Material & Methods
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For present study cases shall be taken from the patients attending Ear, Nose and Throat OPD and also from the in-patients admitted in ENT/Surgical wards of MLB Medical College Hospitals, Jhansi, U.P. and all the cases of nasal masses from the old records of the histopathology section of the department of pathology, MLB medical College Hospitals, Jhansi, U.P. Shall be collected and analysed.

1) Material in the form of Biopsies shall be obtained from patients in the form of incisional biopsy.

2) Biopsies shall be preserved in 10% formal saline solution.

3) Biopsies thus procured shall be subjected to following –
   a. Tissue blocks shall be prepared by paraffin embedding technique.
   b. Section shall be cut on microtome at 4-6 μm thickness.
   c. The section thus prepared shall be subjected to following staining procedure.
      i. Routine haematoxylin and eosin staining (Clayden, 1971).
      ii. Special staining methods – following special staining methods shall be applied wherever found suitable –
          1. Reticulin (Gorden and Sweet) stain
          2. Periodic –acid Schiff's stain
          3. Mucicarmine stain
          4. Grams staining (to demonstrate bacteria)
FOR H&E STAINING FOLLOWING METHOD WAS USED (CLAYDEN – 1971)

METHOD

- Sections of 4μ thickness were cut and gently lowered on the surface of water bath having a temperature 5-10°C lower than the melting point of wax.
- These sections were taken on egg albumin smeared slides.
- These slides were warmed on hot plate at a temperature so that wax just melts. The removal of wax then done in xylene.
- The xylene was washed by 95% alcohol. The sections were then brought to water by washing in descending order of alcohol i.e. 90%, 70% and then in deionised water.
- Section is then stained with hematoxylin for 10 to 20 minutes.
- Tip off stain and wash thoroughly with water.
- Differentiate with 1% acid alcohol for 10-20 secs.
- Wash of acid alcohol with tap water.
- Blue in warm tap water for atleast 55 mins.
- Counterstain with 1% aqueous eosin for 1-5 minutes.
- Wash in tap water. The length of time depends on the amount of eosin that is desired to be left in the section.
- Wash in 95% alcohol for few seconds. Dehydrate with absolute alcohol for few seconds.
- Clear by washing with xylene.
- Mount in DPX.
RESULT –

Cell nuclei stained blue
Cytoplasm stained eosinophilic
Muscle fibres are stained red
Collagen fibres are stained pink
Red blood cells are stained bright red.

GORDEN AND SWEETS METHOD FOR RETICULIN (CLAYDEN – 1971)

METHOD

• Prepare paraffin wax sections.
• Bring sections down to distilled water.
• Oxidize in acidified permanganate solution for 1 to 5 minutes.
• Wash in distilled water for 0.5 to 1 mins.
• Bleach until white in 1% oxalic acid usually a few secs.
• Wash in tap water for 1 to 5 minutes.
• Wash in two changes of distilled water each for 1 min.
• Mordant in 2.5% iron alum for a minimum of 15 minutes but not longer than 2 hrs.
• Wash in 2-3 changes of distilled water.
• Impregnate with diamino silver solution by flooding the slide for 10-40 minutes.
• Rinse in distilled water.
• Reduce in 10% formalin for 1 min.
• Wash in water.
• Tone if desired in 0.2% gold chloride for 0.5 to 2 mins.
• Wash in tap water.
• Place in 5% sodium thiosulphate solution for 5 mins.
• Wash thoroughly with tap water.
• Dehydrate with absolute alcohol.
• Clear with xylene.
• Mount in DPX.

RESULTS
Reticulin fibres are stained brownish black in untoned and dark purple in toned preparation.

FOR PERIODIC ACID–SCHIFF’S STAINING (CLAYDEN – 1971)

METHOD
• Prepare paraffin wax sections
• Bring section in 70% alcohol for 10 minutes.
• Treat with alcoholic periodic acid solution for 5-10 minutes.
• Rinse in 70% alcohol for 1 minute.
• Treat with acid reducing rinse for 1 minute.
• Rinse in 70% alcohol.
• Treat with Schiff’s reagent for 10-30 minutes.
• Wash in running tap water for 10 minutes.
• Place in celestine blue for 3 minutes.
• Bring section in Mayer’s hemalum for 5 minutes.
• Differentiate in 1% acid alcohol for 10-20 seconds.
• Wash in running water till blue.
• Counter stain with orange G for 10 seconds.
- Wash in water for 20 to 30 seconds.
- Dehydrate with absolute alcohol.
- Clear with xylene
- Mount in DPX.

RESULT

Rose to pink – is acid mucopolysaccharide, glycogen, mucin, colloid droplets, hyaline deposits of glomeruli, epithelial mucin, basement membrane, colloid of thyroid, amyloid and fungi.

FOR SOUTHGATES MUCICARMINE STAINING FOLLOWING METHOD WAS USED (CLAYDEN – 1971)

METHOD

- Prepare paraffin wax sections.
- Bring section to distilled water.
- Stain in Weigert's iron haematoxyline stain for 5 minutes.
- Differentiate in acid alcohol and blue in tap water.
- Stain for 30 minutes in the staining solution (mucicarmine solution) diluted to four times with distilled water.
- Wash in tap water.
- Dehydrate with absolute alcohol.
- Clear with xylene.
- Mount in DPX.

RESULT

Mucin : Reddish
Nuclei : Blue
FOR GRAM’S STAINING FOLLOWING METHOD WAS USED

(CLAYDEN – 1971)

METHOD

- Prepare paraffin wax sections
- Bring section down to water.
- Stain for 1 minute in gram’s crystal violet (0.5% solution in distilled water).
- Rinse in water
- Mordant in Lugol’s iodine for ½ minute.
- Rinse
- Differentiate in acetone till no more clouds of stain come out (about 3 seconds).
- Rinse in water.
- Counterstain in neutral red fuchsin.
- Dehydrate quickly in alcohol.
- Clear with xylene.
- Mount in DPX.

RESULT

Gram positive organism - Blue Black
Other tissue structures - Shades of Red
WORKING PROFORMA

TITLE OF THESIS: “Histopathological study of tumour and tumour like nasal masses - A prospective and retrospective study”.

CLINICAL DATA

Patient’s Name : 
Age/ Sex : Male/ Female
Address : 
Religion : Hindu/ Muslim/Others .......... 
Occupation : 
Clinician I/C : 
Clinical diagnosis

Chief/ Presenting complaints : (a) Nasal Blocked
(b) Nasal mass
(c) Bleeding
(d) Any other

Clinical findings :
P/NS :

PATHOLOGICAL DATA

1. Gross Examination :

2. Histopathological findings and Diagnosis (H & E) :

3. Special staining procedures if any :
   a. PAS :
   b. Reticulin :
   c. Mucicarmine :
   d. Gram’s staining :

4. Clinico-Pathological Correlation :

5. Pathological Diagnosis :

6. Remarks :