MATERIAL AND METHODS

The material for present retrospective study comprised of 540 cases of breast diseases received in the Histopathology section of Pathology Department, M.L.B. Medical College, Jhansi during a period of 15 years from 1982 to 1996.

Majority of these specimens were received from the Department of Surgery, M.L.B. Medical College and Hospital, Jhansi and some cases were received from other associated hospitals.

The present study was carried on the basis of -

1. The forms from the medical record section of M.L.B. Medical College & Hospital, Jhansi.

2. Old Paraffin embedded blocks of the cases along with Haematoxylin and eosin stained sections available in the Pathology department were examined and evaluated for study.

3. The fresh sections wherever needed, were cut from the old paraffin blocks at 4 u - 5 u thickness and were subjected to routine haematoxylin and eosin staining for better diagnosis, evaluation & typing etc.

4. Special staining procedures like PAS (Hotchkiss, 1948), and Reticulin (Gordon & Sweet, 1935) were also applied in selective cases for differential studies.
5. The relevant findings & observations or analysis were recorded on a pre-set proforma for final observation, correlation and discussion.

6. Categorization of breast diseases as per World Health Organisation Classification (1968 & 1982) shall be compared with reference to criteria laid down.

**Routine Haematoxyline & Eosin Staining Procedure (Culling, 1975):**

**Fixative**  - 10% formal saline.

**Reagent**  -  
  - Harris/Ehrlich's Haematoxylin,
  - Aquous Eosin.

**Steps**  - 1. Sections were deparaffinized and brought to water through graded solution of alcohol.

2. Stained in a solution of Harris Haematoxyline for 10–20 minutes.

3. Washed thoroughly with water for 15–30 seconds.

4. Decolourized with 1% acid (HCl) alcohol for 10–20 seconds.

5. Again wash with tap water.

6. Kept in warm tap water for 5 minutes for blueing.

7. Then counterstained with 1% aqueous Eosin for 1–5 minutes.

8. Washed rapidly in water to remove desired amount of eosin.

9. Dehydrated by several changes of increasing graded alcohol solutions e.g. 70%, 80%, 90% and absolute alcohol.

10. Cleared in Xylene.

PERIODIC ACID-SCHIFF REACTION

To stain:

1. Bring the section to distilled water.

2. Immerse in the 1 percent Periodic acid for five minutes on no account sections should remain in longer than ten minutes, nor should heat be used as chemical changes may take place.

3. Wash in running tap water for fifteen minutes.

4. Stain with Schiff’s reagent for ten to fifteen minutes.

5. Place in Sulphurous acid solution for 5 minutes.

6. Place in another change of sulphurous acid for five minutes.

7. Wash in running tap water for ten minutes.

8. Counter-stain with fast green F.C.F. for thirty seconds or tartrazine for three to five minutes.

9. Dehydrate with absolute alcohol or 74 C.P. spirit.


11. Mount in balsam or D.P.X.

Result:

P.A.S. Positive substances ............ Red.
GORDEN AND SWEETS SILVER IMPREGNATION STAIN FOR RETICULIN

(CULLING, 1975):

Fixative - Formal saline.

Reagents -

1. Silver solution to 5 ml of 10% silver nitrate, 3 percent sodium hydroxide was added, titration was done with liquid ammonia until clear. Total volume was made up to 50 ml double distilled water.

2. Acidified permanganate solution consisting of 47.5 ml of 0.5 percent aqueous potassium permanganate added to 2.5 ml of 3 percent sulphuric acid.

3. 1% oxalic acid.

4. 2% aqueous Iron alum

5. 10% aqueous neutral formalin

6. 0.2% yellow gold chloride

7. 5% sodium thiosulphate.

TO STAIN (TECHNIQUE):

1. Sections were brought to water through graded alcohols.

2. Oxidized for 1 to 5 minutes in acidified permanganate solution.

3. Washed with water.

4. Bleached in 1% oxalic acid for 3 to 5 minutes or until white.

5. Washed with two changes of distilled water.
6. Kept in 2% aqueous iron alum for 2 to 15 minutes.
7. Washed with 2 to 3 changes of distilled water.
8. Treated with silver solution until section was transparent (30 seconds).
9. Washed well in several changes of distilled water.
10. Reduced in 10% aqueous formalin neutral (about 30 seconds).
11. Washed in water.
12. Toned in 0.2% gold chloride for 1 to 3 minutes.
13. Washed in tap water.
14. Fixed in 5% sodium thiosulphate for 5 minutes.
15. Washed in water for 1 to 2 minutes.
16. Counter stained with 1% safranine.
17. Dehydrated in absolute alcohol, cleared in Xylene and mounted in DPX.

The relevant clinical data of the cases, including pathological data, histopathological correlation findings were recorded on a pre-set proforma for final analysis and evaluation (Appendix - I).

The W.H.O. Classifications of breast tumours (1968) and with subsequent modification (1982) are the basis for categorisation of the breast tumours and tumour-like lesions in this present retrospective study.
**W.H.O. CLASSIFICATION (1968)**

**A. Benign Mammary Dysplasias:**

1) Chronic cystic disease – a) Simple cyst
   b) Papillary cyst
2) Adenosis
3) Duct Ectasia
4) Fibro scleros is
5) Gynaecomastia
6) Other non-specific proliferative lesions

**B. Benign or apparently Benign Tumors:**

1) Adenoma of breast
2) Adenoma of nipple
3) Duct papilloma
4) Fibroadenoma – Pericanalicular fibroadenoma
   – Cellular intracanalicular fibroadenoma
5) Benign soft tissue tumours

**C. Carcinoma:**

1) Intraduct & intralobular non-infiltrating carcinoma
2) Infiltrating carcinoma
3) Special histological variants of carcinoma
   a) Medullary carcinoma
   b) Papillary carcinoma
   c) Cribriform carcinoma
   d) Mucus carcinoma
   e) Lobular carcinoma
   f) Squamous cell carcinoma
   g) Paget's disease of breast
   h) Carcinoma arising in cellular intracanalicular fibroadenoma
D. Sarcoma - arising from cellular intracanalicular fibroadenoma & other types of sarcoma

E. Carcinosarcoma

F. Unclassified tumours


It aims at promoting uniformity in recording and reporting diagnosis in order to facilitate international and other comparisons (Azzopardi et al, 1982).

W.H.O. MISTOLOGICAL CLASSIFICATION (1982)

I. Epithelial Tumours :

A. Benign - (1) Intraductal papilloma
   (2) Adenoma of nipple
   (3) Adenoma - (i) Tubular (ii) Lactating

B. Malignant -
   (1) Non-invasive - (i) Intraductal carcinoma
      (ii) Lobular carcinoma in situ
   (2) Invasive -
      (i) Invasive ductal carcinoma
      (ii) Invasive ductal carcinoma with a predominant intraductal component
      (iii) Invasive lobular carcinoma
      (iv) Mucinous carcinoma
      (v) Medullary carcinoma
vi) Papillary carcinoma
vii) Tubular carcinoma
viii) Adenoid cystic carcinoma
ix) Secretory (Juvenile) carcinoma
x) Apocrine carcinoma
xi) Carcinoma with metaplasia - (1) Squamous type
   (2) Spindle cell type (3) Cartilagenous & Osseous
   (4) Mixed
xii) Others

C. Paget's disease of nipple

II. Mixed connective tissue & epithelial tumours -
   A. Fibroadenoma
   B. Phyllodes tumour (Cystosarcoma phyllodes)
   C. Carcinosarcoma

III. Miscellaneous Tumours -
   A. Soft tissue tumour
   B. Skin tumour
   C. Tumours of haemopoietic and Lymphoid tissues

IV. Unclassified Tumours

V. Mammary dysplasia / Fibrocystic disease

VI. Tumour like lesions :
   1. Duct ectasia
   2. Inflammatory Pseudo-tumours
   3. Haemartoma
   4. Gynaecomastia
   5. Others.

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