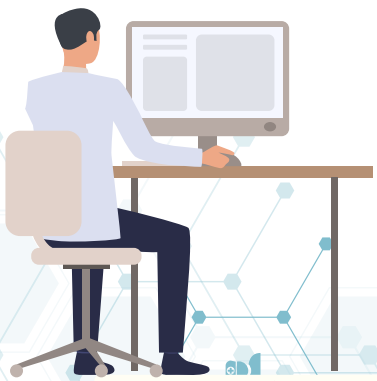

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1. Case Series of Ochronotic Arthritis

Dr. Veena Singh
Consultant Pathologist HLM-RHCC

Abstract:

Ochronotic arthropathy is a rare hereditary metabolic disease found in patients with alkaptonuria. It is associated with deposition of homogentisic acid derivatives in various connective tissues of the body. Joint involvement especially hip and knee destruction is seen. We present two cases of alkaptonuria resulting in ochronotic hip and knee arthritis treated with total hip and knee arthroplasties.

Case report 1

A 58 years old female presented to the emergency department of our hospital with complaints of pain in her right hip after a simple fall , and not able to bear weight on her leg. The anteroposterior radiography of hip joint showed a fracture of neck of the femur (Figure1). Because of a lucency at the base of femur neck, Orthopedic Surgeon came to the conclusion that a pathologic fracture had occurred at that region. She had no other underlying disease except a chronic low back and hip pain. Patient was taken up for Total Hip Replacement and the sample was sent for histopathological examination which revealed ochronotic arthritis (Figure 3).

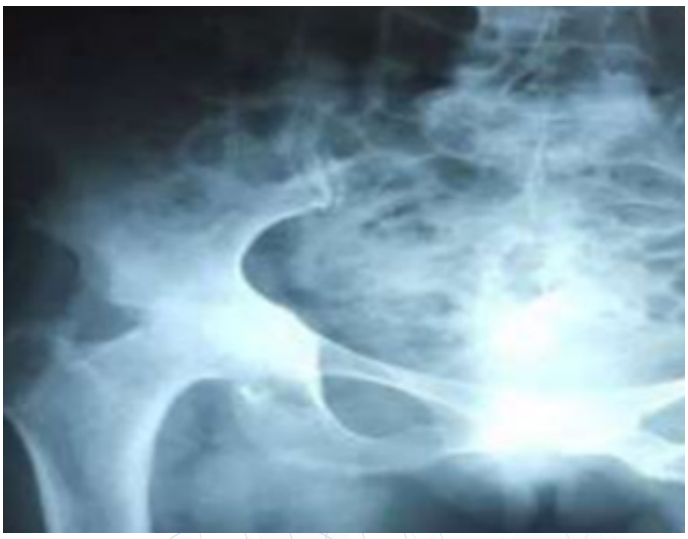

Fig-1: Xray of the hip joint showing AP View

Fig -2: Excised head of femur showing black discoloration

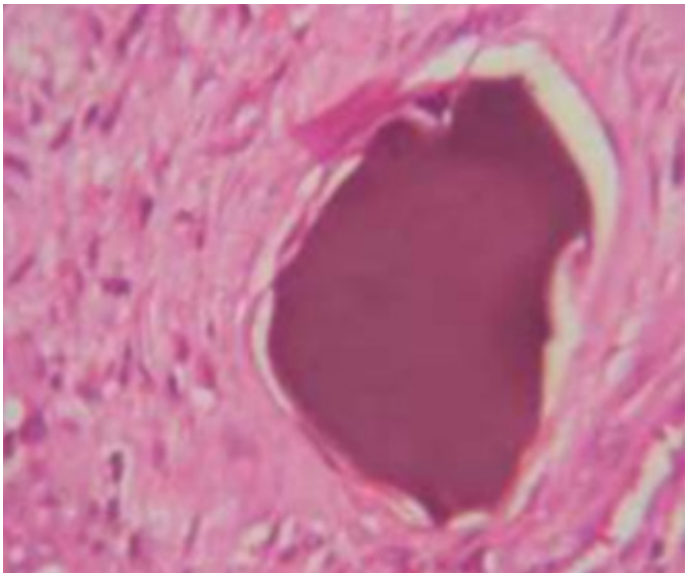

Fig -3: 40 x view showing deposition of brownish material

Case report 2

A 71 year old male came to Orthopaedics department with complaints of multiple joints pain and lowback ache for past 1 year. Pain was non-traumatic, progressive, dull-aching in nature, and aggravated on exertion. X-rays of the cervical and lumbosacral spine showed advanced degenerative changes with intervertebral disk space narrowing and

osteophyte formation. X-rays of the knee joints showed reduced joint space, osteophyte formation with loose bodies, suggestive of osteoarthritis knees (Figure 5). Patient was taken up for total knee replacement (Figure 6). The sample was sent for histopathological examination which revealed ochronotic arthritis (Figure 7)

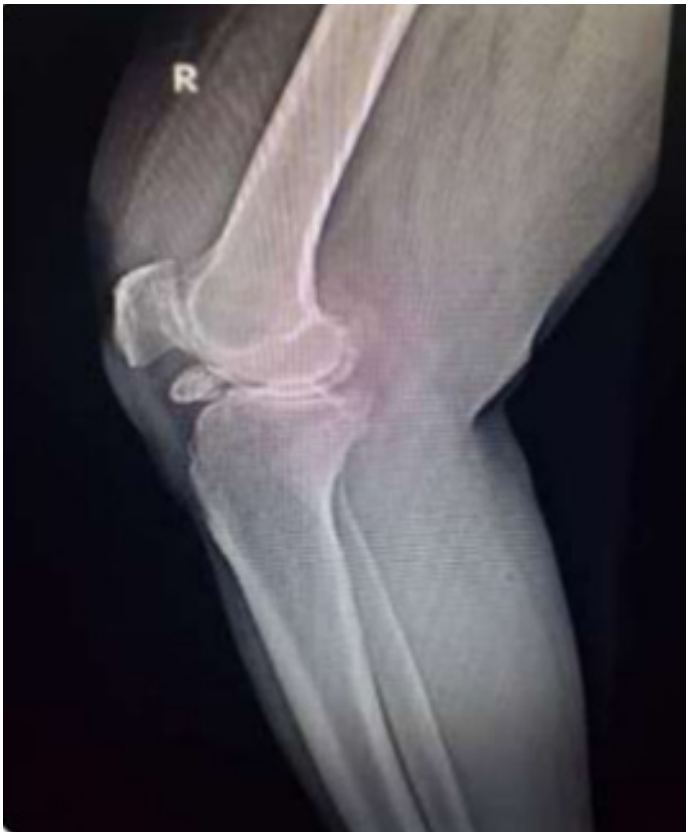

Fig -5: Xray knee joint showing Lateral view

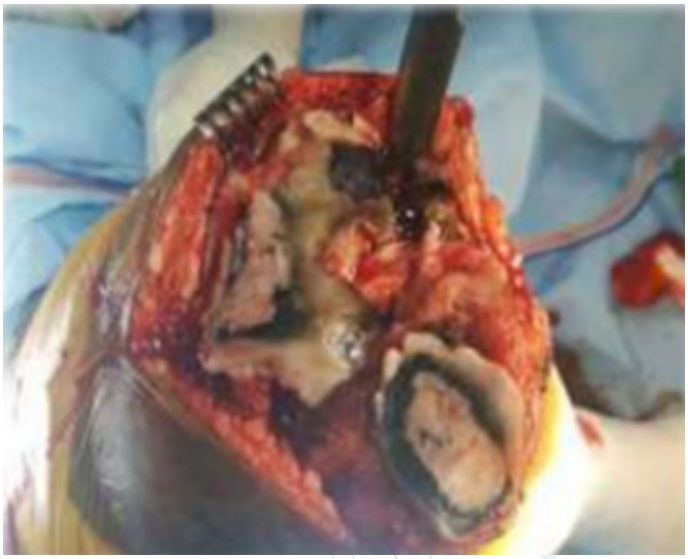

Fig -6: Intra-op blackish discoloration over the patella

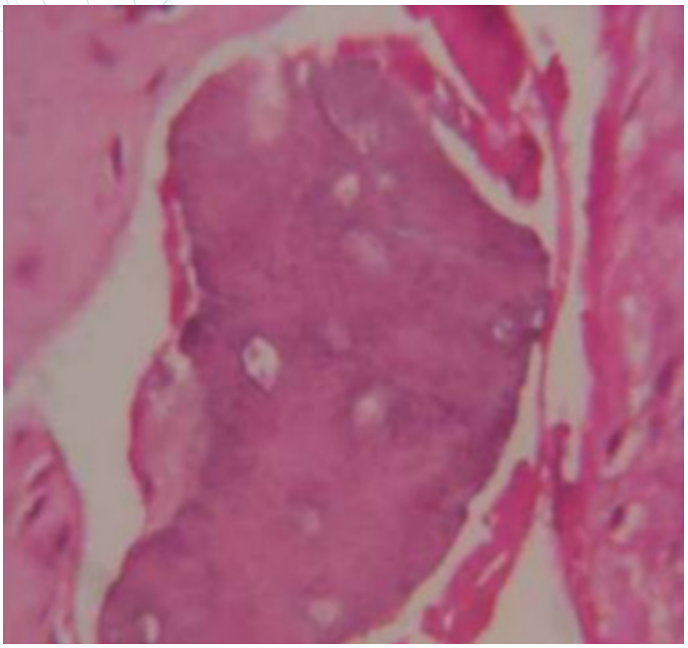

Fig -7: 40 x view showing deposition of brownish amorphous material

Discussion :

Alkaptonuria is a rare disorder with an incidence of 1:125,000 to 1:1 million worldwide (1-4).

It was one of the first conditions in which the law of Mendelian recessive inheritance was proposed and one of the conditions in the group of inborn errors of metabolism. The term ochronosis was first coined by Virchow in 1866 (5) when he found pigmentation of tissues that appeared ochre, meaning yellow, when examined microscopically (6). In alkaptonuria, ochronotic pigment is deposited in all connective tissues, especially cartilage. These pigmentary changes are termed ochronosis. Ochronosis can occur in ligaments, tendons, heart valves, the intima of blood vessels, sclera, and the skin (3). The majority of alkaptonuria symptoms are not detected until the fourth decade (3,7-9).

Conclusion :

The management of ochronotic arthropathy in alkaptonuria patients is usually conservative, but replacement surgery is recommended for severely affected hip and knee joints. This report describes two cases of ochronotic hip and knee arthritis treated with total hip and knee arthroplasties. Since alkaptonuria is a rare disease, it may be beneficial to pre-screen patients with degenerative disease for signs of

ochronosis, as this could aid in the pathologic diagnosis. Additionally, an early diagnosis can be made and an effective conservative management can be started to improve the quality of life. At follow-up of 6 months, both patients with total hip and knee replacement are normal. In these patients, morbidity and complications are significantly decreased by early diagnosis and management.

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2. Rare chromosomal abnormalities in products of conception – A rare case of Dandy Walker syndrome reported in Apollo Diagnostics Global Reference Laboratory, Hyderabad.

Shubhangi Miryala, Preethi Pattamshetty, Dr.Vasavi Narayanan
Department of Genetics. Global Reference Laboratory, Hyderabad.

Case presentation:

29-year old, primigravida with gestational hypothyroidism, at 13+ weeks on scan, fetal weight < 5th centile

Fetal head was disproportionately large, VSD (1.1mm) with single outflow tract.

Diagnosis :- Dandy-walker malformation with occipital encephalocele.

It is rare for a Dandy-Walker malformation to present with an occipital encephalocele. Neural tube defects such as Myelomeningoceles are described in Arnold-Chiari malformation rather than Dandy- Walker malformation.

Dandy-Walker malformation is characterised by an enlarged posterior fossa, a rudimentary cerebellar vermis & a midline ependymal cyst.

Chromosome analysis of the stimulated and cultured cells in this case revealed a triploid chromosome number (3n).

Karyogram: Cytogenetic analysis of cultured cells from POC show 58-70, XXX karyotype with triploidy seen in the cells scored.

Concise discussion:

- Triploidy syndrome is a chromosomal disorder in which a fetus has three copies of every chromosome instead of the normal two. If this occurs in only some cells, it is called mosaic triploid and is less severe.
- The triplication of the chromosomes is caused by the fertilization of an egg by two sperms or the fertilization of an egg by a sperm that has an extra set of chromosomes (diandric) or by the fertilization of an egg that has an extra set of chromosomes by a normal sperm (digynic).
- Pregnancies caused by digynic fertilization are more likely to end, right before a baby is due for full term. Pregnancies caused by diandric fertilization result in a miscarriage towards the first trimester.
- Diandric triploid pregnancies have twice the risk of recurrence as compared to the general population while the same is not established with regard to digynic pregnancies.
- Triploidy affects approximately 1–3% of pregnancies and most embryos with triploidy are known to miscarry early in development.A few affected babies have been reported to have survive beyond childbirth to a couple of months, but suffered from developmental delay, learning difficulties, seizures, hearing loss and other abnormalities.
- At birth, males with triploidy are 1.5 times more common than females. The pregnant mother carrying a triploid fetus sometimes experiences preeclampsia with increase in blood pressure, edema, and albuminuria.
- Single nucleotide polymorphism (SNP)-based NIPT can identify triploidy by detecting the presence of the additional haploid chromosome set. For results

with an extra haplotype, ultrasound is recommended to establish viability, evaluate for viable or vanished twins and detect findings consistent with triploidy.

- Review of patient history, serum screening, and ultrasound will reduce the number of CVS or amniocenteses necessary to confirm a diagnosis of triploidy.
- Abnormal levels of specific maternal blood proteins such as alpha-fetoprotein, human chorionic gonadotropin, estriol and pregnancy-associated plasma protein-A have been associated with an increased risk for triploidy.

Take home message:

All pregnancies must be evaluated by mid trimester ultrasound scan for fetal structural anomalies.

50–60% of spontaneously aborted products of conception have been detected with chromosomal abnormality

Parental balanced translocations or inversions can produce abnormal gametes and present with recurrent miscarriages.

Hence, karyotype testing is recommended as an important part of genetic evaluation of parents with recurrent pregnancy loss.

Genetic testing in early pregnancy will be essential in all these cases especially where fetal anomalies are recorded.

It is important to counsel these families about recurrence risk after suitable testing, for further pregnancies.

Pre-test counselling is needed to discuss the various limitations and diagnostic scope of specific genetic tests, for optimal benefit to the patient.

3. Adjectives of the Pathology lexicon

Dr.Marquess Raj
ZTC, Tamilnadu & Pondicherry

Foreword:

Pathology terminology has evolved over time since Virchow coined the term 'leukemia' after being impressed by the large buffy coats that he was diligent enough to observe in leukemic samples. Unique puns, words & figure of speeches line the avenues of the Pathology lexicon. These subtle nuances of rhetoric, wordplay & grammar find their way to our day-to-dayreporting. The creativity & imagination of the observer result in such typical findings with striking terminology.

While clinical medicine is replete with triads, aphorisms & signs named after the doyens of medicine, pathology does not lag behind. In fact, the 'father of pathology' features in many a medical eponym (1).

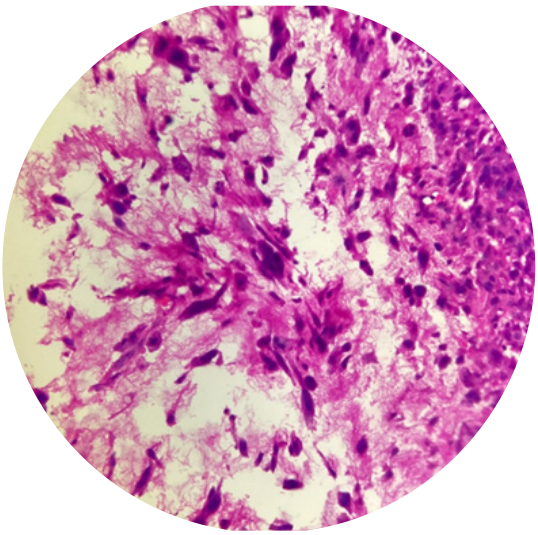

Fig: Bizarre nuclei in a sarcoma (Image from Apollo Diagnostics, RRL, Chennai)

Histopathologist's use words such as 'interspersed' & 'amidst', while describing blood vessels present in between stromal cells. While adjectives such as 'brisk' & 'bizarre' find their way to describe mitoses or the nuclei of certain sarcomas, terms such as 'hyaline'& 'myxoid' can be challenging to interpret, prompting the reader to fish about in a search engine(2&3).

Tongue twisters such as 'ciliocytopthoria' & 'pulmonary placental transmogrification' is very much part of pathology parlance. While the former can be prosaically described as loss of cilia & latter definitely deserves more elaboration(4).

Metaphors of sophisticated prose are often used by sagacious teachers so that the corresponding morphology stays entrenched in the minds of their budding wards. 'Carrot shredded collagen','staghorn vessels'& 'raindrops falling from the sky' are typical analogies (5).

The recognition of patterns on low power is a key skill without which tumour's can whisk away undetected in a wink. Spindle cell lesions in histopathology are akin the 'Aladdin's cave' in pathology & harbor boundless treasure. A multitude of patterns are described, whilestoriform & fascicular are commonplace,' herring bone' & 'tissue culture like pattern' border on the exotic(6).

Considering that the microscopic images are essentially a window to a world in which a chosen few revel. A gamut of awe evoking appearances such as 'starry sky appearanceto the poetic 'butterflies sitting on a fence appearance'can evoke memories of sonnet 18(7).

There could be a separate write up on bodies alone as pathology is well stocked with the same. Many bodies are named after people who described them first, such as Mallory body. Some could be meekly abbreviated but yet be profoundly important in the context of reporting(8).

Footnotes :

1. Eponyms named after Rudolf Virchow : Virchow's triad, Virchow – Robin space, Virchow node, Virchow cells, Virchow –Seckel syndrome, Virchow's theory, Virchow's technique of autopsy & Virchow's law

2. The numbers of mitoses are commonly reported per high power field (HPF).The malignant cells of certain sarcomas such as undifferentiated pleomorphic sarcoma can have bizarre nuclei.
3. Hyaline is terms used to describe acellular material which is eosinophilic & glassy. Myxoid is used to describe tissue rich in mucopolysaccharides which on routine H & E sections takes up a distinct pale blue staining which is best appreciated when seen than described.
4. Ciliocytopthoria is a degeneration of the respiratory epithelium characterized by loss of cilia & decapitation of the apical part of the ciliary epithelium, following adenovirus infection.Placental transmogrification or placentoid bullous lesion of the lung is an unusual condition in which the alveoli develop a peculiar villous configuration that resembles placental villi at low microscopic magnification.
5. Carrot shredded collagen is used to describe the stroma of neurofibromas. The stromal vessels in hemangiopercytoma resemble the antlers of a deer.The vertically oriented melanocytes of a Spitz nevus bear semblance to raindrops falling from the sky!
6. Many spindle cell tumours can have a storiform pattern, but it is typically seen in solitary fibrous tumour. Tissue culture like pattern is described in nodular fasciitis.
7. Sonnet 18 – William Shakespeare Shall I compare thee to a summer's day?

   Thou art more lovely and more temperate:

   Starry sky appearance is described in Burkitt's lymphoma.

   Butterflies sitting on a fenceare noted in bronchoalveolar carcinoma!
8. Mallory bodies are named after Frank Burr Mallory. They are seen in many other conditions apart from the commonly described alcoholic liver disease.EGB is a common abbreviation used to denote 'eosinophilic granular bodies'.

4. Can LC-MS everoutbeat the immunoassays ?

Dr. Eswari Dodagatta-Marri
Molecular biologist, GRL Hyderabad

Foreword:

The measurement of molecules of miniscule proportions in laboratories has evolved over time. The latest technology on the horizon, LC-MS is being adopted by most referral laboratories owing to superior quantification of matter & other distinct advantages.

The advent of immunoassays helped laboratories diverge from traditional RIA's which were time consuming as well as carried lab & environmental safety hazards due to the use of radioactive material in RIA based tests. RIA performed using radioactive materials with long incubation time points has become a redundant procedure nowadays & has been largely replaced by safer methods such as immunoassays (Fig 1) (1).

ELISA is a common plate-based immunoassay platform performed for detecting and quantifying soluble substances like proteins, peptides, antibodies and hormones (Fig 2a). The antigen is immobilized on to a solid surface and then either directly or indirectly captured using the antibody on the antigen(Fig 2b) (2). The detection is measured by the activity of reporter enzyme after incubating with appropriate substrate.

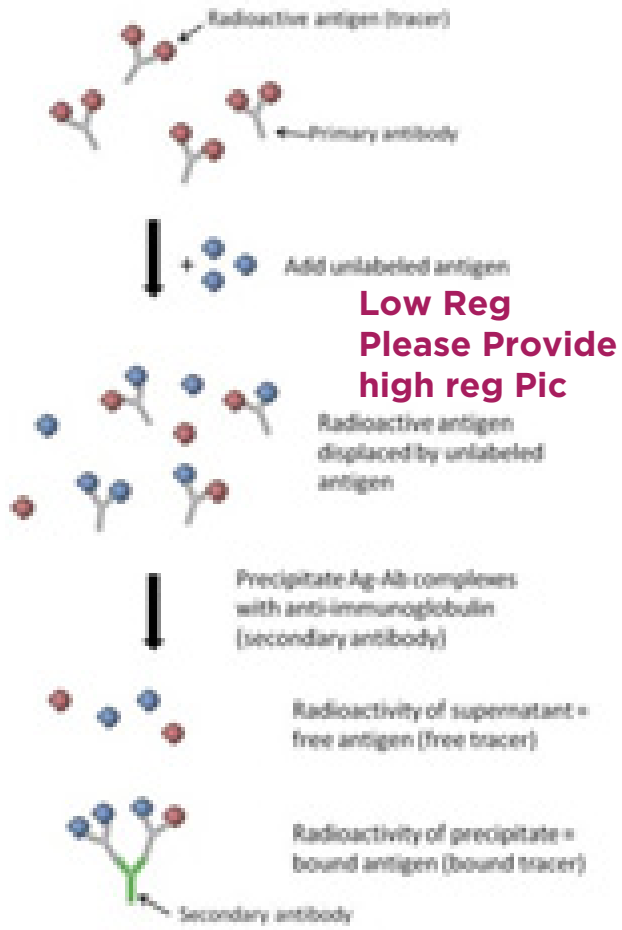

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Figure 1 (Perkin Elmer) Figure 2a

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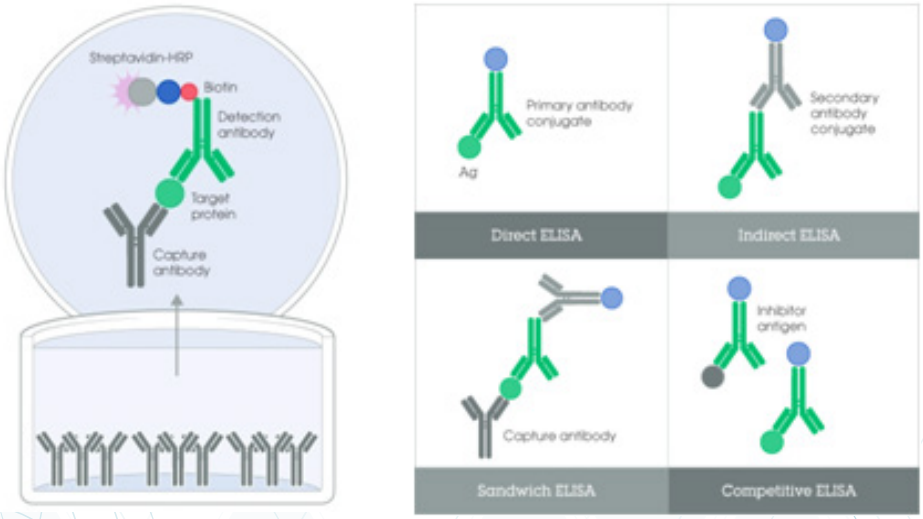

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Figure 2b

Although the immunoassays have been in the field for over 50 years and have advantages of ease, acceptance, cost-effectiveness, throughput and sensitivity, the limitations when compared to latest technology is dependent on the specificity of the antibodies used, sample volume required (i.e,100-200 QL) also is higher.

Liquid Chromatography (LC) is an analytical technique based on the physical separation of analytes facilitated only with few microliters of sample.The sample which is injected into a flowing stream of a solvent called the mobile phase. Depending on the chemical composition, physical properties and the reaction between the analytes in the mobile phase and the stationary phase in the column, LC separation takes place as follows;

1. Partition Chromatography- Differences in solubility and hydrophobicity
2. Ion-exchange Chromatography- Based on ion charges
3. Size-exclusion Chromatography- based on size difference
4. Affinity Chromatography- Ability to bond with the stationary phase, higher the bonding later is the elution (3).

Mass Spectroscopy (MS) is based on the principle of mass-to-charge ratios which is helpful in the detection and quantification (4). The combination of two techniques, liquid chromatography and mass spectrometry, LC-MS is widespread technology (Fig 3a) (1). in referral laboratories in this era. Laboratories use a LC-MS/MS instrument (Fig 3b) with single quadruple mass spectrometer detector for high analytical specificity, sensitivity, fast scan speed and ease (2,6).

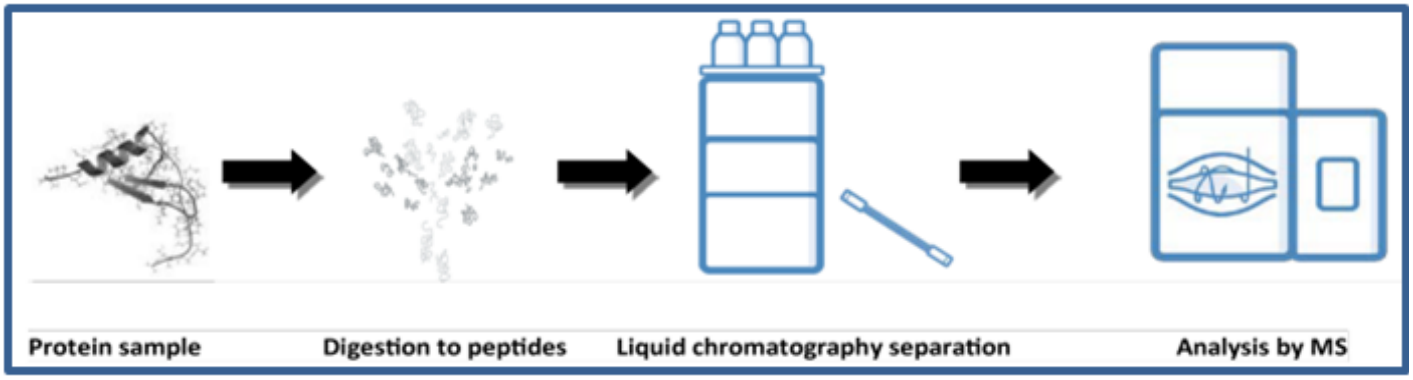

Figure 3a

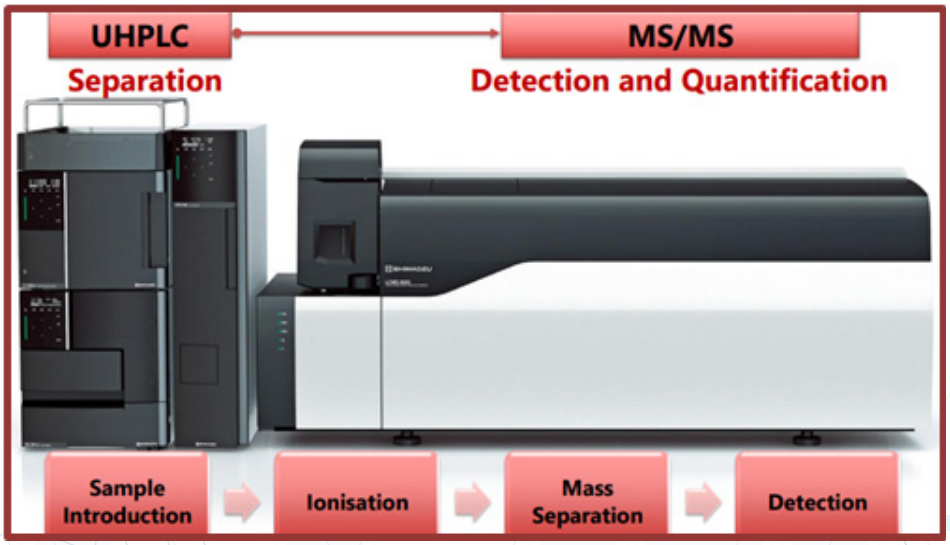

Figure 3b

- **High resolution** separation by LC along with high resolution-accurate mass (HRAM) by MS/MS helps in the identification and quantification of biomolecules with similar structures. The selectivity is high for multiplexing when compared to immunoassays.
- **Sensitivity** of the instrument using the technique is high to atom mole levels making it superior to the immunoassays.
- **High-throughput** is achieved using UHPLC (ultrahigh-performance liquid chromatography) allowing fast processing of separation and concurrently analysis by Mass spectroscopy with the advanced automation and no human intervention requirement in-between.
- **Very low volumes** of samples are required as much as <5 QL for LC-MS/MS making it accessible for precious samples from infants, animal models and so on.
- With advanced HPLC systems and MS, there is a good **reproducibility** of the results are obtained when compared to the immunoassays which are handled mostly manually.
- **Cost-effective** due to the volumes used and in terms of number of separations done with one HPLC (7).

Even though immunoassays have been the gold standard for the past few decades, with the advanced technologies like LC-MS/MS which are not only futuristic with specificity, accuracy, and efficiency, there is also growing acceptability amongst the clinical as well as laboratory professionals. These technologies cover a gamut of tests ranging from New Born Screening (NBS), therapeutic drug monitoring, various other diseases, metabolites, hormones as well as vitamins (8).

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5. Histopathology slide of the month - An uncommon variant of meningioma

Dr. Marquess Raj
ZTC Tamilnadu & Pondicherry & Dr. Chidhambharam.C (Consultant Histopathologist, RRL Chennai)

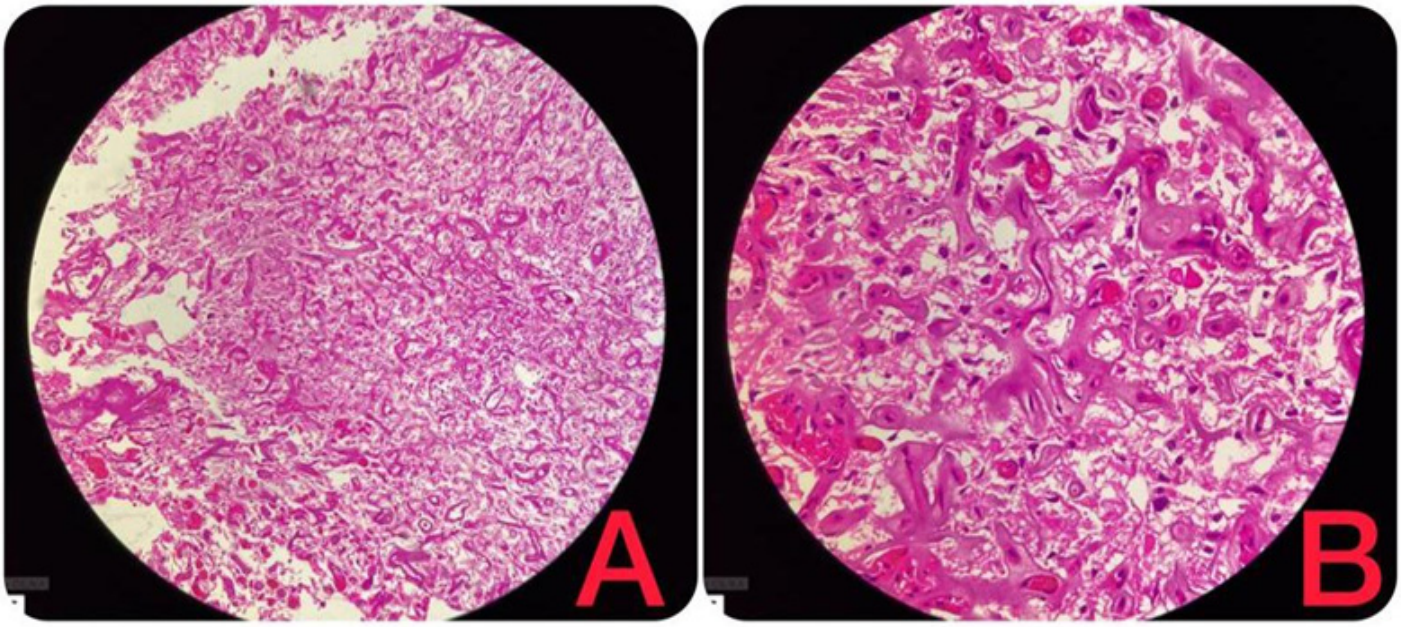
Case history:

A 44 year-old male presented with progressive weakness to the neurosurgeon.

MRI showed a frontoparietal SOL which was attached to the dura & the lesion was biopsied & sent for histopathological examination.

Histopathological examination:

- H & E slides revealed a vascular tumour which had sparse meningothelial cells.
- The morphology correlated with Angiomatous meningioma.



A. Low power view of the lesion shows delicate vessels.

B. On higher power hyaline vessels predominate over the meningothelial elements. Plump meningothelial cells wrapping around the vessels are evident.

Take home points:

Angiomatous meningioma has striking morphology on Histopathological examination. The vascular component exceeds 50 % of the tumour volume.

Angiomatous meningioma's has features of a benign meningioma but in addition contains many small (micro vascular variant) or large vascular channels (macrovascular variant) & these may predominate over meningothelial elements.

The vessels maybe hyalinised.

The main differentials are vascular lesions such as hemanigoendothelioma & vascular malformations such as AVM.

Angiomatous meningioma's are graded as WHO grade 1.

6. Patterns in histopathology

Dr. Marquess Raj & Dr. Shalini Singh
Co-editors AD express

In continuation with article 3 which touches upon the fine details which make the science of histopathology fascinating. We present a few time tested pearls in a quiz format to interest our readers.

1. Match the patterns given with the respective tumours

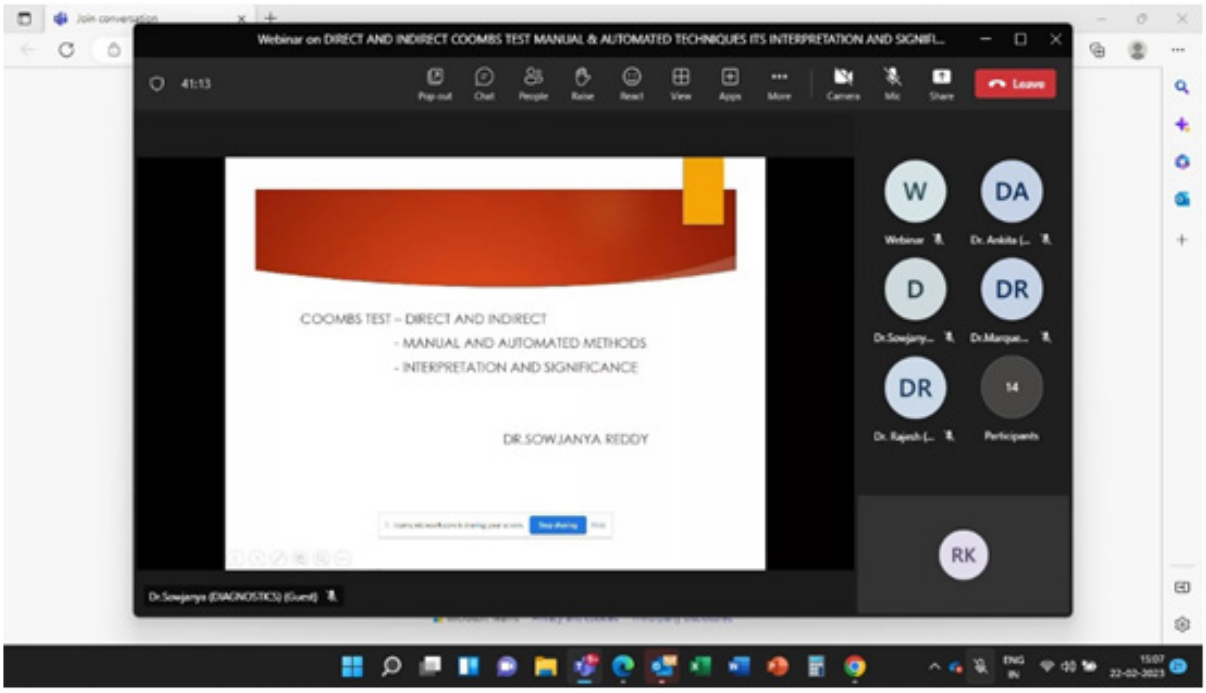
A. Chicken wire pattern	1. Lobular carcinoma of the breast
B. Watered silk pattern	2. GIST
C. Indian-file pattern	3. Chondroblastoma
D. Skeinoid fibres	4. Fibrosarcoma
E. Herring bone pattern	5. Granulosa cell tumour

2. Match the 'giant cells & rosettes' given with the respective tumours/lesions

A. Touton giant cells	1. Neuroblastoma
B. Langhan's giant cells	2. Retinoblastoma
C. Foreign body giant cells	3. Sarcoidiosis
D. Homer wright rosettes	4. Xanthoma
E. Flexner Winter Steiner rosettes	5. Suture granuloma

Recent Events:

Webinar: Coomb's Test: Direct and Indirect by Dr. Sowjanya Reddy



Best Diagnostic award for Global Reference Lab on Founders Day

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