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A CLINICAL HISTOMORPHOLOGICAL AND
IMMUNOHISTOCHEMICAL ANALYSIS OF BREAST NEOPLASMS

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CERTIFICATE

This is to certify that this dissertation entitled **"A CLINICAL HISTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF BREAST NEOPLASMS"** is the bonafide record work done by **Dr. S. Jenita Christiana Ranjana** submitted as partial fulfillment for the requirements of M.D. Degree Examinations Branch III Pathology to be held in March 2008.

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INTRODUCTION

Breast cancer is the most common carcinoma in females which accounts for 22% of all female cancers. It is more than twice the occurrence of carcinoma in females at any other site.[29] In southern India, breast cancer is the second most common cancer among women.[28] The incidence of the disease had been increasing in both developed and developing countries until 1980, but still continues to increase in the developing countries.

Breast disorders encompass a heterogenous group of lesions that may be presenting as a palpable mass, non-palpable abnormality detected on breast imaging or an incidental microscopic finding. Women who have undergone breast biopsies reflect a spectrum of histologic conditions from normal breast tissue of varying physiologic states at one extreme to changes approximating to carcinoma at the other end.[60]

Etiology of breast cancer is multifactorial. It includes diet, reproductive life style, exogenous and endogenous hormones, body weight, alcohol, smoking and physical activity. However, more than most other neoplasms, breast cancer shows familial clustering.[29] Two high penetrance genes BRCA1 & BRCA2 greatly increase the cancer risk. Multigenic traits also play a significant role in the inherited susceptibility to cancer. Literatures say carcinoma breast is a disease of affluent societies which have acquired the western style characterized by high calorie diet combined with lack of exercise.[29]

There have been two general approaches to prognostication via histopathologic analysis. The first categorizes carcinomas based on specific features, recognizing the so called special type carcinoma. Histopathologic features have been recognized as a necessary element for appropriate management of breast carcinoma. The second approach evaluates individual characteristics of the carcinoma – grading which is shown to be robust determinant of outcome of breast carcinoma.[15] Establishment of a uniform system of grading will increase the frequency of grading by pathologists, significantly reduce observer variation and strengthen the predictive value of histologic grade.

The most widely accepted system for grading invasive breast carcinoma is the Elston-Ellis system which represents the modification of the Scarf-Bloom-Richardson system established in the middle of the last century. It is performed by combining tissue architecture (tubule formation), cell morphology (nuclear pleomorphism) and assessment of cell proliferation rate (Mitotic count).[24,29,35,36,52.]

Histological grading has been considered as too subjective to be used clinically and grading may be associated with lack of reproducibility even when performed by experienced pathologists. On the other hand, numerous studies have shown a significant association between histological grade and survival in breast cancer and there is no doubt that grading is simple, quick and economical to perform. Low histological grade is significantly related to recent or current use of combined hormone replacement therapy.[42] Histological grading identifies patients with low risk of breast cancer recurrence. Since it is associated with minimal cost, its use in clinical decision making may result in substantial savings. Omission of grading from clinical decision making results in overseas of adjuvant therapies.

Histological grade was correlated strongly with survival. Women with well differentiated node negative cancer had 97% 5year distant disease free survival rate as compared with 78% for women with poorly differentiated cancer.[39] The relative importance of histologic grade in multivariate analysis may depend on many factors such as the selection process of breast cancers for the series being analyzed, the quality of assessment, end points chosen and personal skill of the pathologist responsible for grading.[39]

It is important to evaluate the prognostic value of histologic grade in relation to novel molecular genetic markers. It remains to be seen whether some of the novel biologic factors can replace histologic grade as a simple and powerful prognostic variable and produce superior result when performed in routine clinical setting. A recent study indicates that grade may yet be a more powerful predictor than intensively studied molecular genetic factors such as P53 and Cerb-2.[39]

This study is undertaken in view of evaluating the actual incidence of breast neoplasms in semi-urban areas like Thanjavur with particular attention to demographic characteristics, clinical presentation, histopathology and grading.

AIM OF STUDY

1. To evaluate the incidence and prevalence as well as trend of Breast

    neoplasms in semi-urban area.

2. To evaluate the clinical features, symptoms and signs associated with

    Breast malignancies.

3. To evaluate the malignant tumours of breast by histological grading.

4. To evaluate the initial role of histochemical stains in diagnosis of Breast

    neoplasms.

5. To study the role of immunohistochemistry in doubtful cases.

MATERIALS AND METHODS

The study group comprises of 267 breast neoplasm received from January 2005 to December2006 at Thanjavur Medical College, treated by either lumpectomy or mastectomy (simple/modified radical). The detailed history with particular attention to family history, socio-economic status, radiation, nutrition and parenteral occupation were elicited for each case. A thorough clinical evaluation, routine haematological investigations, ultrasonogram and CT scan (in proportion of cases with possibility of distant metastasis) was done in every case.

In proportion of cases, initial evaluation with fine needle aspiration (FNA) was also done. FNA smears were fixed in isopropyl alcohol and processed with both routine H & E and Giemsa stains (Appendix I, II).

The received specimens, both lumpectomy as well as mastectomy were bisected initially and fixed properly with neural buffered formalin. Small lumpectomy specimens of size less than 1cm were submitted to toto. In larger specimens, multiple sections were taken at 1cm interval. Mastectomy specimens were opened at 1cm interval like opening a book with special attention to include base, both lateral margins, skin and ulcerated areas. The tissue sections were processed routinely, 3-5u sections were cut and stained with H & E.

In all cases cytomorphological evaluation was done to grade the neoplasm. In mucinous/colloid carcinoma of the breast, histochemical stains were also done to identify mucin (Appendix III). In doubtful cases, immunohistochemical evaluation with markers such as smooth muscle actin and myoglobin was also done.

REVIEW OF LITERATURE

Breasts are often described as modified sweat glands resting on pectoral muscle.The breast epithelium is arranged in the form of 10-15 segments, each consisting of a branching structure that has been likened to a flowering tree. The lobules drain into ductules and ducts which in turn drain into the collecting ducts onto the surface of the nipple. Just below the nipple the ducts are expanded by lactiferous sinuses.
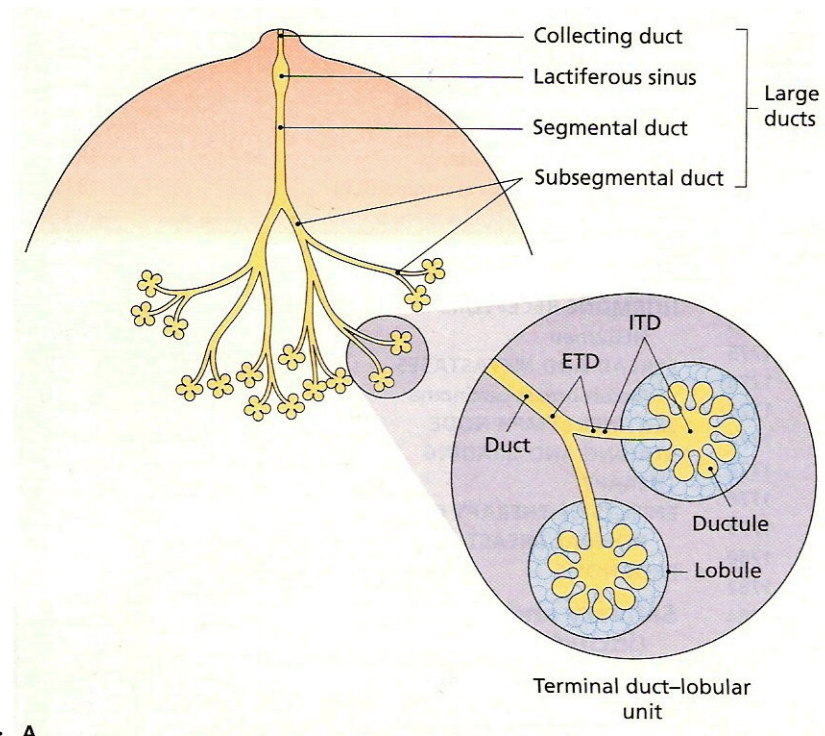
The breast epithelium is arranged in the form of a lobule. The lobule together with its terminal duct has been called the terminal duct lobular unit (TDLU).Most pathologic lesions arise from this area. The normal lobule consists of a variable number of blind ended terminal ductules alternatively called acini which has a typical double epithelial cell layer. The inner epithelium with its secretory and

absorptive functions is cuboidal or columnar. The outer layer is the basal or myoepithelial layer. The acini are surrounded by loose fibro vascular intralobular stroma which contrasts with the dense interlobular stroma.The nipple has large collecting (lactiferous) duct associated with sebaceous apparatus opening onto the surface.

At puberty, the female breast develops its characteristic adult structure. Minor variations occur during the menstrual cycle, but major physiological changes are seen during pregnancy and lactation. During menopause there is a major regression of breast tissue which merges with the atrophy associated with aging. Physiological changes result in radically different histological appearances at varying ages. These changes should be differentiated and compared with the pathological processes which occur in breast.

BENIGN NEOPLASMS

FIBROADENOMA

Fibrodenoma occurs most frequently in women of child bearing age, especially under 30.Grossly, fibrodenoma is a sharply demarcated firm mass grayish in colour with a slightly lobulated pattern and slit-like spaces.

Histopathology comprises of admixture of stromal and epithelial proliferations in two distinct patterns – pericanalicular pattern as a result of proliferation of stromal cells around ducts in a circumferential fashion and intracanalicular pattern due to compression of ducts into clefts by the proliferating stromal cells.

The stromal component may sometimes exhibit hypercellularity, atypical bizarre multinucleated giant cells, extensive myxoid changes or hyalinization with dystrophic calcification and rarely ossification.[29]

Fibrodenomas that contain cysts larger than 3mm, sclerosing adenosis, epithelial calcifications or papillary apocrine change have been called complex fibroadenomas. Complex fibroadenomas were associated with a greater risk for subsequent breast cancer than fibroadenomas lacking such changes.[52]

Malignant changes in fibroadenomas were found only in 0.1% of cases[35]. The risk of invasive cancer was 2.17 times higher among patients with fibrodenoma and relative risk increased to 3.10 among patients with complex fibrodenoma.[61] They usually involve the epithelial component Sarcomatous transformation of the stroma of fibrodenoma is an even rarer phenomenon. Possibly only one case had a focal appearance of an osteosarcoma [35]

Juvenile fibroadenomas are characterized by increased stromal cellularity and epithelial hyperplasia. It should be distinguished from phyllodes by absence of leaf like projections and periductal stromal cellularity. Distinction between the two is important since Juvenile fibroadenoma is treated by excision with preservation of surrounding breast tissue whereas in phyllodes, a rim of normal tissue should be included in the excision.[52]

TUBULAR ADENOMA

They occur mainly in young adults. They are firm, well circumscribed and homogenous with a uniform yellowish cut surface. Histological examination shows proliferating tubules closely packed together with little or no surrounding fibrous stroma. The tubules are lined by a double cell layer. The tubular lumen is small, often empty but eosinophilic proteinaceous material can be present. Combined tubular adenoma and fibroadenoma has been described.[29,35,52.]

LACTATING ADENOMA

This presents as a solitary or multiple freely movable breast mass during pregnancy or puerperium. It may develop in ectopic locations such as axilla, chest wall or vulva.[35,52] Grossly the lesion is well circumscribed with grey or tan cut surface. Microscopically, proliferated glands are seen lined by actively secreting cuboidal cells.Hyperplastic lobules show marked cytoplasmic vacuolization.[35,52]

INTRA-DUCTAL PAPILLOMA

It can arise in large or small ducts in the subareolar region with bloody nipple discharge. Microscopically, papillomas are intricately arborescent with the presence of two cell types, normochromatic and often oval nuclei, scanty mitotic activity and absence of necrosis. Multiple grossly detectable papillomas have been found to be either associated with or develop into carcinoma at a frequency higher than that expected from chance alone.[35,52.]The relative risk of invasive carcinoma for women with papillomas containing atypical hyperplasia was more than four times than that of without atypical hyperplasia.[22,49.]

PHYLLODES TUMOUR

Phyllodes tumour forms firm lobulated masses of varying sizes between 2 and 40cm diameter mostly seen in middle aged women. They are usually well circumscribed   with a characteristic whorled pattern with visible clefts.
Phyllodes tumours exhibit an enhanced intracanalicular growth pattern with leaf like projections into dilated lumens. The epithelial component consists of luminal epithelial and myoepithelial cells. There is increased periductal stromal cellularity. Hyalinization or myxoid changes may be seen.

Malignant phyllodes have infiltrative rather than pushing margins. Stroma usually shows fibrosarcomatous changes. Heterologous differentiation such as liposarcoma, osteosarcoma, chondrosarcoma or rhabdomyosarcoma may occur.

Due to overgrowth of sarcomatous components, epithelial component can be identified only after multiple sections.[29] Norris and Taylor used 3 characters together as determinants of malignant potential. Those are stromal cellular atypia, mitotic activity and nature of tumour margin (pushing Vs infiltrating).

Pretraszka and Barnes refined the criteria of mitosis with classification as

Benign: (0 – 4 mitosis / 10hpf)

Borderline: (3 – 9 mitosis / 10 hpf, pushing or infiltrating margins, stromal cellular atypia).

Malignant : (10 or more mitosis / 10 hpf), infiltrating margins, moderate to marked stromal cellular atypia.[26]

MYOEPITHELIAL LESIONS

These are lesions either derived from or composed of dominant to pure population of myoepithelial cells.

CLASSIFICATION OF MYOEPITHELIAL LESIONS

1) Myoepitheliosis
    a. Intraductal
    b. Periductal
2) Adenomyoepithelial adenosis
3) Adenomyoepithelioma
    a. Benign
    b. With malignant change
        Myoepithelial carcinoma arising in an adenomyoepitheloma
        Epithelial carcinoma arising in an adenomyoepitheloma
        Malignant epithelial and myoepithelial components.
        Sarcoma arising in adenomyoepitheloma.
        Carcinosarcoma arising in an adenomyoepitheloma.
4) Malignant myoepithelioma (myoepithelial carcinoma)

ADENOMYOEPITHELIOMA

This is composed of a predominantly and usually solid proliferation of phenotypically variable myoepithelial cells around small epithelial lined spaces.

The tumour may display a spindle cell, tubular or most often a lobulated growth pattern. Myoepithelial cells range from clear to eosinophilic and hyaline (plasmacytoid) types.[29]   Microscopically there is a   balanced proliferation of round, oval or lobular glandular elements with intervening islands and bands of polygonal myoepithelial cells with clear cytoplasm. In some tumours, the clear myoepithelial cells are numerous resulting in extensive zones virtually devoid of glands. Epithelial cells tend to have sparse, darkly staining cytoplasm and hyperchromatic nuclei.

Squamous, apocrine and sebaceous metaplasia may be present focally.In very rare instances epithelial component exhibits exaggerated proliferations accompanied by cytologic atypia and mitosis that may constitute carcinoma arising in adenomyoepithelioma. Sarcomas and carcinosarcomas also can occur in this setting. Rarely both components may develop into either separate malignancies or a single malignant infiltrative tumour.[29]

NIPPLE ADENOMA

This is also known as florid papillomatosis of the nipple ducts. It usually occurs in the fourth of fifth decade and is accompanied by serous or bloody discharge.[29,35,52.]Microscopically it is composed of haphazardly arranged proliferating tubular structures surrounded by varying amounts of fibrous stroma. It shows an abrupt junction with stratified squamous epithelium. The tubules are bilayered. The epithelial cells are usually cuboidal or columnar but apocrine and squamous metaplasia can occur.

MYOFIBROBLASTOMA

This spindle cell tumour is a very rare tumour consisting of well circumscribed nodular mass of haphazardly arranged bundles of spindle cells interspersed with bundles of collagen.[29,35,52]

FAT NECROSIS

Fat necrosis assumes importance because it can simulate carcinoma both clinically and mammographically.[47] Fat necrosis more commonly results from prior surgical intervention or radiation therapy while a history of antecedent trauma may also be obtained. The lesion excised early in the course of its evolution contains histiocytes with foamy cytoplasm, as well as larger lipid filled cysts and occasional multinucleated giant cells. An infiltrate of chronic inflammatory cells including lymphocytes, plasma cells and cosinophils are present. As the process evolves, the lesion becomes surrounded by dense fibrosis with progressive encapsulation and foci of calcification.

CARCINOMA BREAST

Breast carcinoma is the most common malignant tumour and the leading cause of carcinoma death in women with more than 1,000,000 cases occurring world wide annually.[35,52] Several risk factors for the development of breast cancer have been established such as family history, exogenous or endogenous estrogen and nulliparity. The common denominator for these factors is strong and prolonged oestrogen stimulation operating on a genetically susceptible background.[24,29,35,36,52] The genes responsible for hereditary predisposition have been identified on chromosomes 17q and 13q and named as BRCA1; BRCA2 respectively[29,35]

INSITU CARCINOMA

The term insitu carcinoma is used to describe a proliferation of presumably malignant epithelial cells that remain at their site of origin confined by a basement membrane.Insitu carcinoma is divided into ductal and lobular types based on the architectural and cytologic features of proliferation rather than on its anatomic location within the ductal-lobular system because it is thought that both types arise from the terminal ductal lobular units[35, 52]

DUCTAL CARCINOMA IN SITU

It is a neoplastic intraductal lesion characterized by increased epithelial proliferation with subtle to marked cellular atypia. The incidence rate is 3 – 5% of breast cancers [4,40]. Traditionally DCIS has been classified on the basis of architectural pattern but now the recent International consensus conference has recommended that grading of DCIS should form the basis of classification [35,52].

Low grade DCIS is composed of small monomorphic cells growing in arcades, micropapillae, cribriform or solid patterns. The nuclei are of uniform size with regular chromatin pattern and inconspicuous nucleoli. Mitotic figures are rare. Microcalcification are of the psammomatous type[29,52].

Intermediate grade DCIS form solid, cribriform or micropapillary patterns with some containing intraluminal necrosis. Nuclei exhibits occasional nucleoli and coarse chromatin[29]. The recurrence rate is 10% [44].

High grade DCIS is composed of markedly pleomorphic, poorly polarized cells with irregular contour and distribution forming micropapillae, cribriform and solid patterns with characteristic comedo necrosis[29]. This is associated with local recurrence of 19% [44]. Unusual variants of DCIS are composed of spindled, apocrine, signet ring, neuroendocrine, squamous or clear cells

VAN NUYS PROGNOSTIC INDEX SCORING OF DCIS [24,52.]

	Gross and Microscopic finding	**Score**
Lesion size	≤ 1.5cm	1
	1.6 – 4cm	2
	≥ 4.1cm	3
Margins	≥ 1.0cm	1
	0.1 – 0.9cm	2
	< 0.1cm	3
Grade	Non High grade nuclei, no necrosis	1
	Non High grade nuclei with necrosis	2
	High grade nuclei with or without necrosis	3

Lesions with score of 3 or 4 receive only surgical treatment. A score of 5 –7 receive local surgical and radiation treatment. Scores of 8 and 9 receive local surgical and radiation treatment with the caveat that recurrence rate may be as high as 40%. Careful follow up for patients at increased risk for local recurrence is warranted [12.]

PAPILLARY DUCTAL CARCINOMA IN SITU

This lesion is located within a variably distended duct and characterized by multiple papillary processes with thin fibrovascular stalks and are totally devoid of a myo-epithelial cell layer. The malignant epithelial cells usually have a monomorphic appearance with tall oval nuclei lying perpendicular to the core[35,50.] Concomitant DCIS may be present in the surrounding breast tissue.

Solid and transitional type of variants have been described [29.]

LOBULAR CARCINOMA IN SITU

In LCIS, lobular architecture is retained and individual acini are enlarged and distended in contrast to surrounding lobules. The lumina are completely obliterated . The monomorphic cells filling the lumina are smaller than those in DCIS but larger than normal epithelial cells with less pronounced nuclear pleomorphism and they exhibit notable feature of lack of cellular cohesion.[34,52]The reported incidence of subsequent breast cancer ranges from 17% to 37%.

Invasive carcinoma that develops in these patients is mostly of ductal origin than of lobular origin [41.]

INVASIVE CARCINOMA

Infiltrating breast cancers constitute a heterogenous group of lesions that differ in clinical presentation, radiographic characteristics, pathologic features and biologic potential.

The most common histologic type of invasive breast cancer by far is invasive (infiltrating) ductal carcinoma "not classified into any of the other categories of invasive mammary carcinoma". The specific histologic types are invasive lobular, tubular, mucinous, medullary and other rare types. In general special type cancers comprise approximately 20% to 30% of invasive carcinomas and atleast 90% should demonstrate the defining histology characteristics of a special type cancer before it is designated as being of that histologic type.

INFILTRATING DUCTAL CARCINOMA – NOS

It is a type of carcinoma that is without any special feature that would allow it to be classified as one of distinctive subtypes.

Gross appearance has an irregular stellate outline.

Microscopically architectural arrangement may be in cords, clusters and trabeculae while some are characterized by predominantly solid or syncytial infiltrative pattern. Glandular differentiation may be present as tubular structures with central lumina. Carcinoma cells have variable appearance of uniform to pleomorphic nuclei with abundant eosinophilic cytoplasm. Degree of mitotic activity varies. Necrosis may be focal or extensive. Foci of elastosis may be present in periductal or perivenular regions.

Mixed type carcinoma comprises of ductal NOS pattern in 10% - 40% of tumour and the rest being of a recognized special type [29] Pleomorphic carcinoma is a rare variant of high grade ductal NOS characterized by pleomorphic and bizarre giant cells in more than 50%of tumour cells in a background of adenocarcinoma or adenocarcinoma with spindle and squamous differentiation .Carcinoma with osteoclastic type of giant cells and choriocarcinomatous features have also been reported [29]

INFILTRATING LOBULAR CARCINOMA

These tumours frequently present as irregular and poorly delimited tumours.The malignant cells are composed of single lines of cells and lack cohesion (Indian file pattern). In the targetoid or bull's eye pattern, the tumour cells are arranged in concentric rings around ducts and lobules. " Skip areas" i.e foci of infiltrating carcinoma separated by areas of uninvolved mammary tissue are a common feature of lobular carcinoma.Solid, tubulolobular, alveolar and pleomorphic variants are some of the special variants that have been described [52]

TUBULAR CARCINOMA

Tubular carcinoma is usually smaller than 2cm and there are two morphologic types, 'pure type' with stellate nature and 'sclerosing type' with more diffuse ill defined structure [29.]

Microscopically, it consists of irregularly arranged tubules lined by a single layer of epithelial cells with little pleomorphism and low mitotic rate. The tubules are characteristically angulated and have open glandular lumina [35,52.]

MUCINOUS CARCINOMA

Mammary mucinous carcinomas are also known as colloid, mucoid or gelatinous carcinomas. They have rounded outline and soft texture with characteristic glistening gelatinous appearance. Histologically the tumour consists of small islands or clusters of epithelial cells floating in lakes of extracellular mucin divided by delicate fibrous septae. Intraepithelial component is characterised by micropapillary to solid pattern. The lakes of mucin are positive for mucicarmine and periodic acid Schiff (PAS) stains. Traditionally pure and mixed variants of mucinous carcinoma have been described. Pure type is further divided into cellular variant with intracytoplasmic mucin and hypocellular variant.

MEDULLARY CARCINOMA

Medullary carcinoma are soft, fleshy and circumscribed.On microscopic examination, syncytial growth pattern, circumscription and lymphoplasmocytic response are most important of the diagnostic criteria .Nuclear pleomorphism, prominent nucleoli and frequent mitotic figures are present. Tumour giant cells are not uncommon. Those tumours that do not conform precisely to these criteria has been classified as 'atypical medullary carcinoma'[52].

INVASIVE PAPILLARY CARCINOMA.

Invasive papillary carcinomas are diagnosed predominantly in postmenopausal patients. Microscopically they are characteristically circumscribed, show delicate or blunt papillae with focal solid areas of tumour growth. They may exhibit apical snouting of cytoplasm. The nuclei of tumour cells are typically intermediate grade. DCIS is present in more than 75% of cases and usually but exclusively has papillary pattern. Calcification are commonly seen histologically but usually are present in associated DCIS [29.] They have an excellent prognosis [35,37.]

INVASIVE MICROPAPILLARY CARCINOMA

Micropapillary carcinoma consists of hollow aggregates of malignant cells which on cross section have the appearance of tubules with diminished or obliterated lumens. Tumour cell clusters lie within artefactual stromal spaces caused by shrinkage of the surrounding tissue. . Nuclear pleomorphism is moderate, mitotic activity is low and there is neither necrosis nor lymphocytic reaction [29.]

METAPLASTIC CARCINOMAS

Carcinomas showing extensive metaplastic change to spindle cells, squamous cells and heterologous mesenchymal elements have been applied the term metaplastic carcinoma.

CLASSIFICATION (WHO)*[29]

- Purely epithelial
- Squamous
  - ❖ Large cell keratinizing
  - ❖ Spindle cell
  - ❖ Acantholytic

- Adenocarcinoma with spindle cell differentiation.

- Adenosquamous including muco-epidermoid.

- Mixed epithelial and mesenchymal

  ❖ Carcinoma with chondroid metaplasia.

  ❖ Carcinoma with osseous metaplasia.

  ❖ Carcinosarcoma.

The metaplastic spindle cell and squamous cell carcinomas may present in a pure form without any admixture with a recognizable adenocarcinoma. Extensive sampling of metaplastic tumours should be done to identify carcinomatous foci and distinguish them from true sarcomas because of differences in biologic behaviour and response to therapy. Matrix producing carcinomas are those which show an apparently abrupt transition from carcinoma to an osseous or cartilaginous matrix without an intervening transitional phase[35,52.]

Spindle cell carcinoma is composed of predominantly bipolar spindle cells of relatively bland appearance with only mild or moderate atypia arranged in interweaving bundles. Such tumours frequently also contain areas of squamous differentiation. Matrix producing carcinomas and spindle cell carcinomas have generally favourable prognosis[52.]When the mesenchymal component is malignant the designation of carcinosarcoma is used, which has an aggressive behaviour[59.] Among squamous cell carcinomas, acantholytic variant is a very aggressive tumour[29.]

OTHER RARE TYPES

Various entities such as infiltrating cribriform type, lipid rich carcinoma, secretory carcinoma, oncocytic carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, glycogen rich clear cell carcinoma, apocrine carcinoma, sebaceous carcinoma,inflammatorycarcinoma,neuroendocrinetumours,Adenomyoepithelioma and malignant myoepithelioma have been reported in the literature[29] Hematological malignancies can also affect the breast. Diffuse large cell NHL is the most common[14.]

OTHER MALIGNANT STROMAL TUMOURS

Stromal sarcoma is the generic term given to malignant breast tumours thought to arise from the specialized stroma of this organ but lacking an epithelial component with a phyllodes pattern.Microscopically, most of them have the features of fibrosarcoma, focal osseous metaplasia can occur. Stromal tumours with an appearance equivalent to various types of sarcomas include liposarcoma[7], leiomyosarcoma[6], rhabdomyosarcoma, fibrosarcoma,[34] malignant fibrous histiocytoma, chondrosarcoma,[8]osteosarcoma, follicular dendritic cell sarcomas and Ewing's sarcoma / PNET.

HISTOLOGICAL GRADING

HISTORY

The power of histological grading in breast cancer is evident form the outset since Greenhough and a colleague reviewed histological sections and assessed eight morphological factors namely, the amount of gland formation, presence of secretory vacuoles, cell size, nuclear size, variation in size of both cells and nuclei, degree of nuclear hyperchromatism and number of mitoses and allocated to one of 3 grades grade – I low malignancy grade II – medium malignancy and grade III – high malignancy.

The next landmark provided by studies carried out by Scarff and his colleagues placed most emphasis on amount of tubule formation, inequality in size of nuclei, hyperchromatism and mitosis.

Bloom, a radiotherapist revived interest in histological grading. He chose to follow the Patey and Scarff method but seem to have paid more attention to mitotic figures and found a clear correlation with prognosis.

Together with Richardson, a surgical research fellow, Bloom is responsible for the next advance in histological scoring system. Scarff Bloom Richardson method was adopted as preferred method by WHO[24,26,29.]

Alternative methods that concentrated on nuclear characteristics such as Black's method has been used almost exclusively in the United States where it is referred as nuclear grading[24.] They devised four grades of malignancy based on the regularity of nuclear outline, delicacy of chromatin strands, presence or absence of nucleoli and mitotic figures. Black and colleagues reversed the numerical order to their grades in comparison to Scarff Bloom Richardson so that nuclear grades 0 –1 apply to poorly differentiated carcinomas and grades 3 – 4 to well differentiated tumours.

The method devised by Harveit was based on the definition of cell borders, nuclear crowding, nuclear lobulation and nuclear diameter. Mitoses were not considered.

Fisher and associates combined the histological grade devised by Patey and Scarff and nuclear grade. Le Doussal and colleagues confirmed that the SBR method together with nodal status were the most important factors in predicting metastasis- free survival. They also found the nuclear pleomorphism and mitotic count were the most predictive elements. They arranged the scores to produce five

modified SBR categories (MSBR) which produced better separation of prognostic groups.

It can be concluded that selection of particular grading method is to a large extent a matter of personal choice. In practice the choice of method till the present has emerged mainly along geographical lines, the nuclear grading technique of Black, being more popular in United States and the SBR method in Europe and Australia.

Elston has been the most vocal champion of this approach to use architecture and cytology in conjunction to correlate with prognosis and is usually referred to as the Nottingham modification of the Bloom – Richardson system. This system also incorporates the evaluation of mitotic activity.

Preparation of specimen

The first pre-requisite for accurate histological grading is careful specimen preparation.10% phosphate buffered formalin and other fixatives such as B5s are used.With a 6hr delay in fixation, the number of mitosis was reduced in assigned histological grade. This data insists the importance of incising tumour masses in the fresh state immediately after resection. Adequate tumour sampling is equally important. At least 3 - 4 blocks should be examined. Sections are to be cut 4 - 6µm. Nuclear detail is obscured if sections are too thick.

Parameters of grading

Histological grading is therefore based on assessment of three morphological features, tubule formation, nuclear pleomorphism and mitotic counts [24,29,35,36,52.]

Qualitatively tubular structures must exhibit central lumina. Evaluation of nuclear pleomorphism is the least satisfactory element of any grading system. For complete accuracy morphometry or image analysis can be used but they are expensive, time consuming and impractical for routine diagnostic practice.

In order to introduce degree of objectivity, normal epithelial cells in breast tissues adjacent to tumour can be used as reference point for assessing differences in nuclear features Inflammatory cells such as lymphocytes can also be used for comparative purposes[26]. Regarding the assessment of mitotic counts, a hyper chromatic nucleus that indicates individual cell necrosis should be excluded from the counts. Confusion with apoptic nuclei and intratumoral lymphocytes can be avoided by strict application of criteria for prophase nuclei. The size of so called high power field varies upto six fold from microscope to microscope, hence our counts should be standardized to a defined field area. [16,26,35.] Using this convention any microscope can be calibrated to produce comparable data. Mitotic count evaluated under strict quality control conditions seems to be an accurate and feasible prognostic variable. [5.]

Microscopic grading of breast carcinoma:

Nottingham Modification of the Bloom-Richardson system

Tubule formation

1 point : Tubular formation in > 75% of the tumor.
2 points : Tubular formation in 10 to 75% of the tumor.
3 points : Tubular formations in < 10% of the tumor.

Note : For scoring tubule formations, the overall appearance of the tumor has to be taken into consideration.

Nuclear pleomorphism

1 point : Nuclei with minimal variation in size and shape.

2 points : Nuclei with moderate variations in size and shape.

3 points : Nuclei with marked variation in size and shape

Note: the tumor areas having cells with greatest atypia should be evaluated. For

Mitotic count

1, 2 or 3 points, are assigned as per the table adapted from Diagnostic Histopathology of Tumours by Christopher D.M Fletcher – 3rd edition.

TABLE
ASSIGNMENT OF POINTS FOR MITOTIC COUNTS ACCORDING TO THE MICROSCOPE FIELD AREA

Field diameter in mm	Number of mitoses corresponding to		
	Score 1	Score 2	*Score 3*
0.40	Up to 1	5 to 8	9 or more
0.41	Up to 4	5 to 9	10 or more
0.42	Up to 4	5 to 9	10 or more
0.43	Up to 4	5 to 10	11 or more
0.44	Up to 5	6 to 10	11 or more
0.45	Up to 5	6 to 11	12 or more
0.46	Up to 5	6 to 11	12 or more
0.47	Up to 5	6 to 12	13 or more
0.48	Up to 6	7 to 12	13 or more
0.49	Up to 6	7 to 13	14 or more
0.50	Up to 6	7 to 13	14 or more
0.51	Up to 6	7 to 14	15 or more
0.52	Up to 7	8 to 14	15 or more
0.53	Up to 7	8 to 15	16 or more
0.54	Up to 7	8 to 16	17 or more
0.55	Up to 8	9 to 16	17 or more
0.56	Up to 8	9 to 17	18 or more
0.57	Up to 8	9 to 17	18 or more
0.58	Up to 9	10 to 18	19 or more
0.59	Up to 9	10 to 19	20 or more
0.60	Up to 9	10 to 19	20 or more
0.61	Up to 9	10 to 20	21 or more
0.62	Up to 10	11 to 21	22 or more
0.63	Up to 10	11 to 21	22 or more
0.64	Up to 11	12 to 22	23 or more
0.65	Up to 11	12 to 23	24 or more
0.66	Up to 11	12 to 24	25 or more
0.67	Up to 12	13 to 25	26 or more
0.68	Up to 12	13 to 25	26 or more
0.69	Up to 12	13 to 26	27 or more
0.70	Up to 13	14 to 27	28 or more

Mitotic figures are to be counted only at the periphery of the tumor. Counting should begin in the most mitotically active area ; 10 high-power fields are to be counted in the same area (but not necessarily contiguous). The fields should be filled with as much tumor as possible ; poorly preserved areas are to be avoided. .

Point scoring system was not meant to ascribe mathematical accuracy to the method but it is certainly a step on the way to providing greater objectivity in an essentially subjective method and for creating reproducible treatment algorithms. [5,26.]

The scores for each factor are added together giving a possible total of 3 –9 points. Tumour grade is then allocated on the following basis.

3.5 points – grade 1 – well differentiated.

6-7 points – grade 2 – moderately differentiated.

8-9 points – grade 3 – poorly differentiated.

An acceptable degree of interobserver reproducibility has been achieved such that a strict protocol as recommended by Elston and Ellis is followed.

Significance of grading

Histologic grading is performed in all cases of invasive carcinoma of breast regardless of morphological types[29.] Special types such as tubular, invasive cribriform and mucinous carcinoma carry excellent prognosis comparable with their grade I status.[26,42.] Infiltrating lobular carcinoma especially those of classical subtypes are designated grade 2 [26,42.] However a minority fall into grade 1 or grade 3 category. By definition medullary carcinomas are considered as grade 3 but have been found to have a more favourable prognosis than this degree of differentiation would imply [26,42.]Grading and typing should be carried out in all invasive cancers.

In the majority of breast carcinomas of special type, the grade does not represent a prognosis factor. Validity of grading of lobular carcinoma requires further evaluation. In lobular carcinomas, the diffuse distribution of tumour structures represents the main unfavourable prognostic factor in addition to lymph node and pleomorphic cytologic features[42.]

Analysis of a range of prognostic factors using the multiple regression technique of Cox showed that only tumour size, histological lymph node status and histological grade have significant correlation with overall survival.

Based on the co-efficient of significance produced in the Cox analysis, a simple composite prognostic index has been devised as follows :

NPI = $0.2 \times$ tumour size + lymph node (1-3) stage + histological grade (1-3)

The higher the value for NPI, the worse the prognosis. Three groups have been identified by employing (arbitrary)cut off points <3.4 for the good prognostic group (GPG), 3.41-5.4 for the moderate group (MPG) and >5.4 for the poor group (PPG)[26.]

NPI has become the most widely used and its strength depends on relatively simple data, which can be provided in any routine histopathology laboratory. It is a powerful and reproducible method of assessing prognosis and is the only integrated index, which has been confirmed, in prospective studies. In future more objective methods for estimating tumour differentiation and invasiveness may become available, but currently other techniques including molecular markers do not achieve significance in multivariate analysis when compared with histological grade. It is likely that a molecular classification of breast cancer will only become commonplace when treatment strategies are based on specific gene or biological events.

Studies on the significance of grading early minimal or stage I breast carcinomas have generated controversial results[42.] in contrast with the official statement in the WHO book that tumour grading has prognosis value even in breast cancers of 1cm and smaller. One may criticize that the failure in demonstrating significant results is a consequence of not applying the strict criteria of Elston-Ellis system. However the report of James et al indicates that the invention of this grading system failed to confirm the prognostic significance of

Elston-Ellis grade in small carcinomas. Thus the value of Elston-Ellis grading system seems to be limited to certain subgroups of breast carcinomas. This system is not applicable for insitu carcinomas accounting for approximately 10 – 20% of breast carcinomas.

According to the results of above mentioned studies, the rationale for grading tumour smaller than 15mm, which should account for at least 50% of invasive carcinomas in a screened population according to the current mammography screened quality assurance guidelines may be questioned. Carcinomas larger than 15mm should be graded with exception of tumours with predefined grade as mentioned above[42.]

Grading of tumours exhibiting metastasis at the time of diagnosis may also be questioned, as metastasis may be a more significant determinant of the outcome than the grade itself. Thus grading seems to be meaningful in approximately one-third cases of breast carcinoma; the large. "Not otherwise specified" ductal carcinomas.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is useful in breast tumours in two main sections. First, dealing with antibodies it is useful in diagnosis and recognition of various conditions. Secondly it presents an overview of markers which can be used as predictive or prognostic markers in breast cancer.

DIAGNOSTIC MARKERS

The tumour cells show reactivity for low molecular weight Keratin – cyt 8, 18, 19, EMA, antigen obtained from human milk fat globule membrane and lactalbumin. CEA, B72.3 and BCA 225 are positive in majority of cases.

Identification of myo-epithelial cells in combination with luminal epithelial cells is generally regarded as benign breast conditions.

The myoepithelial cell markers are smooth muscle actin (SMA), S100 protein, calponin, caldesmin, smooth muscle myosin and cytokeratins 5, 6, 14 [33].

- Distinguishing insitu from invasive carcinoma by using antibodies to myo-**epithelial cell markers** and **basement membrane proteins** (eg. Type IV collagen, Laminin).
- Encapsulated papillary carcinoma is distinguished from intracystic papillary carcinoma by using **myoepithelial cell markers** [37]
- To identify neuroendocrine differentiation using neuron specific enolase, chromogranin and synaptophysin.
- Evaluation of spindle cell lesions (metaplastic Vs mesenchymal lesion) by using antibodies to **cytokeratin** and **mesenchymal markers** (eg. Vimentin, CD 34).
- Distinguishing ductal from lobular insitu carcinomas by using antibodies to **E.cadherin.**
- Assessment of metastatic lesions for possible breast origin by using antibodies to estrogen receptor, gross cystic disease fluid protein, cytokeratin 7, 20 and other markers depending on clinical circumstances, anatomic location and histological differential diagnosis [52].

Cell adhesion molecules such as integrins, cadherins, proteases, metalloproteinases, growth factors and their receptors, tumour suppressor genes such as P53, oncogene expression including C-erb B-2 status have been assessed [16].

Steroid hormone receptors such as oestrogen and progesterone receptors are also assayed . They predict response to hormonal therapy . Likewise expression of c-erb-2 protein predicts the response to chemotherapy using monoclonal antibody trastuzumab (Herceptin)[33.]

PROLIFERATIVE INDICES

Proliferative rate of tumour can be determined by combining measurements of growth fraction and cell cycle time through sequential sampling. Estimate of S-phase fraction with thymidine or 5 Bromodeoxy uridine labeling tests and other growth fraction markers such as MIB/Ki-67 and proliferating cell nuclear antigen (PCNA) or cyclin has emerged [33.]Quantitation of KI-67 in CAPSS(Columnar Cell Alteration with Prominent Apical Snouts and Secretions) has increased compared with normal breast but lower than insitu or invasive cancer.

OBSERVATION AND RESULTS

This prospective study of breast neoplasms cover a total of 267 cases in which 120 cases were observed as benign neoplasms and 147 cases as malignant neoplasms.

The incidence of breast neoplasms from January 2005 to December 2006 in correlation with total number of specimens – females is given in following table 1.

TABLE . 1

INCIDENCE OF BREAST NEOPLASMS

S.No.	Period	Total No. of Female Neoplasm	No. of Breast Neoplasm	Percentage
1.	Jan 2005 – Jun 2005	333	69	20.72 %
2.	Jul 2005 – Dec 2005	316	68	21.51 %
3.	Jan 2006 – Jun 2006	221	50	22.62 %
4.	Jul 2006 – Dec 2006	247	80	32.38 %
	TOTAL	1117	267	24.30%

The overall incidence of breast neoplasm is 24.30%

Then considering the incidence of malignancies alone, the overall percentage of breast malignancies is 17.50% as given in following table 2.

TABLE . 2

INCIDENCE OF BREAST MALIGNANCIES

S.No.	Period	Total No. of Female Neoplasm	No. of Breast Neoplasm	Percentage
1.	Jan 2005 – Jun 2005	237	38	16.05 %
2.	Jul 2005 – Dec 2005	274	41	14.96 %
3.	Jan 2006 – Jun 2006	166	31	18.67 %
4.	Jul 2006 – Dec 2006	177	36	20.33 %
	TOTAL	854	146	17.50%

When age specific incidences are divided into eight groups (i.e. <10yrs to > 70yrs),there was an increased incidence of breast neoplasms observed in 31-50yrs (116 cases, 43.44%) followed by 10-20yrs (54cases, 20.22%) and 21-30yrs (53cases, 19.85%). The incidence is very low in paediatric age group less than 10yrs (1case, 0.37%) followed by 61-70yrs (8cases, 2.99%) as given in following table 3.

TABLE . 3

AGE INCIDENCE OF BREAST NEOPLASMS

S.No.	Age Group	No. of Cases	Percentage
1.	<10yrs	1	0.37 %
2.	10 – 20yrs	54	20.22 %
3.	21 – 30yrs	53	19.85 %
4.	31 – 40yrs	58	21.72 %
5.	41 – 50yrs	58	21.72 %
6.	51 – 60yrs	35	13.10 %
7.	61 – 70yrs	8	2.99 %
8.	>70yrs	–	–

Like, age specific incidences of malignant neoplasms are given in following Table 4

TABLE . 4.

S.No.	Age Groups	Epithelial	Fibroepithelial	Mesenchymal	Lymphoma
1.	< 20yrs	1	–	–	–
2.	21 – 30yrs	9	–	–	–
3.	31 – 40yrs	39	2	2	–
4.	41 – 50yrs	47	3	–	1
5.	51 – 60yrs	31	1	1	–
6.	61 – 70yrs	9	–	–	–

The table shows, like overall incidence, the incidence of malignant neoplasms are also common between 41 – 50yrs (51cases, 34.93%) followed by 31 – 40yrs (43cases, 29.45%) and 51 – 60yrs (33cases, 22.60%).

Most of the females with lump breast detected by routine clinical examination were from the surrounding villages with low socio economic status. They presented with the following symptoms as given in table 5.

TABLE . 5

CLINICAL EVALUATION OF CASES WITH MALIGNANT TUMOURS

S.No.	History / Clinical Features	No. of cases	Percentage
1.	Early menarche	140	95.89 %
2.	Age of first child birth		
	Early	137	93.83 %
	Late	9	6.16 %
3.	Parous women	141	96.57 %
4.	Nulliparous women	5	3.42 %
5.	Breast fed	141	96.57 %
6.	Menstrual status		
	Menopausal	93	63.69 %
	Menstruating	53	36.30 %
7.	Family history	4	2.73 %
8.	Treatment history		
	Hormonal therapy	0	0 %
	Previous biopsy for benign disease	2	1.36 %
9.	Breast lump	146	100 %
10.	Discharge	60	41.09 %
11.	Skin changes		
	Erythema	31	21.23 %
	Peaude orange appearance	46	31.50 %
	Ulceration	19	13.01 %
12.	Nipple retraction	40	27.39 %
13.	Axillary nodes	45	30.82 %
14	Location		

	Upper outer quadrant	85	58.21 %
	Upper inner quadrant	11	7.53 %
	Lower outer quadrant	8	5.47 %
	Lower inner quadrant	6	4.10 %
	Diffuse – involving all quadrants	36	24.65 %

All the cases presented with breast lump (146 cases, 100%) and the risk factor like early menarche was seen in most cases. Most of the women were multiparous with history of regular breast feeding. Skin changes like erythema, peau-de-orange appearance, erosion/ulceration of nipple, nipple retraction were seen in one fourth of cases. The table also shows most of the neoplasms were observed in the upper outer quadrant followed by diffuse involvement and upper inner quadrant. The neoplasms were rarely seen in lower outer/inner quadrant. Axillary Lymphadenopathy and nipple discharge were also observed in a proportion of cases.

The overall distribution of breast neoplasms is given in the following table 6.

TABLE . 6

DISTRIBUTION OF BREAST NEOPLASMS

S.No.	Type of Neoplasm	No. of Cases	Percentage
1.	Benign	120	44.94 %
2.	Insitu	0	0 %
3.	Invasive with insitu	12	4.49 %
4.	Epithelial tumours	124	46.44 %
5.	Fibroepithelial tumours	7	2.24 %
6.	Mesenchymal tumours	3	1.12 %
7.	Lymphoma	1	0.37 %

Malignant neoplasm predominates with 147 cases (54.66%) when compared with benign neoplasms (120 cases, 45.31%)

The associated features – Proliferative and nonproliferative changes observed in received specimens are presented in the following table 7.

TABLE . 7

S.No.	Histopathological changes	No. of cases
1.	Non Proliferative breast changes	
	a) Duct ectasia	0
	b) Cysts	4
	c) Apocrine change	0
	d) Mild hyperplasia	6
	e) Adenosis	4
	f) Fibroadenoma without complex features	107
2.	Proliferative disease without atypia	
	a) Moderate or florid hyperplasia	10
	b) Sclerosing adenosis	5
	c) Papilloma	1
	d) Radial scar	–
	e) Fibroadenoma with complex features	–
3.	Proliferative disease with atypia	
	a) Atypical ductal hyperplasia	1
	b) Atypical lobular hyperplasia	0
4.	Carcinoma Insitu	
	a) Lobular carcinoma insitu	0
	b) Ductal carcinoma insitu	0
5.	Other Neoplasms	
	a) Tubular adenoma	2
	b) Lactating adenoma	1
	c) Nipple adenoma	1
	d) Myofibroblastoma	1
	e) Adenomyoepithelioma	1
	f) Duct papilloma	1

Conventional fibroadenoma(Fig7-10&24) without complex features predominates with 107 cases. Florid hyperplasia was observed in 10 cases and sclerosing adenosis in 5 cases.

The epithelial and stromal changes in fibroadenoma are given in the following table 8.

TABLE . 8

S.No.	Histopathological changes	No. of cases
1.	Pattern	
	1) Pericanalicular	60
	2) intracanalicular	35
	3) Both	12
2.	Epithelial changes	
	1) Mild hyperplasia	6
	2) Moderate to florid hyperplasia	10
	3) Atypical ductal hyperplasia	1
	4) Sclerosing adenosis	5
	5) Adenosis	4
	6) Cystic change	4
	7) Apocrine change	–
3.	Connective tissue (stromal) change	
	1) Increased cellularity	13
	2) Hyalinisation	13
	3) Myxoid changes	16

The age incidences of individual benign neoplasms are given in following table 9.

TABLE . 9

INCIDENCES OF INDIVIDUAL BENIGN NEOPLASMS

S.No.	Type of benign neoplasm	Age Groups				
		< 20	21 – 30	31 – 40	41 – 50	51 – 60
1.	Fibroadenoma	53	56	13	3	2
2.	Fibroadenoma with benign phyllodes	1	3	–	–	–
3.	Benign phyllodes	–	–	1	1	–
4.	Others a) Nipple adenoma	–	1	–	–	–
	b) Tubular adenoma	1	1	–	–	–
	c) Lactating adenoma	–	1	–	–	–
	d) Myofibroblastoma	–	–	–	1	–
	e) Duct papilloma(Fig13)	–	–	1	–	–
	f) Adenomyoepithelioma	–	–	–	–	1

Most of the fibroadenomas are seen in the early reproductive age group, less than 20yrs (53 cases, 50%) followed by 21 – 30yrs (36cases, 33.96%). Special forms of adenomas such as tubular adenoma(Fig.11) and lactating adenoma(Fig.12) were also observed in the same age group. One case of adenomyoepithelioma and myofibroblastoma were observed in the postmenopausal age group.

Distribution of epithelial / Non epithelial malignant neoplasms of the breast are given in the following tables 10A and 10b.

TABLE 10A

DISTRIBUTION OF EPITHELIAL MALIGNANT TUMOURS

S.No.	Type of tumour	No. of cases
1.	Infiltrating ductal carcinoma (IDC) with ductal carcinoma insitu (DCIS)	12
2.	Infiltrating ductal carcinoma – NOS	103
3.	Invasive lobular carcinoma(Fig.26)	2
4.	Mucinous carcinoma(Fig.2,18&19)	4
5.	Medullary carcinoma(Fig.25)	1
6.	Invasive papillary carcinoma(Fig.20&21)	5
7.	Invasive micropapillary carcinoma(Fig.22)	1
8.	Infiltrating cribriform carcinoma(Fig.29)	1
9.	Adenoid cystic carcinoma(Fig.27)	1
10.	Neuroendocrine – carcinoid tumour(Fig.28)	1
11.	Metaplastic carcinoma(Fig.30-32)	4
12.	Adenomyoepithelioma with malignancy (epithelial) (Fig.33&34)	1

TABLE 10B

DISTRIBUTION OF NON-EPITHELIAL MALIGNANT TUMOURS

Type of tumour	No. of cases
Mesenchymal	
Pleomorphic sarcoma	1
Fibrosarcoma	1
Angiosarcoma(Fig.3)	1
Lymphoma	
Non Hodgkin's Lymphoma	1

Most of the cases were infiltrating ductal carcinoma-NOS type(Fig.1) followed by IDC with DCIS(Fig.17). 5 cases of invasive papillary carcinoma and 4 cases of mucinous and metaplastic carcinoma were also observed. In our study 4 nonepithelial malignant neoplasms such as i.e. pleomorphic sarcoma(Fig.43), fibrosarcoma(Fig.44), angiosarcoma (Fig.45)and lymphoma(Fig.46&47) were also observed.

The histomorphological patterns seen in DCIS are given in the following table 11.

TABLE .11

HISTOPATHOLOGICAL PATTERNS IN DCIS ASSOCIATED WITH IDC.

S.No.	HPE Pattern	No. of Cases
1.	Solid	3
2.	Comedo	8
3.	Micropapillary	1
4.	Cribriform	2

Comedopattern with or without necrosis(Fig.23)is seen in 8 cases followed by solid pattern in 3 cases.

The histopathological pattern in infiltrating ductal carcinoma is given in following table 12.

TABLE .12

HISTOPATHOLOGICAL PATTERNS IN IDC

S.No.	HPE Pattern	No. of Cases
1.	Solid(Fig.16)	27
2.	Trabecular(Fig.15)	65
3.	Tubular(Fig.14)	7
4.	Comedo	23
5.	Cribriform	2
6.	Papillary	–

The trabecular pattern is commonly seen (65cases, 56.52%) followed by solid (27cases, 23.47%) and comedo pattern (23cases, 20%)

The following table 13 shows the distribution of fibroepithelial lesion.

TABLE .13

DISTRIBUTION OF FIBRO-EPITHELIAL TUMOURS

S.No.	Type of tumour	No. of cases

1.	Fibroadenoma
	    - Nos
	    - Juvenile
		
		103
		4

2.	Benign Phyllodes(Fig.4&35)	2
3.	Phyllodes with fibroadenoma	4
4.	Borderline phyllodes	1
5.	Malignant phyllodes(Fig.36&37)	4
6.	Malignant phyllodes with heterologous differentiation(Fig.5,6,38&42)	2

The age specific grading of malignant neoplasms of breast is given in following table 14.

TABLE. 14

GRADING AND AGE

S.No.	Age Groups	Grade I	Grade II	Grade III
1.	< 25	–	1	2
2.	25 – 29	–	1	1
3.	30 – 34	–	8	2
4.	35 – 39	2	12	6
5.	4 0 – 44	–	15	7
6.	45 – 49	2	21	8
7.	50 – 54	2	14	1
8.	55 – 59	3	8	1
9.	60 – 64	2	10	4
10.	65 – 69	2	3	1
11.	≥ 70	–	–	1
		13 (9.2%)	93 (66.42%)	34 (24.28%)

Like, the nodal positivity and staging are given in following table15A and 15B.

TABLE .15A

AGE AND NODAL POSITIVITY

S.No.	Age Groups	No. of Nodes		
		1 – 3	4 – 9	≥ 10
1.	< 25	–	–	–
2.	25 – 29	1	–	–
3.	30 – 34	4	1	–
4.	35 – 39	5	1	–
5.	40 – 44	6	2	–
6.	45 – 49	2	2	–
7.	50 – 54	4	2	–
8.	55 – 59	3	1	–
9.	60 – 64	5	3	–
10.	65 – 69	2	–	–
11.	≥ 70	–	–	–

TABLE .15B
STAGING AND GRADING

S.No.	Stage	Grade I	Grade II	Grade III
1.	Stage I	–	3	–
2.	Stage II A	5	38	16
	Stage II B	7	32	9
3.	Stage III A	–	13	3
	Stage III B	–	1	1
4.	Stage IV	–	–	–

The overall grading of epithelial tumours according to modified Bloom and Richardson Method. Elston & Ellis is given in the following table 16.

TABLE .16
GRADING OF EPITHELIAL TUMOURS – MODIFIED BLOOM &
RICHARDSON METHOD – ELSTON & ELLIS

S.No.	HPE No.	Scores			Total Score	Grade
		Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts		
1.	24/05	1	2	1	4	I
2.	52/05	3	2	1	6	II
3.	96/05	3	2	1	6	II
4.	124/05	3	2	1	6	II
5.	166/05	3	2	1	6	II
6.	195/05	3	2	1	6	II
7.	295/05	3	2	1	6	II
8.	345/05	3	2	1	6	II
9.	347/05	3	2	2	7	II
10.	352/05	3	2	1	6	II
11.	433/05	3	2	3	8	III
12.	505/05	3	2	1	6	II
13.	530/05	3	2	1	6	II
14.	533/05	3	2	1	6	II
15.	538/05	3	2	1	6	II
16.	559/05	3	3	2	8	III
17.	627/05	3	2	1	6	II
18.	663/05	3	2	1	6	II
19.	749/05	3	2	1	6	II

20.	765/05	3	2	1	6	II
21.	816/05	3	3	3	9	III
22.	859/05	3	2	1	6	II
23.	881/05	3	2	1	6	II
24.	917/05	3	3	1	7	II

S.No.	HPE No.	Scores			Total Score	Grade
		Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts		
25.	1055/05	3	2	1	6	II
26.	1068/05	3	2	1	6	II
27.	1098/05	3	2	1	6	II
28.	1101/05	3	2	1	6	II
29.	1104/05	3	3	2	8	III
30.	1174/05	3	3	1	7	II
31.	1228/05	3	3	1	6	II
32.	1344/05	3	2	1	6	II
33.	1352/05	3	2	2	7	II
34.	1359/05	3	2	1	6	II
35.	1501/05	3	2	1	6	II
36.	1505/05	3	2	3	8	III
37.	1654/05	3	2	1	6	II
38.	1772/05	2	2	1	5	I
39.	1783/05	3	2	1	6	II
40.	1805/05	3	2	1	6	II
41.	1820/05	3	3	2	8	III
42.	1886/05	3	2	1	6	II
43.	1970/05	3	2	1	6	II
44.	1972/05	3	2	1	6	II
45.	2091/05	3	2	1	6	II

46.	2111/05	3	2	1	6	II
47.	2174/05	3	2	1	6	II
48.	2204/05	3	2	1	6	II
49.	2264/05	3	3	2	8	III
50.	2216/05	3	2	1	6	II
51.	2319/05	3	3	2	8	III
52.	2362/05	3	3	2	8	III
53.	2401/05	3	2	1	6	II
54.	2455/05	3	2	1	6	II
55.	2496/05	3	2	1	6	II
56.	2497/05	3	2	1	6	II
57.	2540/05	3	2	2	7	II
58.	2541/05	3	2	2	7	II
59.	2788/05	3	2	1	6	II
60.	2801/05	3	2	1	6	II
61.	2817/05	3	2	1	6	II
62.	3035/05	3	2	2	7	II
63.	26/06	3	2	2	7	II
64.	129/06	3	2	1	6	II
65.	324/06	3	2	1	6	II

S.No.	HPE No.	Scores			Total Score	Grade
		Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts		
66.	386/06	3	2	1	6	II
67.	400/06	3	2	1	6	II
68.	475/06	3	2	1	6	II
69.	481/06	3	2	1	6	II
70.	555/06	3	3	2	8	III
71.	608/06	3	2	3	8	III
72.	753/06	3	3	3	9	III
73.	770/06	3	3	2	8	III
74.	931/06	3	2	1	6	II
75.	1041/06	3	2	2	7	II
76.	1114/06	3	2	1	6	II
77.	1160/06	3	2	1	6	II
78.	1207/06	3	2	3	8	III
79.	1268/06	3	2	1	6	II
80.	1464/06	3	3	2	8	III
81.	1499/06	3	3	2	8	III
82.	1618/06	3	3	2	8	III
83.	1645/06	3	3	2	8	III
84.	1682/06	3	3	2	8	III
85.	1710/06	3	2	1	6	II
86.	1732/06	3	3	2	8	III

87.	1785/06	3	2	1	6	II
88.	1902/06	3	2	1	6	II
89.	1938/06	3	2	1	6	II
90.	1939/06	3	3	2	8	III
91.	1988/06	3	2	1	6	II
92.	2104/06	3	2	1	6	II
93.	2131/06	1	2	1	4	I
94.	2147/06	3	2	3	8	III
95.	2314/06	3	3	2	8	III
96.	2355/06	3	2	1	6	II
97.	2412/06	3	2	1	6	II
98.	2449/06	3	3	2	8	III
99.	2455/06	3	3	2	8	III
100.	2497/06	3	2	1	6	II
101.	2538/06	3	3	2	8	III
102.	2575/06	3	3	1	7	II
103.	2643/06	3	2	1	6	II
104.	2659/06	2	2	1	5	I
105.	2666/06	3	3	2	8	III
106	2799/06	2	2	1	5	I

S.No.	HPE No.	Scores			Total Score	Grade
		Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts		
107.	2815/06	3	2	1	6	II
108.	2890/06	3	2	1	6	II
109.	2996/06	3	2	1	6	II
110.	3105/06	3	2	1	6	II
111.	3106/06	3	2	1	6	II
112.	3126/06	3	2	1	6	II
113.	3128/06	3	3	2	8	III
114.	3360/06	2	2	1	5	I
115.	3361/06	3	2	1	6	II

The grading for epithelial special types, nonepithelial neoplasms as well as Malignant Phyllodes tumour are given in the following tables 17A, 17B and 18

TABLE .17A

GRADING OF SPECIAL TYPES – EPITHELIAL

S.No.	HPE No.	Diagnosis	Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts	Total Score	Grade
1.	295/05	Metaplastic carcinoma	3	3	2	8	III
2.	794/05	Invasive lobular	3	2	1	6	II

		carcinoma					
3.	1745/06	Invasive papillary carcinoma	3	2	1	6	II
4.	1933/05	Papillary carcinoma with cystic changes	3	2	1	6	II
5.	2405/05	Metaplastic carcinoma	3	3	2	8	III
6.	2469/05	Mucinous carcinoma	3	1	1	5	I
7.	2471/05	Infiltrating cribriform carcinoma	3	1	1	5	I
8.	2898/05	Adenoid cystic carcinoma	3	2	1	6	II
9.	2909/07	Invasive lobular carcinoma	3	2	1	6	II

S.No.	HPE No.	Diagnosis	Tubule and Glandular formation	Nuclear pleomorphism	Mitotic counts	Total Score	Grade
10.	3027/05	Intracystic papillary carcinoma	3	2	1	6	II
11.	3076/05	Invasive papillary carcinoma	3	2	1	6	II
12.	3085/05	Metaplastic carcinoma	3	3	2	8	III
13.	545/06	Invasive micropapillary carcinoma	3	2	1	6	II
14.	695/06	Medullary carcinoma	3	2	3	8	III
15.	732/06	Mucinous carcinoma	3	1	1	5	I
16.	1106/06	Invasive papillary carcinoma	3	2	1	6	II
17.	1876/06	Metaplastic carcinoma	3	3	2	8	III
18.	1965/06	Mucinous carcinoma	3	1	1	5	I
19.	2670/06	Adenomyoepithelioma with malignancy	3	2	1	6	II
20.	2774/06	Mucinous carcinoma	3	1	1	5	I
21.	987/06	Neuro endocrine tumour	3	1	1	5	I

TABLE .17B

GRADING OF NON-EPITHELIAL TUMOURS

S.No.	HPE No.	Diagnosis	Grade
1.	801/05	Non Hodgkin's Lymphoma	II
2.	2281/05	Fibrosarcoma	II
3.	3013/05	Pleomorphic sarcoma	III
4.	916/06	Angiosarcoma	I

When grading the epithelial tumours, tubule and glandular formation, nuclear pleomorphism and mitotic counts were included for scoring.

EPITHELIAL TUMOURS WITH SCORE 3 – 5 WERE GRADED AS GRADE I, 6 – 7 AS GRADE II AND 8 – 9 AS GRADE III.

TABLE. 18

S.No.	Features	Case I 764/05	Case 2 1475/05	Case 3 802/06	Case 4 983/06	Case 5 2195/06	Case 6 1028/06
1.	Stromal Hypercellularity	Moderate	Marked	Marked	Marked	Moderate	Marked
2.	Cellular pleomorphism	Marked	Moderate	Marked	Marked	Marked	Marked
3.	Mitosis	> 8/hpf	> 7/hpf	> 10hpf	7/hpf	6/hpf	> 10/hpf
4.	Margins	Pushing/ intermediate	Pushing/ Intermediate	Invasive	Invasive	Pushing/ Intermediate	Invasive
5.	Stromal pattern	Stromal expansion	Stromal overgrowth	Stromal overgrowth	Stromal expansion	Stromal expansion	Stromal overgrowth
6.	Heterologous stromal differentiation	–	–	+	–	–	+

In phyllodes tumour, morphological factors such as stromal hypercellularity, cellular pleomorphism, mitosis, margins, stromal pattern and Heterologous stromal differentiation were taken for scoring.

DISCUSSION

Breast cancer is the commonest cancer among females in developed countries. It is the second most common cancer among women in south India but it is the most frequent cancer among women in western India (Bombay)[15]. In several countries worldwide, there has been a steady increase in breast cancer.

There is a wide variation in incidence between countries, and also within any country for which several causes are cited. Some of the increased diagnosis is due to more widespread screening programmes and alterations in registration procedures in certain countries, but it appears that there is a genuine increased incidence not accounted for by these factors[1]. These two aspects of breast cancer emphasize the importance of early diagnosis and appropriate management.

The incidence as well as prevalence of breast cancer by various studies conducted as per the literature and journals are given in the following table no 19.

TABLE. 19

S.No.	Name and Year of Study	Prevalence of Breast Cancers
1.	Indian Cancer Society, Mumbai (2001-2003)	27.47%
2.	Kidwai Memorial Institute of Oncology, Bangalore (2001-2003)	24.58%
3.	Nargis Dutt Memorial Cancer Hospital, Barshi (2001-2003)	16.85%
4.	Gandhi Medical College, Bhopal (2001-2003)	24.91%
5.	Gujarat Cancer and Research Institute, Ahamadabad (2001-2003)	19.32%
6.	Dr. B.R. Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi. (2001-2003)	25.11%
7.	National Cancer Registry, Bangalore Dibrugarh District (2003-2004)	18.78%
8.	National Cancer Registry, Bangalore Kamrup Urban District (2003-2004)	18.6%
9.	National Cancer Registry, Bangalore Silchar town (2003-2004)	16.5%
10.	National Cancer Registry, Bangalore Imphal West District (2003-2004)	14.3%
11.	National Cancer Registry, Bangalore Mizoram State (2003-2004)	11.9%
12.	National Cancer Registry, Bangalore Sikkim State (2003-2004)	14.2%
13.	WHO, Annual Report 2002	20%
14.	Cancer Institue (WIA), Adyar Chennai (2001-2003)	26.07%
15.	Study of Thanjavur Medical College Hospital (2002 -2003)	12.3%
16.	Present Study	17.50%

In our study the prevalence is 17.50%, which is in close correlation with other studies as well as the annual report by WHO. The table also shows that the prevalence at Mumbai is 27.47%, which denotes breast cancer being the most common malignant neoplasm in females surpassing carcinoma cervix. The table also shows that the incidence at semi-urban area-Thanjavur medical college is

increasing from 12.3% to 17.50%, which is about 40%. This study also shows that in females, breast neoplasms alone constitute 25%.

The age specific incidence of breast neoplasm ranges from 20-70 yrs as per the studies, journals and literature which also show increased incidence at postmenopausal age group i.e. 50-70 yrs. In contrast, in our study the highest incidence of breast neoplasms are noticed between 21-50 yrs and the incidence of breast neoplasms after 60yrs constitute less than 3%. Like, the age specific incidences of malignant neoplasms are also common between 40-50 yrs in contrast. Our study also shows that incidence of epithelial neoplasms (94%) outnumber the mesenchymal neoplasms.

The risk factor evaluation shows that presence of breast lump and early menarche are found in almost all cases but in contrast to the literature most of them are multipara and with early age at first child birth. Most of the neoplasms are found in the upper outer quadrant which is in correlation with literature.

Studies in Nordic countries have shown an increased breast cancer risk in women of low parity and those who have their first child late[28], which is in contrast to our study. Likewise in studies in Norway it has been found that high parity is associated with an overall reduced risk of breast cancer but in our study parous women contribute to 96.57% of malignant breast tumour cases. Similarly in Norway studies, the protective effect of high parity was particularly strong among women with first child birth before age of 20 years and rather weak among those with first child birth at the age 30 years or more[28]. This is in contrast to our study where early age at first child birth contributes to 93.83% of cases and late age at first birth to only 6.16% of cases.

Studies in Nordic countries and Norway have shown increased risk of breast cancer among women who had oral contraceptives and hormone

replacement therapy for prolonged periods but in contrast, our study has no association with the above mentioned risk factors[28].

Nipple discharge is considered to be pathologic, if it is spontaneous, arises from a single duct, is persistent and contains gross or occult blood. As per the studies by Richard J.Santen et al, among female patients referred to physicians because of symptoms of breast disorder, 6.8% have nipple discharge[50]. This is in contrast with our study where 41.09% of cases presented with nipple discharge.

Among women who have had a benign breast biopsy, the risk for developing subsequent carcinoma is related to the histologic components of antecedent biopsy. A higher relative risk is associated with atypical hyperplasia than with other proliferative lesions. Page et al found the relative risks to be 4.7 and 5.8 respectively for women with atypical duct and atypical lobular hyperplasia when compared with women who had non proliferative biopsies[12]. In our study women who had previous biopsy for benign disease account for 1.36% of cases but relevant details about histologic components of antecedent biopsy are not available.

The interaction of atypical hyperplasia with family history is so strong in the study of David L. Page et al[54], that it is relevant to consider women with atypical hyperplasia who have a family history of breast cancer separately from those who do not. Women with a positive family history experienced a risk of invasive breast cancer of about 20% at 15 years. This strong interaction has been supported in two recent studies[18]. In our study family history contributes to 2.73% of cases but in contrast no interaction of atypical hyperplasia is found.

In our study, benign neoplasms constitute 45% of cases with predominantly fibroadenoma exhibiting conventional pattern without complex features.   Few cases of non-proliferative breast changes i.e hyperplasia and cystic changes are

also observed in this study. As in the literature precancerous lesions like sclerosing adenosis and florid hyperplasia are also observed in this study.

Most of the cases with benign breast neoplasms presented as nontender, firm, mobile masses varying in size from 1 to 15 cms. Almost all cases are evaluated initially with fine needle aspiration and subsequently subjected for excision biopsy.

Fine needle aspiration remains   as a single initial evaluative tool in breast lump with sensitivity and specificity of 90 to 95%.

The major goals in evaluation of breast biopsy is to distinguish benign from insitu, invasive breast cancers and to assess the risk of subsequent breast cancer associated with benign lesions identified.

The benign disorders are categorized according to the criteria of Dupont Page and Rogers as   Nonproliferative diseases, proliferative diseases without atypia and proliferative diseases with atypia [60] Dupont and page quantified   the association between subtypes of benign breast disease and breast carcinoma. They have   observed that the risk is minimal   for non-proliferative   disease, modestly   increased   for proliferative disease   without   atypia and greatest   for atypical-hyperplasia [18.] Women with   nonproliferative   lesion associated with strong family history are also at increased risk.

According to Timothy W.Jacobs et al, for women with proliferative disease without atypia the relative   risk of breast cancer is 3.0 for those with radial scars and 1.5   for those without radial   scars. Among   women with   atypical hyperplasia the relative risk for breast cancer is 5.8 for those with radial scars and 3.8 for those without radial scars.[57] David L page et al also pointed out that women with atypical lobular hyperplasia associated with family history have the

relative risk of 8.4 and those with atypical ductal hyperplasia and family history have the relative risk of 9.7 [23.].

In fibroadenomas, the morphological patterns i.e. pericanicular, intracanalicular and mixed fashions are observed even though they have little importance in prognosis of the patient. According to the criteria of Page et al, the risk of breast cancer after benign disease is slightly elevated in the presence of proliferative disease without atypia and is substantially increased in women with atypical hyperplasia. The magnitude of the association between atypical hyperplasia and risk of breast cancer (RR 3.7) is in the range reported by Dupont and Page (RR, 5.3) and Carter et al (RR - 3.0).[18] Epithelial changes like florid hyperplasia and sclerosing adenosis are also followed up for a period of 2 years and no evidence of malignant transformation are observed in this study. As in the literature as well as studies by various resource people, fibroadenoma is commonly observed in the age group of 15-30 years in our study.

As a result of population - based mammographic screening , the panorama of the diagnosed breast carcinomas has shifted from the clinically detectable advanced tumours to the clinically silent but radiologically detectable early forms of breast cancer in most of the developed countries. A reduction in breast cancer mortality has been achieved.[42.]

In our study infiltrating ductal carcinoma NOS, type observed in 103 cases stands as the most common malignant neoplasm of the breast. 15% of the cases also show associated DCIS changes. In our study, the epithelial malignant neoplasms predominates with 136 cases, 50.93%. Few rare neoplasms like invasive lobular carcinoma, medullary carcinoma, mucinous carcinoma, invasive papillary carcinoma, invasive micropapillary carcinoma, infiltrating cribriform cardinoma, Adenoid cystic carcinoma, Neuro-endocrine -

carcinoid tumour and metaplastic carcinoma are also observed in this study. One case of adenomyoepithelima with epithelial malignant transformation exhibited as epithelial cells dispersed in solid sheets with absence of myo-epithelial layer and the individual cells having increased nuclear atypia accompanied by atypical mitosis is also observed in this study.

In our study 4 cases of non-epithelial malignant tumours are also observed. Pleomorphic sarcoma in which the neoplastic cells are undifferentiated small cells admixed with bizarre cells are dispersed in solid sheets. There is increased atypical mitosis and areas of necrosis. In fibrosarcoma the individual cells are spindle cells arranged in the form of herring bone pattern. Angiosaroma consists of interanastamosing vascular channels dissecting the interlobular stroma and some replacing the terminal ductal lobular units. The nuclei of the endothelium lining the neoplastic vessels are prominent and hyperchromatic. Primary breast lymphoma is a rare clinical entity that accounts for less than 1% of all patients with Non-Hodgkin's lymphoma and approximately 1.7% of all patients with extralymph node NHL. Nearly all patients with lymphomatous involvement of the breast had their disease detected as palpable breast masses rather than by mammography. The tumours are comprised of large cells with round to irregular vesicular nuclei variably prominent nucleoli and scant to moderate amounts of cytoplasm. Wiseman and Liao, first defined clinical criteria for the classification of primary breast lymphoma including 1) adequate pathologic evaluation 2) mammary tissue in close association with lymphomatous infiltrate 3) no evidence of disseminated lymphoma other than simultaneous ipsilateral lymph node involvement and 4) no prior diagnosis of lymphoma[55.].

In our study, 9 cases of Phyllodes tumour are observed in which 6 cases are observed as malignant phyllodes / malignant phyllodes with heterologous differentiation.

Histological grading is one of the traditional histological prognostic factors in breast carcinoma. It is acknowledged that there are atleast three situations in which prognostic factors are useful; to identify a group of patients whose prognosis is so good that adjuvant therapy would not be beneficial ; to identify patients whose outlook is so poor that aggressive adjuvant therapy is warranted; to identify patients who are likely to be responsive or resistant to a particular type of therapy.[25,26.] Omission of Histologic grading from clinical decision making may result in overuse of adjuvant therapies in breast cancer.

Pathology has proudly performed the role of "gold standard" in diagnosing breast carcinoma for a long time. Achieving high resolution of the details of the examined tissue, histopathology is still the most sensitive method in diagnosing breast malignancy compared with the other currently used imaging techniques. The so called first generation morphologic prognostic factors (tumour size, histological grade, and lymph node status) have repeatedly been proven as reliable in large, unselected series of breast carcinomas as well as in series of advanced breast carcinomas. These parameters as assessed by the pathologist represent the basis for staging and are decisive factors in international therapy recommendations. The standards for interpreting high resolution histological images were already established more than half a century ago and the basic principles of diagnosing, grading, typing and measuring breast lesions have not substantially changed for decades.[42.]

The most widely accepted system for grading invasive breast carcinoma is the Elston-Ellis system which represents a modification of the Scarf-Bloom -

Richardson system established in the middle of last century. Most practicing breast pathologist are trained to apply the Elston - Ellis system and the reproducibility of this grading system is well documented.

Some tumour types have a predefined grade e.g.   classic lobular carcinomas are always grade 2, tubular carcinomas always grade 1, and medullary carcinomas always grade 3. The most serious problem in applying any grading system is the intratumoral and intertumoral heterogeneity characterizing   many breast   carcinomas.   This   means that one cannot be convinced that the most aggressive tumour cell population within the tumour determined the results of the grading, although it may determine the outcome of the   disease.   However the advantage   of giving a   numerical parameter for assessing the biologic   potential of a carcinoma seems to clearly exceed the disadvantages above,   especially   when   creating   reproducible   treatment algorithms.

Different methods for histological grade evaluation have been used over time. Although some authors have demonstrated that these approaches have almost the same potential for predicting patient survival. The basic problem remains that the prognostic value of histological grade has been studied in series of patients who are heterogenous in terms of stage and treatment and therefore unsuitable for correctly   determining the prognostic value. Moreover, the few initiatives of interlaboratory quality control   and histological grade evaluations have   mainly highlighted unsatisfactory concordance results with correlation co-efficients more frequently around 0.55 and never higher than 0.75.

A comparative analysis of proportion of grade I, grade II and grade III breast cancers by various studies are given in the following Table 20.

TABLE . 20

COMPARISON OF RELATIVE PERCENTAGE OF CASES IN EACH GRADE

S.No.	Study & Year	Grade		
		I	II	III
1.	Bloom and Richardson 1957	26	45	29
2.	Tough et al., 1957	11	51	38
3.	Champion, Wallace & Prescott 1972	23	52	25
4.	Fisher et al., 1984	17	37	46
5.	Elston 1984	17	37	46
6.	Doris et al., 1986	22	49	22
7.	Contesso et al., 1987	21	30	29
8.	Hopton et al., 1989	29	45	26
9.	Le Donssal et al., 1989	11	55	46
10.	Study – Thanjavur Medical College 2002-2003	38.8	50	11.2
11.	Present Study	9.2	66.42	24.28

In our study, most of the breast cancers are in the grade II in contrast with the western literature where more cases are picked up in the early stages. In our study 24% of cases are seen in well advanced stage, grade III.

Two difficult cases are also subjected for immunohistochemical markers studies as in the following table: 21

Table . 21

S.NO.	HISTOPATHOLOGICAL DIAGNOSIS	MARKER	RESULT	IMPRESSION
1.	Malignant Phyllodes with heterologous rhabdomyosarcomatous differentiation (Fig 48 a,b)	Actin Desmin	POSITIVE POSITIVE	Malignant Phyllodes with heterologous rhabdomyosarcomatous differentiation
2.	Adenomyoepithelioma with ? malignancy (Fig 49)	Smooth muscle actin	Positive	Adenomyoepithelioma

Relative incidence of breast malignancies are given in the following table : 22.

TABLE -22

RELATIVE INCIDENCES OF MALIGNANT TUMOURS

S.No.		Type of Malignancy	Anderson et al.,	Rosen	Fisher et al.,	Ellis et al.,	Tata Memorial, Mumbai	Study at Thanjavur Medical College 2002-2003	Present Study
I		*NON INVASIVE*							
	a)	Ductal	8%					6.45%	
	b)	Lobular	2%						
II		*INVASIVE*							
	a)	Ductal carcinoma-NOS	60%	75%	53%	49%	88%	86.91%	78.23%
	b)	*Special Types*	30%					5.35%	15.44%
		Lobular	15%	10%	5%	16%	4%	0.64%	1.36%
		Medullary	7%	9%	6%	3%	2%	0.64%	0.68%
		Tubular	5%	2%	1%	2%			
		Mucinous	5%	2%	2%	1%		1.94%	2.72%
		Papillary						0.64%	3.4%
		Metaplastic carcinoma							2.72%
		Other Rare Types Infiltrating cribriform Ca							0.68%
		Infiltrating micropapillary Ca							0.68%
		Adenoid cystic carcinoma							0.68%
		Adenomyoepithelioma with malignancy							0.68%
		Neuroendocrine tumour							0.68%
III		*Malignant Phyllodes*						1.29%	4.08%
IV		*Mesenchymal tumours*							2.04%
V		*Lymphoma*							0.68%

CONCLUSION

In the present prospective study of 267 cases of breast neoplasms, evaluated with clinical, light microscopy, histochemical and immunohistochemical markers, following conclusions are made and presented.

1. The average incidence of breast neoplasms is 24.30%.
2. The average incidence of breast malignancies in relation to total number of cancers in females is 17.50%.
3. The incidence of breast neoplasm is steady and slowly progressive without any abrupt increase.
4. Early menarche and early age of first child birth are the only two clinical parameters found in association with breast cancer.
5. Mammography and fine needle aspiration remains as gold standard in initial evaluation of breast lump.
6. Upper and outer quadrant is the preferable site for all breast lumps
7. Conventional fibroadenoma is the commonest benign breast neoplasm.
8. In conventional fibroadenoma, pericanalicular fashion is common.
9. Benign neoplasms commonly occur between 15-30 years.
10. Infiltrating Ductal Carcinoma (NOS) is the commonest malignant neoplasm with trabecular pattern.
11. Malignant neoplasms commonly occur between 30-60 years.
12. No age is exempt for breast cancer since one case is also observed at the age of five.
13. Non epithelial malignant tumours commonly occur in the post menopausal age group.
14. Fibro epithelial phyllodes tumour commonly present in an advanced stage.

15. Grading is a very useful protocol in evaluation, management and further prognosis.

16. Most of the epithelial malignancies are seen as grade II.

17. Modified Bloom Richardson method - Elston & Ellis grading with tubular and glandular formations, nuclear pleomorphism and mitotic count stands as the best method for grading epithelial tumours.

18. Grading of fibroepithelial phyllodes tumour is done separately by taking morphological factors such as stromal cellularity, cellular pleomorphism , mitosis , margins, stromal pattern and heterologous stromal differentiation for scoring

19. In case of doubtful diagnosis, immunohistochemistry is very useful for further confirmation

The majority of breast carcinomas in young women are invasive, with T2 disease at presentation and of poor histological grade. The recent rise in numbers suggests increased detection, possibly due to improved awareness of breast disease among the younger female population.

The urgent need for improved screening techniques for early detection and for an aggressive health education compaign to increase the awareness of women about the potential risk of breast cancer and early detection by regular testing like mammography and fine needle aspiration studies improves survival.

APPENDIX – I

HAEMATOXYLIN AND EOSIN

Preparation of the solution :

Distilled water	-	1000ml
Ammonium alum	-	100g
Haematoxylin	-	5g
Absolute ethylalcohol	-	50ml
Mercuric Oxide	-	2.5g

100g of ammonium alum dissolved in 1000ml of distilled water by heating nad shaking at 60°C. Add solution of 50g of haematoxylin in 50ml of ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 2.5g of Mercuric oxide. Mix by swirling gently.

EOSIN STAIN

Eosin Y	-	1g
Distilled water	-	20ml
95% ethanol	-	80ml
Glacial acetic acid	-	0.2ml

Dissolve 1g eosin Y in 20ml of water add 06% ethanol and glacial acetic acid.

PROCEDURE

❖ Sections to water.

❖ Harris's hematoxylin for 15 minutes.

❖ Rinse in tap water.

❖ Differentiate in acid alcohol – 3 to 10 quick dips.

❖ Wash in tap water very briefly.

❖ Dip in ammonia water (for 10-20 seconds) saturated lithium carbonate until sections are bright blue.

❖ Wash in running tap water for 10-20 minutes.

❖ Stain with eosin for 15 seconds to 2 minutes depending in the age of the eosin and the depth of counter stain required.

❖ 95% alcohol.

❖ Absolute alcohol – at least 2 changes.

❖ Xylene – 2 changes.

❖ Mount in DPX mountant.

APPENDIX – II

MAY GRUNWALD GIEMSA

1. Air dry the smear
2. Fix by immersing in a jar of methanol   - 5 to10 minutes
3. Transfer to a staining jar containing

    MAY GRUNWALD stain freshly diluted with an   -15 minutes

     equal volume of buffered water
4. Transfer the slide without washing to a jar

    containing giemsa stain freshly diluted with 9   -   10 to15 minutes

    volume of buffered water
5. Wash with buffered water
6. Dry and mount.

APPENDIX-III

PERIODIC ACID SCHIFF TECHNIQUE

Solution required

a) 1.5% periodic acid.

b) Mayer's haemalum

c) Sulphurous acid

Sodium metabisulphite 10%	6 ml
N/I hydrochloric acid 10%	5 ml
Distilled water	100 ml

(d). Schiffs reagent

Basic fuchsin	1 gm
Sodium metabisulphite, anhydrous	1 gm
Distilled water	200 ml
N/I hydrochloric acid	20 ml

Boil the distilled water; add basic fuchsin and Stir, Cool to 50° C.

Then filter and add hydrochloric acid, Cool to 25°C and add the sodium metabisulphite.

This solution is ready for use when it becomes nearly colourless, which may take up to two days in the dark.

(Alternatively activated charcoal may be added to the solution, shaken and filtered). The solution becomes recoloured it should be discarded.

Technique

1) Section to water
2) Periodic acid 0.5%   5 minutes
3) Rinse in distilled water
4) Schiff's reagent   15 minutes
5) Rinse in the three fresh changes of sulphurous acid

            2 minutes in each change   6 minutes
6) Wash in running tap each changes   5 minutes
7) Counterstain in Mayer's haemalum   30 seconds
8) Wash in running tap water   5 minutes
9) Dehydrate, clear and mount

Results

Positive material: reddish – purple

Nucleus: faint grey

APPENDIX – IV

IMMUNO-HISTOCHEMISTRY

Method Used :

1. 5u thick sections were cut from the blocks received (diagnosed a lymphomas) on slides coated with Chrome alum gelatin.
2. Slides were dewaxed and dehydrated in graded alcohol.
3. Slides were immersed in 0.3% H_2O_2 for 20 minutes to block endogenous peroxidase activity.
4. Washed in phosphate buffered saline (PBS).
5. Incubated in Primary Antibody & Pan G (CD20, clove L26 DAKO) and PAN T (CD3, Polyclonal, DAKO) for 20 minutes.
6. Washed in PBS.
7. Biotinylated link was applied for 20 minutes.
8. Washed in PBS.
9. Incubated in streptavin-biotin complex.
10. Washed in PBS.
11. DAB was used as chromogen
12. Washed and can be stained with haematoxylin.
13. Mounted with coverslip.

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