Material & Methods
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The present study was carried out in 217 patients who were selected from those attending the Surgery, Orthopedics, Pediatrics and Cancer outpatient departments of the Pt. J.N.M. Medical College Raipur and associated Dr.B.R.A.M. Hospital Raipur during a period between 1st April 2008 to 30th September 2009 as well as those admitted in the wards. Retrospective study was done from 1st April 2003 to 31st March 2008, and a total of 496 cases were retrieved from the record.

All cases included in present series were taken up for study, irrespective of their age and sex. The history was elucidated as per Performa with special reference to the following:- Name, age, sex, socio-economic status of each patient, the presenting complaints with their duration was noted in chronological order. General examination of patient was performed to assess the general condition of health. Clinical diagnosis was made on the basis of history, relevant clinical findings. Local examination of the swelling was done carefully and the fine needle aspiration was performed on patients with palpable soft tissue lesions.

Correct positioning of the patient is at most important factor in obtaining a good aspiration. It is essential that patient should be positioned properly, so that the mass is prominent on palpation.
TECHNIQUE

Aspiration of soft tissue lesions were performed following the technique of Zajicek et al (1979). A 23 gauge needle fitted on a 10 ml disposable syringe was used for aspiration under full aseptic technique. The procedure was explained to the patients. The skin over the swelling was cleaned with povidone iodine or absolute alcohol. The aspiration was then carried out without any local anesthesia. A sterile glove was worn and the lump was fixed with the left hand. The needle was then introduced into the swelling with the syringe attached to it. After the needle had entered the lesion, strong suction was applied by pulling the plunger of the syringe fully back and the needle was advanced into the lesion. Multiple passes (4 to 5) of the needle in different planes was done, maintaining the suction. Finally, the suction was released and the needle was withdrawn. The puncture site was sealed with a cotton woolball.

PREPARATION OF SMEAR

The needle was detached from the syringe, vacuum was created, the needle was attached again and contents of the needle were expelled slowly and carefully over the dry clean grease free glass slides. The aspirates were lightly spread with the help of another a glass slide. The slides were immediately fixed in 95% ethyl alcohol to ensure fixation. Staining was done with Papanicolaou stain, Haematoxyline and Eosin and dried smears were stained with MGG (May Grunwald- Giemsa) stain.
Histopathological slide preparation:-

After the FNAC is being done, all the cases were followed up as far as possible so that biopsy specimen is available for histopathological examination. The biopsy specimen in 10 % formalin saline solution were received in histopathological section and processed in automated tissue processor, paraffin blocks were made. The 5-6 micron section were cut on rotatory microtome and stained with Harris haematoxyline and Eosin stain.

PAPANICOLAOU’S STAIN:-

Consist of following reagents (Diagnostic cytology and its histopathologic basis by L.G.Koss, second edition 1968).

1) E A 36 (EOSIN ALCOHOL) equivalent to E A 50
2) O G 6 (ORANGE GREEN)
3) HARRIS HAEMATOXYLINE

Preparation of Papanicolaou Stain:-

Formula for E A 36, equivalent to E A 50.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOSIN</td>
<td>10 gms</td>
</tr>
<tr>
<td>BISMARK BROWN Y</td>
<td>10 gms</td>
</tr>
<tr>
<td>LIGHT GREEN SF YELLOWISH</td>
<td>10 gms</td>
</tr>
<tr>
<td>DISTILLED WATER</td>
<td>300 ml</td>
</tr>
<tr>
<td>95% ETHYL ALCOHOL</td>
<td>2000 ml</td>
</tr>
</tbody>
</table>
PHOSPHOTUNGUSTIC ACID 4 gms
SATURATED LITHIUM CARBONATE 20 drops
SOLUTION IN DISTILLED WATER

FORMULA OF OG 6:-

ORANGE G CRYSTALS 10 gms
DISTILLED WATER 100 ml
95% ETHYL ALCOHOL 100 ml
PHOSPHOTUNGUSTIC ACID 0.15 gms

HARRIS HAEMATOXYLINE:-

It is a powerful selective nuclear stain giving sharp delineation of nuclear structure. It is prepared as follows:-

HAEMATOXYLINE 1 gm
ABSOLUTE ALCOHOL 10 ml
AMMONIUM OR POTASSIUM ALUM. 20 gms
DISTILLED WATER 200 ml
MERCURIC OXIDE 0.5 gm

Haematoxyline was dissolved in alcohol and added to the alum, previously dissolved in hot water, boiled quickly, cooled rapidly under tap water, filtered before use.
Staining procedure: (Diagnostic cytology and its histopathologic basis by L.G. Koss second edition 1968).

Slides removed from fixative were immediately placed in 80% alcohol for 8-10 seconds, the rest of the procedure is as follows:-

70% ETHYL ALCOHOL 5 DIPS (8-10 Seconds)
50% ETHYL ALCOHOL 5 DIPS
DISTILLED WATER 5 DIPS
HARRIS HEAMETOXYLINE 6 MIN
DISTILLED WATER 5 DIPS
0.5% AQUEOUS SOLUTION OF HCL 3-5 DIPS
RUNNING TAP WATER 6 MIN
60% ETHYL ALCOHOL 5 DIPS
70% ETHYL ALCOHOL 5 DIPS
80% ETHYL ALCOHOL 5 DIPS
95% ETHYL ALCOHOL 5 DIPS
OG 6 1.5 MIN
95% ETHYL ALCOHOL 5 DIPS
95% ETHYL ALCOHOL 5 DIPS
EA36 1.5 MIN
95% ETHYL ALCOHOL 5 DIPS
95% ETHYL ALCOHOL 5 DIPS
ABSOLUTE ALCOHOL 5 DIPS
ABSOLUTE ALCOHOL 5 DIPS

EQUAL PART OF ABSOLUTE ALCOHOL & XYLOL 5 DIPS

RINSE IN 6 DISHES OF XYLOL 5 DIPS Each Dish

Mount in D.P.X.

RESULT OF STAINING

<table>
<thead>
<tr>
<th>Component</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus</td>
<td>Blue</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Pink</td>
</tr>
<tr>
<td>RBC</td>
<td>Orange</td>
</tr>
</tbody>
</table>

HARRIS HAEMATOXYLINE AND EOSIN STAIN

Preparation of Harris Haematoxyline stain:-

It is a powerful selective nuclear stain giving sharp delineation of nuclear structure. It is prepared as follows:-

HAEMATOXYLINE 1 gm
ABSOLUTE ALCOHOL 10 ml
AMMONIUM OR POTASSIUM ALUM 20 gms
DISTILLED WATER 200 ml
MERCURIC OXIDE 0.5 gm
Haematoxyline was dissolved in alcohol and added to the alum, previously dissolved in hot water, boiled quickly, cooled rapidly under tap water, filtered before use.

**EOSIN STOCK:** - 5% solution was prepared using distilled water as solvent. To prevent the growth of moulds, crystal of thymol or a few drops of formalin was added.

**FOR USE 5% STOCK SOLUTION IS DILUTED TO 1% SOLUTION WITH DISTILLED WATER.**

**Staining procedure:** -

1. **Hydration**

Slides removed from fixative were placed in 80%, 70%, and 50% alcohol 5 to 8 dips in each.

2. **Nuclear Staining**

Slides were transferred to haematoxyline stain for 3-5 minutes.

3. **Differentiation**

Slides were removed from haematoxyline, washed in water, 2 to 3 quick dips given in 0.5% hydrochloric acid and again washed in tap water.

4. **Blueing**

Slides were kept in diluted ammonia water for 2 to 3 minutes or in tap water for 5 to 10 minutes.

5. **Counter Staining**
Slides were washed in distilled water and then stained in working 1% eosin solution for one to two minutes.

6. **Dehydration**

Slides were placed in 50%, 70%, 80% and 95% alcohol, 3 to 5 dips in each and in absolute alcohol for 3 to 5 minutes, transferred to another jar of absolute alcohol for 3 to 5 minutes.

7. **Cleaning**

Slides were transferred from absolute alcohol to xylene for 2-3 minutes and another change of fresh xylene for 5 minutes.

8. **Mounted with D.P.X.**

**MAY GRUNWALD-GIEMSA STAIN:**

Consist of May Grunwalds stain and Giemsa stain -

**MAY GRUNWALD STAIN:**

- Methanol: 100 ml.
- Powder dye: 0.3 gm.

100 ml methanol is added to 0.3 gm of powder dye in conical flask, mixture was warmed to 50°C flask allowed to cool, shaking several time during the day. It is filtered after 24 hours of standing.

**GIEMSA STAIN:**

Stock Giemsa solution:

- Giemsa stain powder: 1 gm
- Glycerol: 84 ml
Methanol - 84 ml

**Preparation method:**

1 gm Giemsa stain powder was dissolved in 84 ml glycerol at 60°C with regular shaking. 84 ml methanol was added and the mixture was shaken well. It was allowed to stand for 7 days and filtered before use.

**Staining procedure:**

1. Air dry slides.
2. Fix in methanol for 2-3 minutes at room temperature.
4. Stain for 10 minutes in Giemsa stain, freshly diluted with buffered distilled water- pH 6.8 (1/9).
5. Wash in running tap-water and the leave for 3-4 minutes in buffered distilled water pH 6.8.
6. Allow to dry.
7. Mount with DPX.