Libyan International Medical University
Faculty of Basic Medical Sciences
Fall 2018-2019/ Year.

Histopathological study of breast cancer in Libyan Patients in eastern region between years (2015 to 2018)

Submitted by:
Zainab Salem Alshlmany

Under supervision of
Dr. Ahmed Benhasouna

September 2019

A thesis submitted to Libyan international medical university in partial fulfillment of requirements for Bachelor of pathology degree.

Acknowledgement

First and foremost, praise and thanks to Allah Almighty for his blessings and enabling me to finish this project and to present it .

The success and final outcome of this project required a lot of guidance and assistance from many people and I am extremely privileged to have got this all along the completion of my project. All that I have done is only due to such supervision and assistance and I would not forget to thank them.

A special thanks to Benghazi Medical Center for allowing an access to all their medical records, and to our dean Dr. Abdulla M Elmansoury, for giving me the great opportunity to do such a project and for his constant support and motivation .

I would also like to express my deep and sincere gratitude to my research supervisor, I am extremely thankful to Benhasouna for providing such a nice support and invaluable guidance. His vision, sincerity and motivation have deeply inspired me. He also taught me the methodology to carry out the research and present the research work as clearly as possible. It was a great privilege and honor to work under his guidance .

I am thankful to all my professors who have helped me throughout my university years and for their time and constant effort .

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, whose love and guidance are with me in whatever I pursue, and for their continuous love, prayers, and support. They are the ultimate role models.

Contents

List of figures

List of tables

List of abbreviation

RER: Rough endoplasmic reticulum.

WHO: World health organization.

DNA: Deoxyribonucleic acid.

DCIS: Ductal carcinoma in situ.

LCIS: Lobular carcinoma in situ.

ILC: Invasive lobular carcinoma.

IDC: Invasive ductal carcinoma.

TDLU: Terminal duct lobular units.

BRCA1: Breast Cancer 1.

IMPCa: Invasive micropapillary carcinoma.

PIR: Prolactin inducible protein.

DPX: Dibutyphthalate Polystyrene Xylene.

PR: Progesterone receptor.

ER: Estrogen receptor.

HER2: Human Epidermal Growth Factor Receptor 2.

FNAC: Fine needle aspiration.

NOS: Nature of specimen.

MRM: Modified radical mastectomy.

AC: Axillary clearance.

SMAC: Simple mastectomy axillary clearance.

WLE: Wide local excision.

SL: Sentinel lymph node.

EDCIS: endocrine ductal carcinoma in situ.

BMC: Benghazi medical center.

1. Abstract

As cancer comes the first cause of death, breast cancer is the most common cancer worldwide, and this research is done to look into the causes and main factors behind this disease. The main aim of this study is to determine the characteristic of breast cancer in female Libyan patient in eastern part of Libya through analysis of several clinco-pathological features including histopathological type, grade and stage of the disease and to correlate these features and their immunophenotypical profile with other clinical data to better understand the biological behavior of breast cancer. To phonotypical characterize breast cancer cases according to ER, PR and Her2 status. Data of 800 cases of breast cancer were collected from archive of oncology department in Benghazi medical center. Most cases were aged between (40-50) years, most cases were grade II and stage III. For the immunohistochemical data results; nearly two-third of the cases were ER and PR positive. We also concluded that there was a significant correlation between the grade of the cancer and metastasis, as well as a significant correlation between grade and stage. Further study including large number of cases with complete histopathological and clincopathological data as well as genomic characterization are recommended to better understand the behavior and prognostic indicator of breast cancer.

CHAPTER 1: General Introduction

1.1 Anatomical structure of the breast

Is secondary sexual organ for females and source of nutrition for infants. The breasts consist of mammary glands and associated skin and connective tissues. The mammary glands are modified sweat glands in the superficial fascia on the anterior thoracic wall between ribs II and VI and overlie the pectoralis major muscle (Figure1.1).[1,2] The breasts are made up of fat, supportive (connective) tissue and glandular tissue that contain lobes. The lobes (milk glands) are where milk is produced These are connected to the nipple by a network of milk ducts. It's common for woman's breasts to be adifferent size or shape from each other. They change throughout a woman's life and often feel different at different times in the month because of hormonal changes. For example, just before a period they may feel lumpy. As a woman gets older, her breasts may become smaller and feel softer Under the skin, an area of breast tissue extends into the armpit (axilla) The armpits also contain a collection of lymph nodes (glands), which are part of the lymphatic system. There are also lymph nodes just beside the breast bone and behind the collar bones. The lymph nodes throughout the body are connected by a network of tiny lymphatic tubes (ducts).[3]

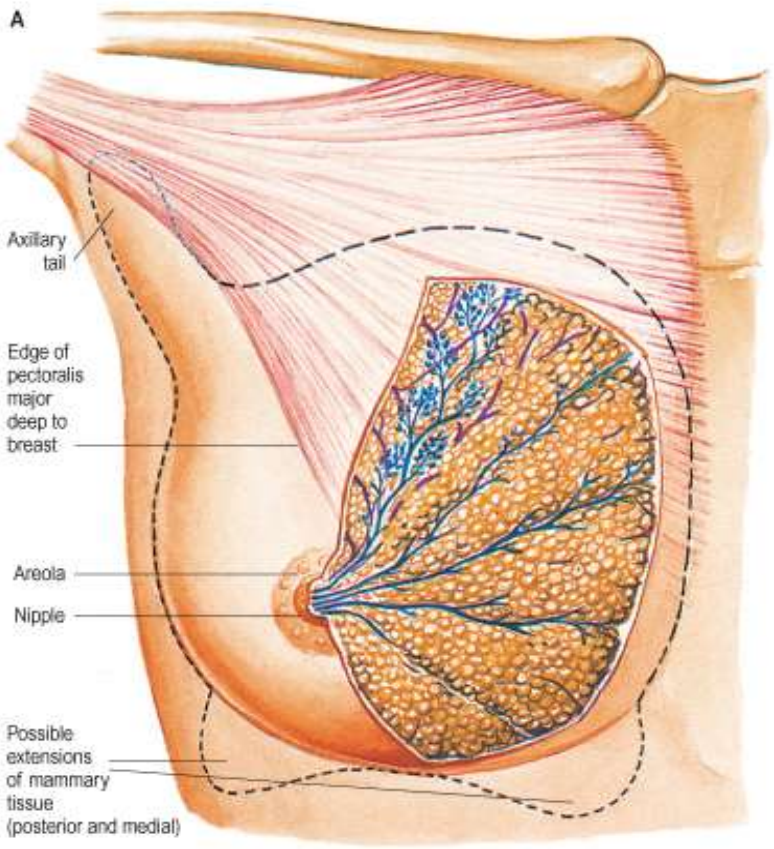

Figure 1.1: The normal breast structure.

1.1.1 Vascular supply

The breasts are supplied by branches of the axillary artery, the internal thoracic artery, and some intercostal arteries. The axillary artery supplies blood from several branches, namely the superior thoracic, the pectoral branches of the thoraco-acromial artery, the lateral thoracic via branches which curve around the lateral border of pectoralis major to supply the lateral aspect of the breast and the subscapular artery. The internal thoracic artery supplies perforating branches to the anteromedial part of the breast. The second to fourth anterior intercostal arteries supply perforating branches more laterally in the anterior thorax. The second

perforating artery is usually the largest, and supplies the upper region of the breast and the nipple, areola and adjacent breast tissue There is a circular venous plexus around the areola. From this, and from the glandular tissue, blood drains in veins which accompany the corresponding arteries that supply the breast i.e. to the axillary, internal thoracic and intercostal veins. Great individual variation may occur .veins unite at the third costal cartilage to ascend medial to the artery, ending in the brachiocephalic vein.[2]

1.1.2 Lymphatic drainage

Approximately 75% of lymphatic vessels drain laterally and superiorly into axillary nodes, the remaining drain into parasternal nodes (Figure1.2) deep to the anterior thoracic wall and associated with the internal thoracic artery.[1] Some drainage may occur via lymphatic vessels that follow the lateral branches of posterior intercostal arteries and connect with intercostal nodes situated near the heads and necks of ribs. Axillary nodes drain into the subclavian trunks, parasternal nodes drain into the bronchomediastinal trunks, and intercostal nodes drain either into the Breast cancer typically spreads by means of lymphatic vessels (lymphogenic metastasis), which carry cancer cells from the breast to the lymph nodes, chiefly those in the axilla. The cells lodge in the nodes, producing nests of tumor cells (metastases). Abundant communications among lymphatic pathways and among axillary, cervical, and parasternal nodes may also cause metastases from the breast to develop in the supraclavicular lymph nodes, the opposite breast, or the abdomen.[4]

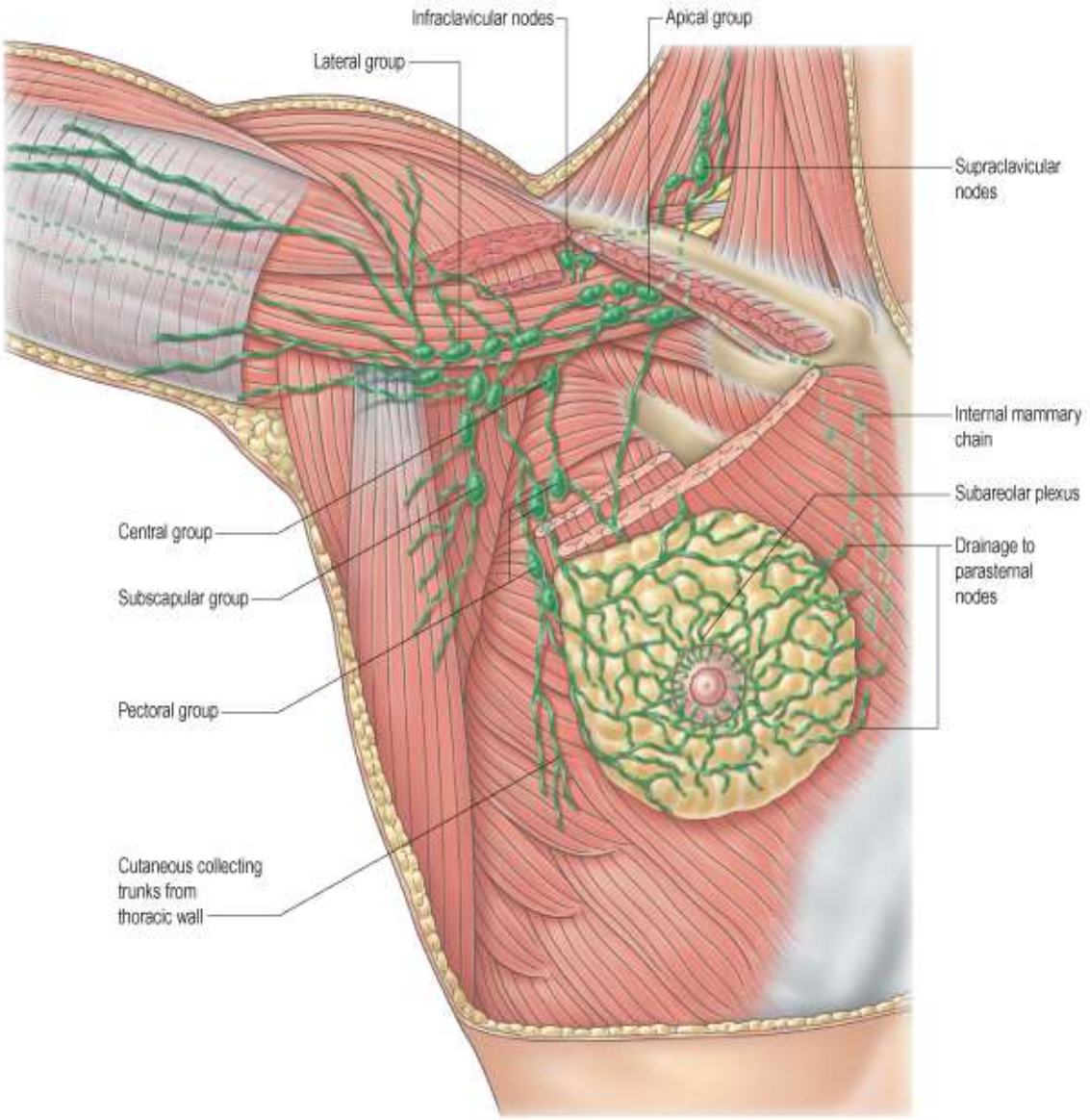

Figure 1.2: Lymph vessels of the breast and the axillary lymph nodes.

1.2 Histological structure of the breast

1.2.1 Mammary gland

The mammary glands are compound tubuloalveolar glands that consist of 15 to 20 lobes radiating out from the nipple and are separated from each other by adipose and collagenous connective tissue. Mammary glands secrete milk, a fluid containing proteins, lipids, and lactose as well as lymphocytes and monocytes, antibodies, minerals, and fat-soluble vitamins, to provide the proper nourishment for the newborn. The mammary glands develop in the same manner and are of the same structure in both sexes until puberty, when changes in the hormonal secretions in females cause further development and structural changes within the glands. Secretions of estrogen and progesterone from the ovaries (and later from the placenta) and prolactin from the acidophils of the anterior pituitary gland initiate development of lobules and terminal ductules. Full development of the ductal portion of the breast requires glucocorticoids and further activation by somatotropin. Concomitant with these events is an increase in connective tissue and adipose tissue within the stroma, causing the gland to enlarge. Full development occurs at about 20 years of age, with minor cyclic changes during each menstrual period, whereas major changes occur during pregnancy and in lactation. After age 40 or so, the secretory portions as well as some of the ducts and connective tissue elements of the breasts begin to atrophy, and they continue this process throughout menopause.The glands within the breasts are classified as compound tubulo alveolar glands, consisting of 15 to 20 lobes radiating out from the nipple and separated from each other by adipose and collagenous connective tissue. Each lobe is drained by its own lactiferous duct leading directly to the nipple, where it opens onto its surface.

Before reaching the nipple, each of the ducts is dilated to form a lactiferous sinus for milk storage and then narrows before passing through the nipple.[5]

1.2.2 Resting (nonsecreting) mammary glands

Alveoli are not developed in the resting mammary gland Lactiferous ducts are lined by a stratified squamous (keratinized) epithelium. The lactiferous sinus and the lactiferous duct leading to it are lined by stratified cuboidal epithelium, whereas the smaller ducts leading to the lactiferous duct are lined by a simple columnar epithelium. Stellate myoepithelial cells located between the epithelium and the basal lamina around the developing alveoli.[6]

1.2.3 Lactating (active) mammary gland

During pregnancy, the ducts branch and grow and develop secretary units known as alveoli.

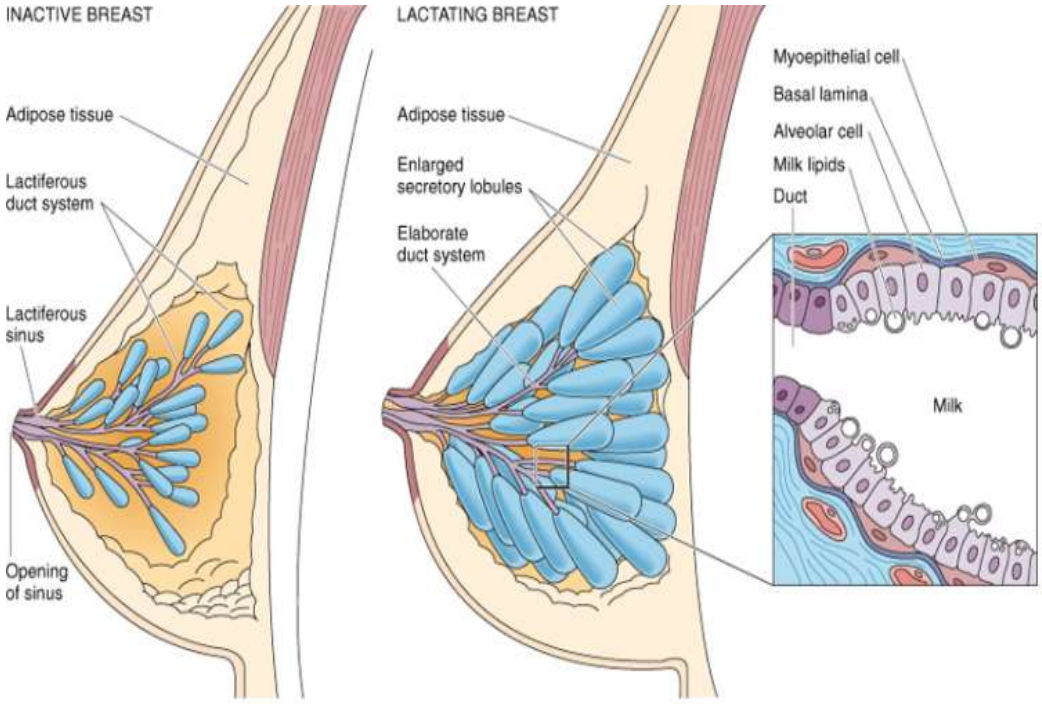

Figure 1.3: Comparison of the glandular differences between an inactive and a lactating breast. Inset shows a longitudinal section of a gland and duct of the active mammary gland.

Mammary glands are activated by estrogen and progesterone during pregnancy to provide milk for the newborn. At this time, the ducts branch and grow and the alveoli develop and mature .As pregnancy progresses, the breasts enlarge due to hypertrophy of the glandular parenchyma and engorgement with colostrum, a protein-rich fluid. Within a few days after birth, prolactin, secreted by the anterior pituitary gland, activates the secretion of milk, which replaces the colostrum. The alveoli of the lactating (active) mammary glands are composed of cuboidal cells partially surrounded by a meshwork (Figure1.3) of myoepithelial cells. These secretory cells possess abundant RER and mitochondria, several Golgi complexes, many lipid droplets, and numerous vesicles containing caseins (milk proteins) and lactose.[6]

1.2.4 Areola and nipple

The undersurface of the epidermis covering the tip of the nipple resembles that found in tactile surfaces; at the sides of the nipple the undersurface is more shallow, and in the areola it has an even, honeycombed appearance. These are distinctive structural signatures of the areas. The nipple is glabrous and the areola largely so. Lactiferous ducts and sebaceous and apocrine glands open only at the tip of the nipple, mostly grouped toward the centre. Glands of Montgomery (accessory mammary glands), clusters of large sebaceous glands, a few scattered eccrine sweat glands, some apocrine glands, and a few vellus hairs toward the periphery are the appendages of the areola. Lactiferous ducts and sebaceous glands contain numbers of melanotic melanocytes among their epithelial cells. Sensory nerve end-organs are found only at the tip of the nipple; there are practically none demonstrable at the sides of the nipple or at the areola. Many nerves are loosely wrapped around the lactiferous ducts, from the lactiferous sinus to the surface. These are probably afferent nerve fibres that convey sensory

impulses. Nipples and areolae have a bountiful framework of elastic fibres that give these structures rigidity and support, anchor smooth muscle fibres, keep the lactiferous ducts and sinuses from collapsing, and attach the epidermis.[7]

1.3 What is breast cancer

Cancer is a group of diseases that cause cells in the body to change and grow out of control. Most type of cancer cells eventually form a lump or mass called a tumor, and are named after the part of the body where the tumor originates**.** Breast cancer is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk.[8] Cancers originating from terminal ducts are known as ductal carcinomas while those originating from lobules are known as lobular carcinomas Breast cancer occurs in humans and other mammals. While the overwhelming majority of human cases are in women, breast cancer can also occur in men.[8] The balance of benefits versus harms of breast cancer treatment is controversial. The characteristics of the cancer determine the treatment, which may include surgery, medication, hormonal therapy and chemotherapy, radiation or immunotherapy**.**[9] Surgery provides the single largest benefit, and to increase the likelihood of remission (no further sign of the cancer), several chemotherapy regimens are commonly given in addition. Radiation is used after breast-conserving surgery and substantially improves local relapse rates and in many circumstances also overall survival**.**[10]According to estimates from the World Health Organization (WHO) in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries [11]

1.4 Epidemiology

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (second most common cancer overall). This represents about 12% of all new cancer cases and 25% of all cancers in women.[12] Breast cancer is hormone related, and the factors that modify the risk of this cancer when diagnosed premenopausally and when diagnosed (much more commonly) postmenopausally are not the same.[13] The Continuous Update Project Panel judged that for premenopausal breast cancer there was convincing evidence that consuming alcoholic drinks increases the risk of this cancer and lactation protects against it. Adult attained height and greater birth weight are probably causes of this cancer and body fatness probably protects against this cancer.[13]The Panel judged that for postmenopausal breast cancer there was convincing evidence that consuming alcoholic drinks, body fatness and adult attained height increase the risk of this cancer and lactation protects against it. Abdominal fatness and adult weight gain are probably causes of this cancer and physical activity probably protects against it.[14] Epidemiology of cancer breast can be described according to two factors which are age and region. Breast cancer is strongly related to age, with only 5% of all breast cancers occurring in women under 40 years old.[15]The incidence of breast cancer varies greatly around the world The highest incidence of breast cancer was in Northern America and Oceania; and the lowest incidence in Asia and Africa. It is lowest in less-developed countries and greatest in the more-developed countries. In the twelve world regions, The annual age-standardized incidence rates per 100,000 women are as follows: in Eastern Asia, 18; South Central Asia, 22; sub-Saharan Africa, 22; South-Eastern Asia, 26; North Africa and Western Asia, 28; South and Central America, 42; Eastern Europe, 49; Southern Europe, 56; Northern Europe, 73; Oceania, 74; Western Europe, 78; and in North America, 90.[16] In Libya, breast cancer is an

important health problem among women. The incidence is 18.8 new cases per 100,000 women per year. Most of the patients present with advanced disease. [17]

1.5 Risk factors

The primary risk factors for breast cancer are female sex and older age .Other potential risk factors include: genetics, lack of breastfeeding, higher levels of certain hormones .certain dietary patterns, and obesity. Recent studies have indicated that exposure to light pollution is a risk factor for the development of breast cancer. The most important risk factor is gender; only 1% of breast cancer cases occur in men. Common risk factors for women identified by epidemiologic studies have been combined into the Breast Cancer Risk Assessment Tool (BCRAT), which now includes information from the Contraceptive and Reproductive Experiences study which provides more accurate information for African American women.[18]

1.5.1 Family history

Women who have a first-degree relative with breast carcinoma have a risk two or three times that of the general population, a risk further increased if the relative was affected at an early age and/or had bilateral disease.[19]

1.5.2 Menstrual and reproductive history

Increased risk is correlated with early menarche, nulliparity, late age at first birth, and late menopause.[20] Breast carcinoma is rare in women who have been castrated; oophorectomy before 35 years of age reduces the risk to one-third. Women who have their first child before the age of 18 years have only one-third the risk of those whose first child is delayed until age 30.[21] A reduction in the risk of breast carcinoma among

premenopausal women who have lactated has been documented, but no such effect was detected among postmenopausal women.[22] Breast carcinoma risk is increased in postmenopausal women with a hyperandrogenic plasma hormone profile.[23]

1.5.3 Exogenous estrogens

In some older series, there has been an overall risk increase (2.5-fold),[24]whereas in others an increased risk (2- to 9-fold) was observed only in patients with a previous diagnosis of fibrocystic disease like benign breast disease.[25] More recently, a large cohort study and a large case-control study have provided strong evidence for a greater increase in breast cancer risk in women using hormone replacement therapy than in those using estrogens alone.[26] Even more recently, highly publicized studies have added to the growing body of evidence that recent long-term use of hormone replacement therapy is associated with an increased risk of breast carcinoma, particularly of the lobular type.[27]increased concentrations of endogenous oestrogens are strongly associated with increased risk for breast cancer in postmenopausal women[28], and trials have shown that the anti-oestrogens tamoxifen and raloxifene reduce the incidence of breast cancer.[29]

1.5.4 Contraceptive agents

The various epidemiologic studies that have been done in this regard have shown no increased risk, or at most a very low increase among young long-term users. The tumors that have developed in this population have not differed qualitatively from those seen in control cases.[30]

1.5.5 Ionizing radiation

An increased risk of breast carcinoma has been documented with exposure to ionizing radiation, particularly if this exposure occurred at the time of breast development.[31]

1.5.6 Breast augmentation

Breast carcinomas (mainly of in situ types) are sometimes detected in women who have undergone augmentation mammoplasty.[32] However, the re-analysis of a previously published linkage study has shown that the incidence of breast carcinoma in that cohort was neither higher nor lower than that among the general population.[33]

1.5.7 Geographic influence

Breast cancer incidence rates in the United States and Europe are four to seven times higher than those in other countries. Unfortunately, the rates are rising worldwide, and by 2020 it is estimated that 70% of cases will be in developing countries.The risk of breast cancer increases in immigrants to the United States with each generation. The factors responsible for this increase are of considerable interest because they are likely to include modifiable risk factors. Reproductive history (number and timing of pregnancies), breastfeeding, diet, obesity, physical activity, and environmental factors all probably play a role.[34]

1.5.8 Relationship between the age of the woman and her risk of contracting the disease

In that in 2005 1 out of 2200 women less than 30 years of age contracted breast cancer, whereas 1 out 54 and 1 out of 23 women less than 50 and 60 years of age, respectively, contracted breast cancer. Although breast cancer is more likely to occur at an older age, younger women tend to have more aggressive breast cancers.[22.34]

1.6 Pathogenesis

1.6.1 Genetic changes

genetic changes have been implicated in the genesis of sporadic breast cancer. As with most other cancers, mutations affecting proto-oncogenes and tumor suppressor genes in breast epithelium contribute to the oncogenic transformation process. Among the best characterized is overexpression of the HER2/NEU proto-oncogene, which has been found to be amplified in up to 30% of invasive breast cancers. This gene is a member of the epidermal growth factor receptor family, and its overexpression is associated with a poor prognosis. Analogously, amplification of RAS and MYC genes has also been reported in some human breast cancers. Mutations of the well-known tumor suppressor genes RB and p53 may also be present. A large number of genes including the estrogen receptor may be inactivated by promoter hypermethylation.[35] Most likely, multiple acquired genetic alterations are involved in the sequential transformation of a normal epithelial cell into a cancerous cell. An important concept resulting from genetic analyses of breast cancers is that it is heterogeneous at the molecular level. Gene expression profiling can stratify breast cancer into four main breast cancer subtypes

have been identified according to estrogen receptor (ER), progesterone receptor (PR), and HER2. These subtypes include luminal types A and B, basal-like, and HER2-enriched subtype.[36] Luminal A is the most common breast cancer subtype and characterized by ER^+ and/or $PR^+/HER2^-$ status, low-grade tumor, and good prognosis.[37] Luminal B subtype accounts for approximately 10% of all breast cancers and is distinguished by ER^+ and/or $PR/HER2^-$ status.[38]Luminal B-like (HER2 positive) is characterized by ER^+, HER2 over expression or amplification, and any Ki-67 or PR.[39] Differentiation of luminal A from luminal $B/HER2^-$ breast cancers results in important therapeutic implications. Hence, the Saint Gallen Guidelines recommended the assessment of the Ki-67 proliferation index.[40] Luminal B breast cancer should show a higher proliferation index than Luminal A; however, the Ki-67 cut-off point for differentiating these two categories has changed over time.[38] Breast cancer subtypes with negative ER, PR, and HER2 status are typically called "triple-negative" breast cancers and approximate the basal-like category. The basal-like subtype is common in premenopausal, young, and overweight patients. This subtype is also associated with high-grade tumors.[37.41] HER2-enriched subtype ($HER2^+/ER^-/PR^-$) is less common but is similarly characterized by high-grade tumors and poor outcomes.[37] Dowsett et al.[42] reviewed that the most common measurement involves immunohistochemical assessment of Ki-67 antigen.Ki-67 is present in all proliferating cells, and its role as a proliferation marker attracts considerable interest. Ki-67 is a nuclear nonhistone protein present in all active phases of cell cycle, except the G_0 phase.[42] Moreover, Ki-67 is among the 21 prospectively selected genes included in the Oncotype DX™ assay used to predict the risk of recurrence and extent of chemotherapy benefits in women with node-negative, ER^+ breast cancers. The proliferation biomarker Ki-67 is also considered a prognostic factor for breast cancer.[44.45]

1.6.2 Hormonal influence

Endogenous estrogen excess, or hormonal imbalance, clearly has a significant role. Many of the risk factors mentioned (long duration of reproductive life, nulliparity, and late age at birth of first child) imply increased exposure to estrogen peaks during the menstrual cycle. Functioning ovarian tumors that elaborate estrogens are associated with breast cancer in postmenopausal women. Estrogens stimulate the production of growth factors by normal breast epithelial cells and by cancer cells. It is hypothesized that the estrogen and progesterone receptors normally present in breast epithelium, and often present in breast cancer cells, may interact with growth promoters, such as transforming growth factor α, platelet-derived growth factor, and fibroblast growth factor elaborated by human breast cancer cells, to create an autocrine mechanism of tumor development. Estrogen may also play a more direct role in carcinogenesis. Metabolites of estrogen can cause mutations or generate DNA-damaging free radicals in cell and animal model systems. It also has been proposed that variants of genes involved in estrogen synthesis and metabolism could increase the risk of breast cancer.[34.35]

1.6.3 Environmental variables

Environmental influences are suggested by the variable incidence of breast cancer in genetically homogeneous groups and the geographic differences in prevalence.[35]

1.7 Classification of breast cancer

Breast cancer classified into:

1-7-1 Non invasive

Ductal carcinoma in situ (DCIS), comedocarcinoma, papillary carcinoma, other forms of DCIS and lobular carcinoma in situ (LCI).

1-7-2 Invasive (infiltrating)

Invasive (infiltrating) ductal carcinoma, invasive lobular carcinoma, classic (NOS) invasive ductal carcinoma, tubular carcinoma, cribriform carcinoma, mucinous carcinoma, medullary carcinoma, inavasive papillary carcinoma, invasive micropapillary carcinoma, apocrin carcinoma, secretory (juvenile) carcinoma and metaplastic carcinoma.

1.7.1.1 Ductal carcinoma in situ (DCIS)

Many morphologic variants of DCIS exist, such as papillary, comedocarcinoma, solid, cribriform, micropapillary, clinging, and cystic hypersecretory. Papillary carcinoma is a very distinct type, thought to arise from large ducts. The others, believed to originate in the TDLU (although often extending to larger ducts), have been traditionally divided into the high-grade comedocarcinoma (characterized by large pleomorphic cells associated with necrosis) and the low-grade solid/cribriform/micropapillary group (composed of smaller uniform cells unassociated with necrosis), with the 'clinging' lesions being included in either of these two categories depending on their cytologic features.[46] In recent times, the trend has been to regard these tumors as part of a continuum and to divide them in a three-grade system largely on the basis of *cytologic* criteria. According to this scheme, classic comedocarcinoma becomes grade 3 DCIS,

classic solid/cribriform/micropapillary lesions become grade 1 DCIS, and those showing intermediate cytologic features are reported as grade 2 DCIS. These criteria apply whether the proliferation is solid, cribriform, micropapillary, or flat (i.e., 'clinging'). They also apply independently of the presence or absence of necrosis and/or calcification,[47] although there is a relationship between the presence and type of calcification and the type of CIS.[48] As many as six different grading systems have been proposed, representing minor variations on the theme. In one study, the highest reproducibility was obtained with Holland's classification. It has been shown that DCIS occurring in younger women is more often symptomatic, is more extensive, and more often accompanied by 'lobular cancerization' than DCIS in older women.[49]

1.7.1.2 Comedocarcinoma

Comedocarcinoma may reach a relatively large size and become palpable. In one series, 28% were over 5 cm in diameter and another 33% were between 2 and 5 cm. Over half of these tumors are centrally located, whereas this is true for less than 20% of invasive tumors.[50] The quoted incidence of multicentricity is approximately 33%, and the incidence of bilaterality is 10%.[51] Grossly, the tumor presents as a cluster of thick-walled ducts with normal breast parenchyma between them. When these ducts are compressed, plugs of necrotic tumor reminiscent grossly of those seen in comedones extrude from them, hence the name comedocarcinoma. If the duct walls are not thickened, the tumor may not be apparent grossly. Microscopically, the ducts show a solid growth of large pleomorphic tumor cells accompanied by generally abundant mitotic activity and lacking connective tissue support. Necrosis is always present and constitutes an important diagnostic sign, whether in the form of a large central focus or of individual tumor cells (Figure1.4). The mean diameter of the ducts containing necrosis is significantly larger than for those lacking this feature, suggesting the

existence of a ‘hypoxic compartment’ in these tumors. Coarse calcification often supervenes in these necrotic areas, and this can be identified by mammography. Myoepithelial cells usually surround ducts involved by comedocarcinoma[52] (Figure1.5). The stroma around the involved ducts shows a characteristic concentric fibrosis accompanied by a mild-to-moderate mononuclear inflammatory reaction, interpreted by some authors as evidence of tumor regression.[53]

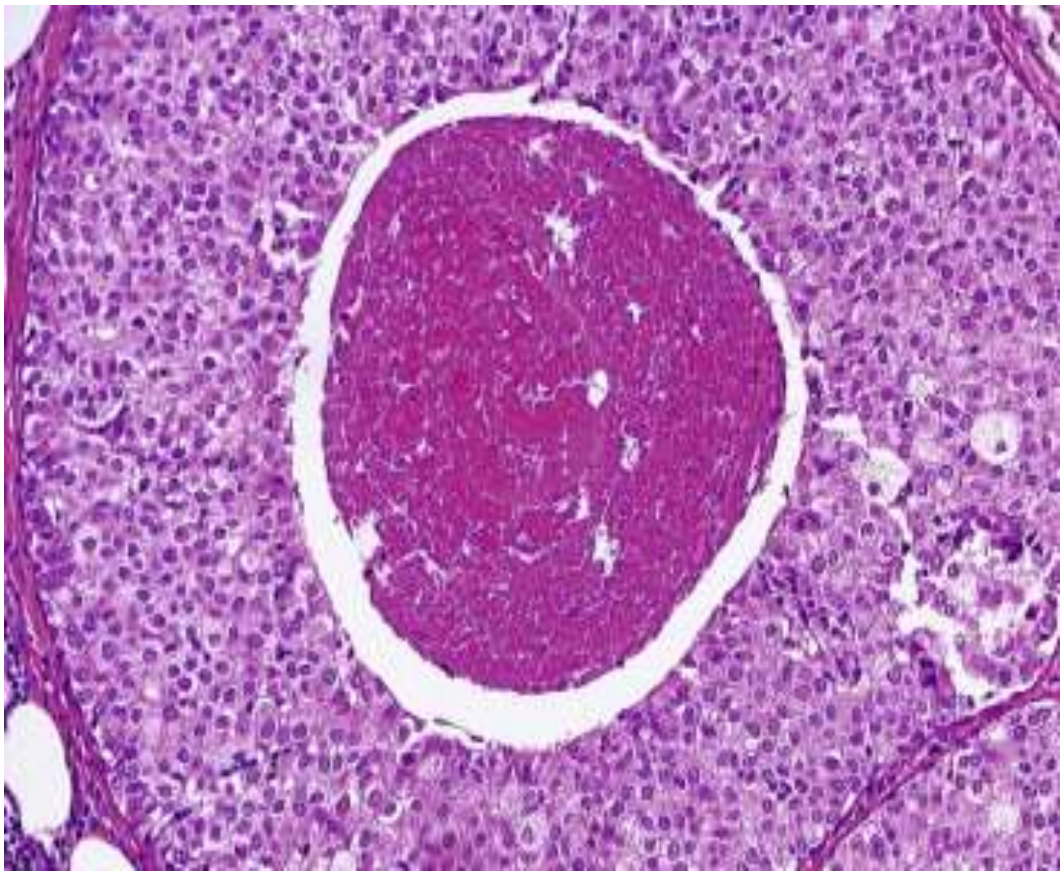

Figure 1.4:In situ ductal carcinoma with comedo-type necrosis.

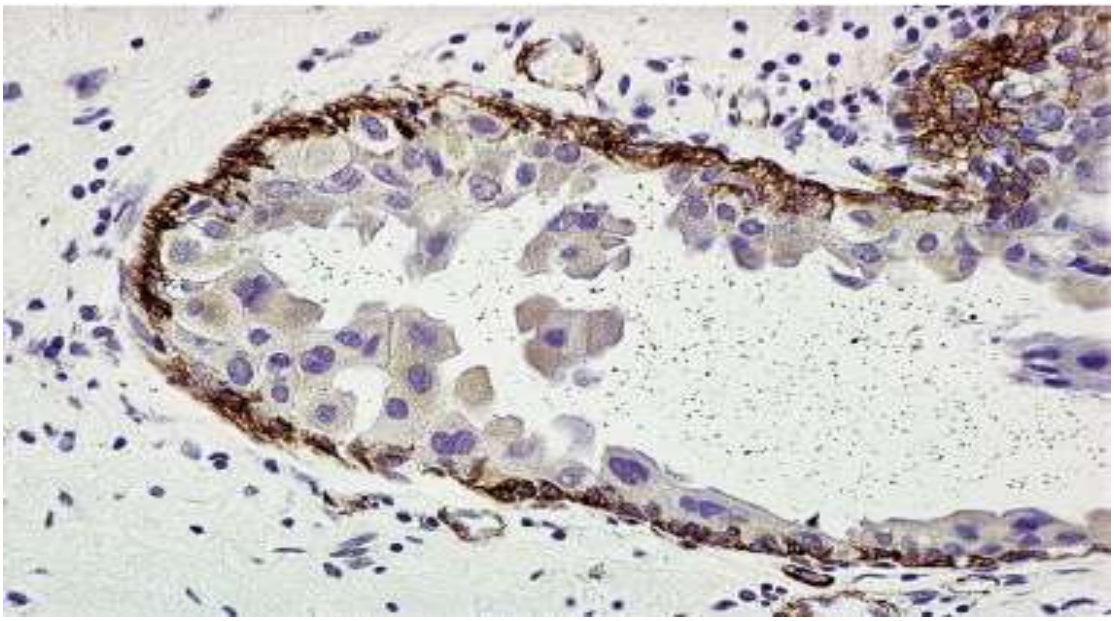

Figure 1.5:Preservation of a myoepithelial cell layer in high-grade intraductal carcinoma. (Smooth muscle actin immunostain)

Once the diagnosis of comedocarcinoma has been established, two additional important determinations need to be made. The first is the degree of intraductal spread, which in some cases may be very extensive and even reach the nipple, resulting in Paget disease[54]. The other is to search for areas of definite stromal invasion and, if these are present, to estimate the relative amounts of in situ and invasive components [55](Figure1.6)

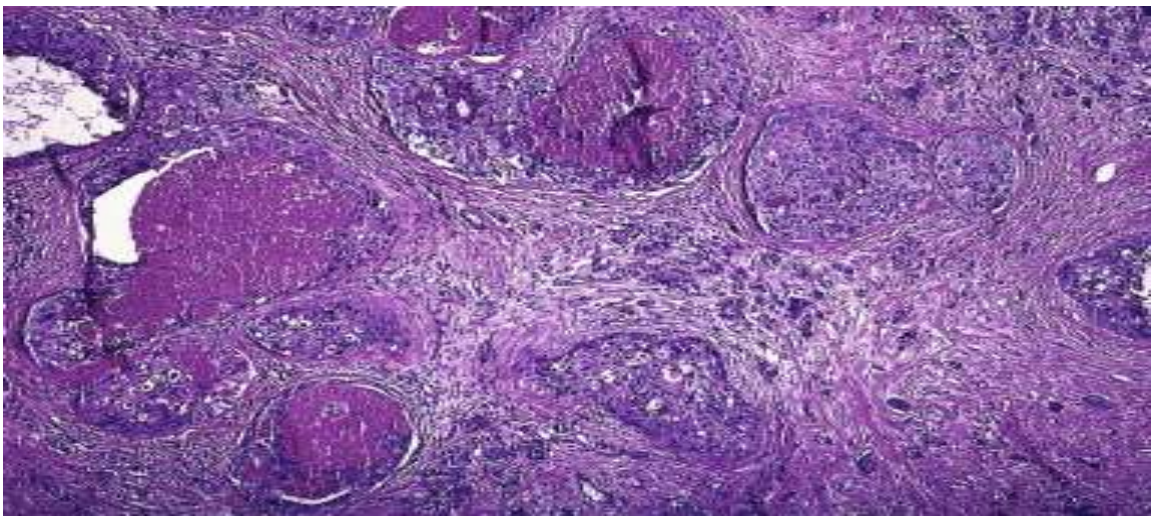

Figure 1.6: Invasive ductal carcinoma associated with extensive intraductal carcinoma component.

1.7.1.3 Papillary carcinoma (In situ)

Papillary carcinoma makes up only a small percentage of breast carcinomas. Grossly, it may present as a well-circumscribed mass, or it may ramify within several ducts to involve an entire breast segment. In the variant known as *intracystic papillary carcinoma*, the tumor appears as a mural nodule within a large cystic space supposedly representing a dilated duct[56] (Figure1.7). The microscopic criteria for the diagnosis must be strict, because most papillary breast lesions are benign. The most important differential features were listed in the classic study by Kraus and Neubeckerand further elaborated (and somewhat modified) by Azzopardi.[57]Microscopically, features favoring carcinoma are (paradoxically) uniformity in size and shape of the epithelial cells (whether round, oval, or spindle, the latter arranged perpendicularly to the duct axis); presence of one cell type only (i.e., lack of myoepithelial cells); nuclear hyperchromasia and high nucleocytoplasmic ratio; high mitotic activity; lack of apocrine metaplasia, cribriform and trabecular patterns; scanty or absent stroma; and lack of benign proliferative disease in the adjacent breast[58] (Figure1.8).

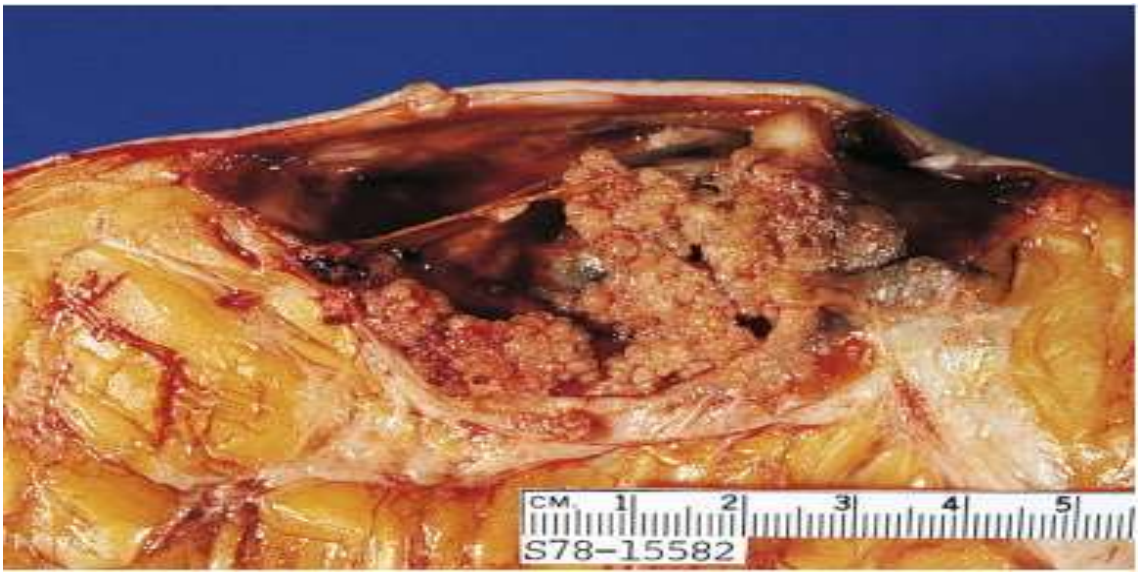

Figure 1.7: Intracystic carcinoma of the breast. The papillary configuration of the tumor is already grossly evident.

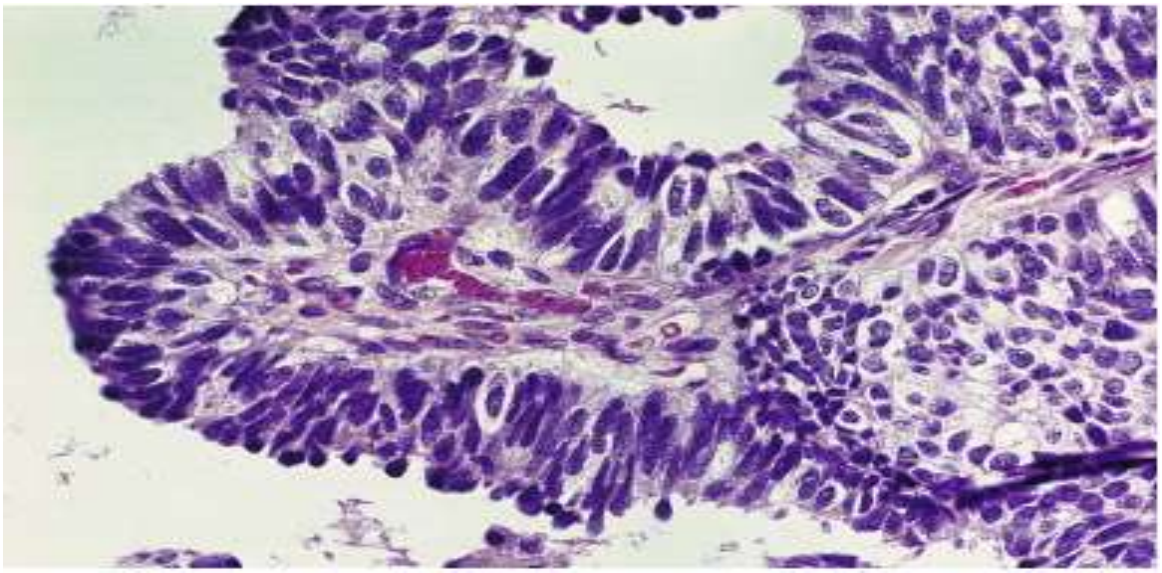

Figure 1.8: High-power view of an in situ papillary carcinoma. Note the layering of cells, loss of nuclear polarity, marked hyperchromasia, and lack of a myoepithelial cell layer.

1.7.1.4 Other forms of ductal carcinoma in situ

In the *solid* form of DCIS, the glandular lumen is filled by the proliferation of medium-sized cells, which are larger than those of LCIS but smaller and more uniform than those of comedocarcinoma[59] (Figure1.9). Azzopardipointed out the sharp cell edges (as opposed to a 'syncytial' quality) and the pallor of the cytoplasm (as opposed to prominent acidophilia) often exhibited by these cells. In the *cribriform* variety, round regular spaces are formed within the glands; the more regular these spaces are in terms of distribution, size, and shape, the more likely the lesion is to be malignant (Figure 1.10). These spaces are often associated with two formations of similar pathogenesis, designated by Azzopardi as trabecular bars and Roman bridges, respectively. Trabecular bars are rigid rows of cells with their long axes arranged more or less perpendicular (or at least not parallel) to the long axis of the bar; these should be distinguished from partial detachments of the duct lining (Figure 1.11).

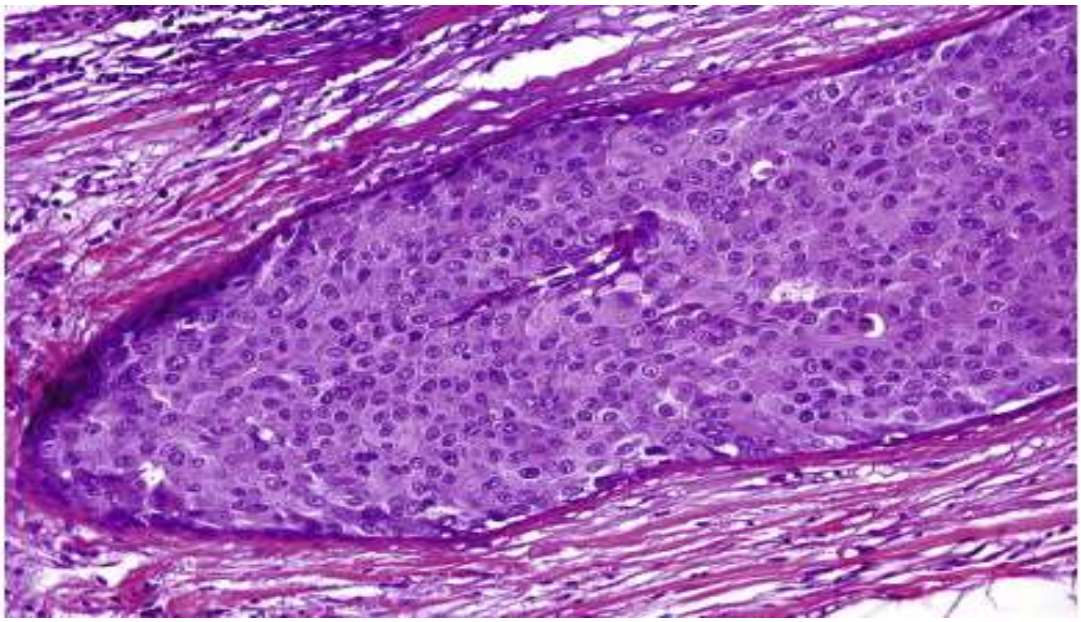

Figure 1.9: Solid type of in situ ductal carcinoma. There is no necrosis.

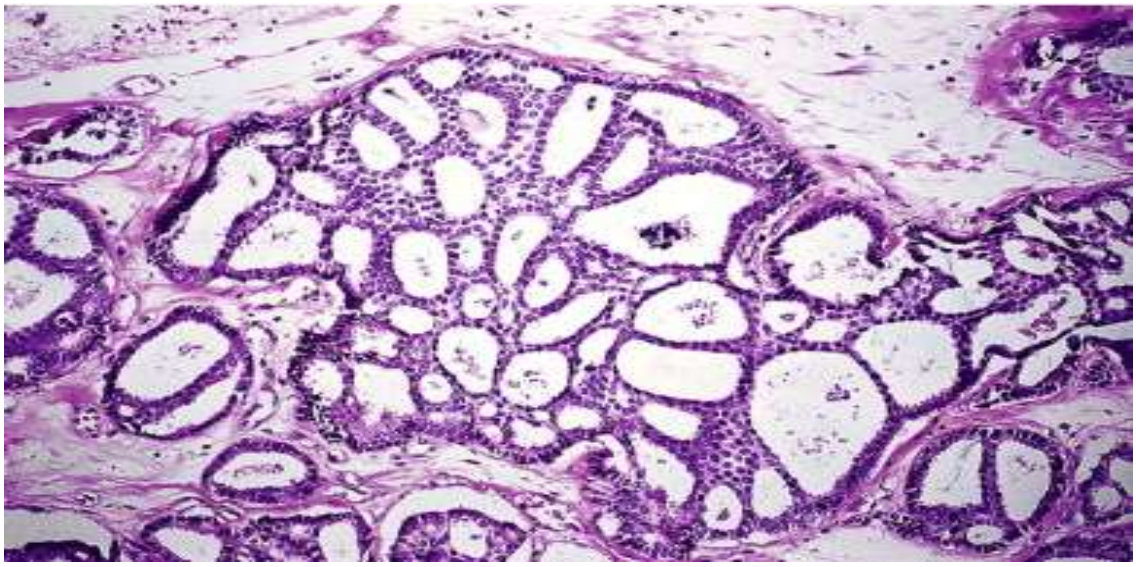

Figure 1.10: Low-grade in situ ductal carcinoma of cribriform type.

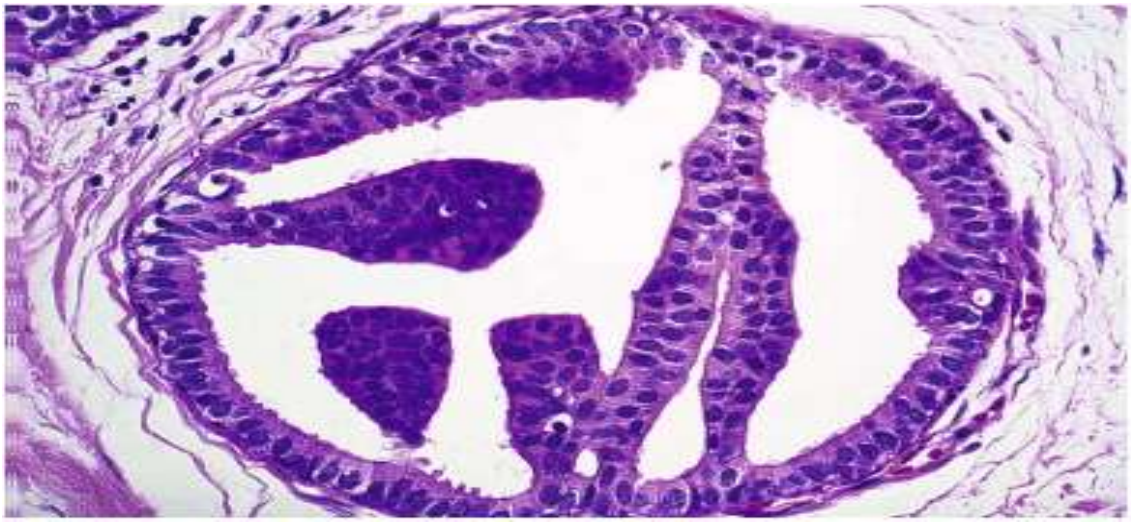

Figure 1.11: Trabecular bars in intraductal carcinoma. Note the perpendicular arrangement of the nuclei in relation to the long axis of the bars.

The *micropapillary* variety (more closely connected to the preceding types of DCIS than to conventional papillary carcinoma) shows elongated epithelial projections projecting into the glandular lumen; these lack connective tissue support, may have a space at the base, and often show a bulbous expansion at the. This variant is more likely than others to involve multiple quadrants of the breast.[60] *Clinging* carcinoma, the more controversial member of this family, shows one or two layers of malignant cells lining a glandular formation with a large empty lumen.[61] In the more easily recognizable (high-grade) forms, the tumor cells are large, highly atypical, and associated with individual cell necrosis, features which suggest a link with comedocarcinoma (Figure1.12). In other instances, the tumor cells are smaller and more regular; these have been interpreted as being related to the low-grade forms of intraductal carcinoma, particularly the micropapillary variety. Indeed, some authors refer to this as the 'flat' variant of micropapillary in situ carcinoma.

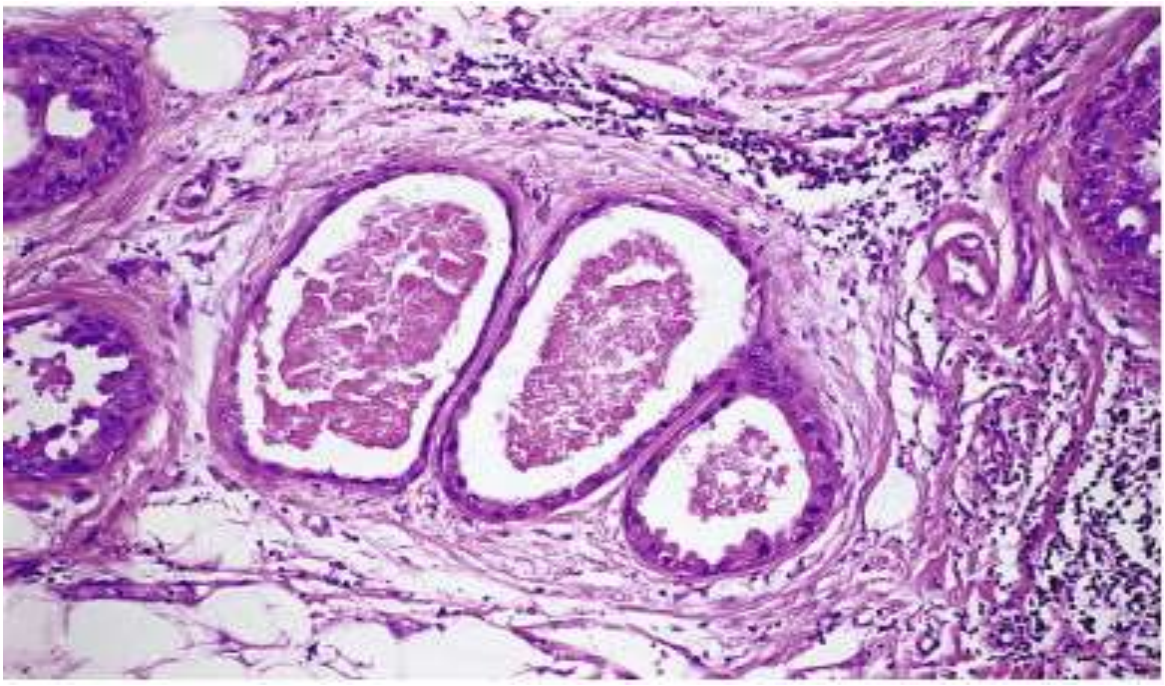

Figure 1.12: Ductal carcinoma in situ of so-called 'clinging type'. One or two layers of atypical cells line dilated glandular structures containing granular intraluminal material in which ghosts of tumor cells are identified.

Adding to the complexity of the situation is the pattern traditionally known as *lobular cancerization*.[62]The term refers to the presence, in a structure easily identifiable as a lobule, of carcinoma with the cytoarchitectural features of DCIS (Figure1.13). The change was first described in connection with the high-grade (comedocarcinoma) form, but it was later realized that it could also be seen with the low-grade types. As the name indicates, the original assumption was that this represented a secondary extension into a lobule of a carcinoma of ductal origin, particularly when this was found associated with a conventional DCIS such as comedocarcinoma.

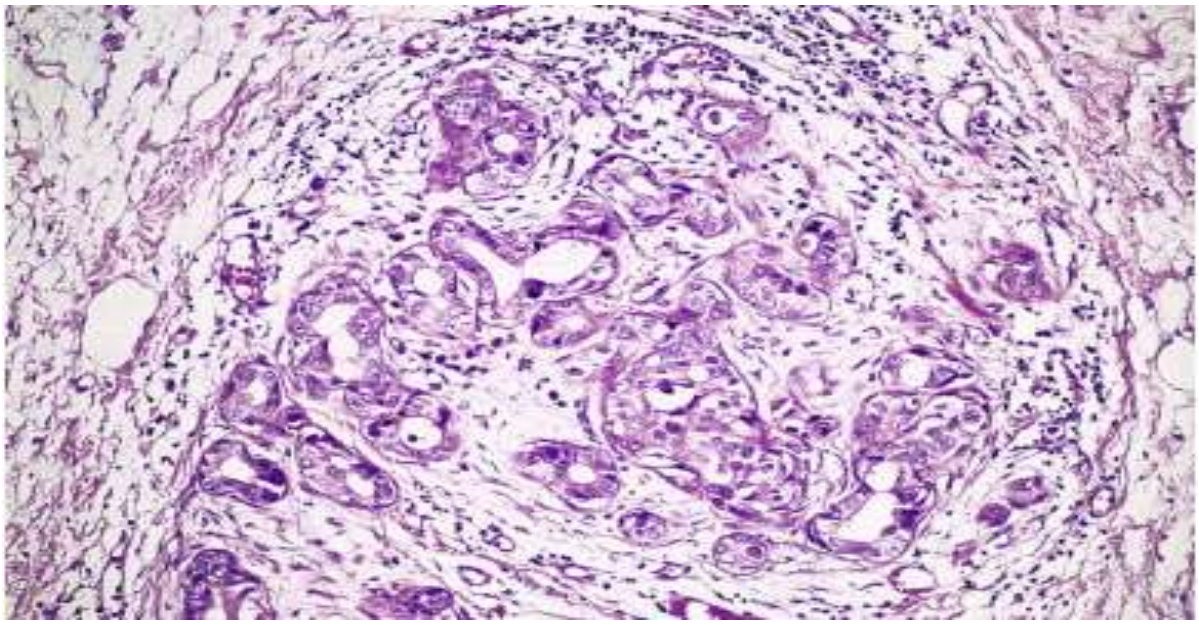

Figure 1.13: So-called 'lobular cancerization'. The lobule is markedly expanded and composed of relatively large tumor cells with the appearance of ductal-type carcinoma. Typical ductal carcinoma was present elsewhere in the specimen.

Rare additional morphologic variations of DCIS include cases with *signet ring cells* (Figure1.14), with *multinucleated giant cells*, with *apocrine-type cytology*, with *squamous features* (squamous cell carcinoma in situ) and those with evidence of (*neuro*)*endocrine differentiation.*[63] The latter tumor, known as (neuro)endocrine DCIS (E-DCIS), is often accompanied by adjacent intraductal papillomas with pagetoid involvement by the carcinoma. Key features for its recognition include the presence of endocrine-type festoons and rosettes, mucin deposition, bland-looking spindle to ovoid nuclei (an important diagnostic clue), and abundant granular eosinophilic cytoplasm (Figure 1.15A). The solid islands of tumor in E-DCIS are frequently traversed by delicate fibrovascular septa[64] Necrosis is usually absent, and neuroendocrine markers such as chromogranin and synaptophysin can be demonstrated[65] (Figure 1.15B).

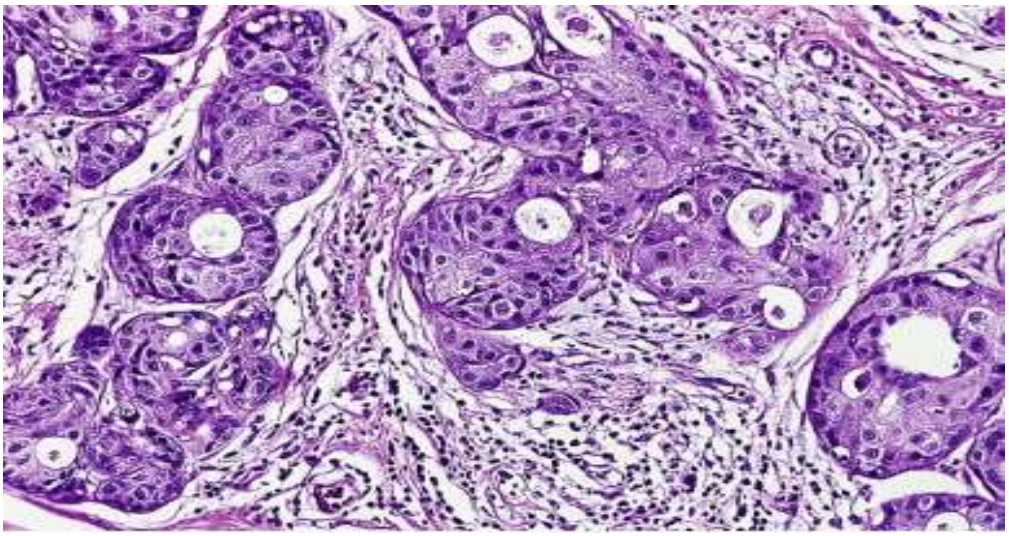

Figure 1.14: Apocrine variant of in situ ductal carcinoma.

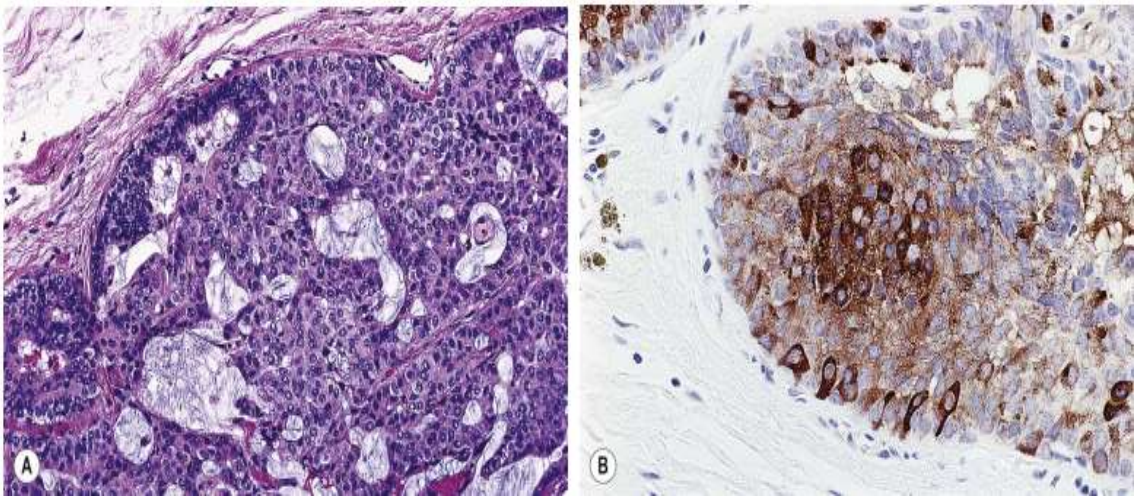

Figure 1.15: Endocrine-type ductal carcinoma in situ: A, hematoxylin–eosin; B, chromogranin.

1.7.1.5 Lobular carcinoma in situ (LCIS)

Lobular CIS, also known as lobular neoplasia, has no distinguishing features on gross examination and is usually found incidentally in breasts removed for other reasons. It is multicentric in approximately 70% of cases [66] and bilateral in approximately 30–40%.Most cases are found within 5 cm of the nipple from the skin surface in either the

outer or inner upper quadrants. Residual tumor foci are found in 60% of breasts removed following a diagnosis of LCIS made from a biopsy specimen.[67] Microscopically, the lobules are distended and completely filled by relatively uniform, round, small-to-medium-sized cells with round and normochromatic (or only mildly hyperchromatic) nuclei. In the typical case, atypia, pleomorphism, mitotic activity, and necrosis are minimal or absent, and there is some lack of cohesiveness among the tumor cells (Figure 1.16 and 1.17). Any of the following minor morphologic variations can occur, singly or in combination: moderate nuclear pleomorphism, larger nuclear size, appreciable mitotic activity, scattered signet ring cells (relatively common), apocrine changes (exceptional), focal necrosis, and variations in the shape of the involved lobules.[68.69.70] When the tumor cells are of medium to large size, with moderateto marked pleomorphism, occasional prominent nucleoli, and moderate to abundant cytoplasm, the lesion is referred to as *pleomorphic LCIS*.[71]

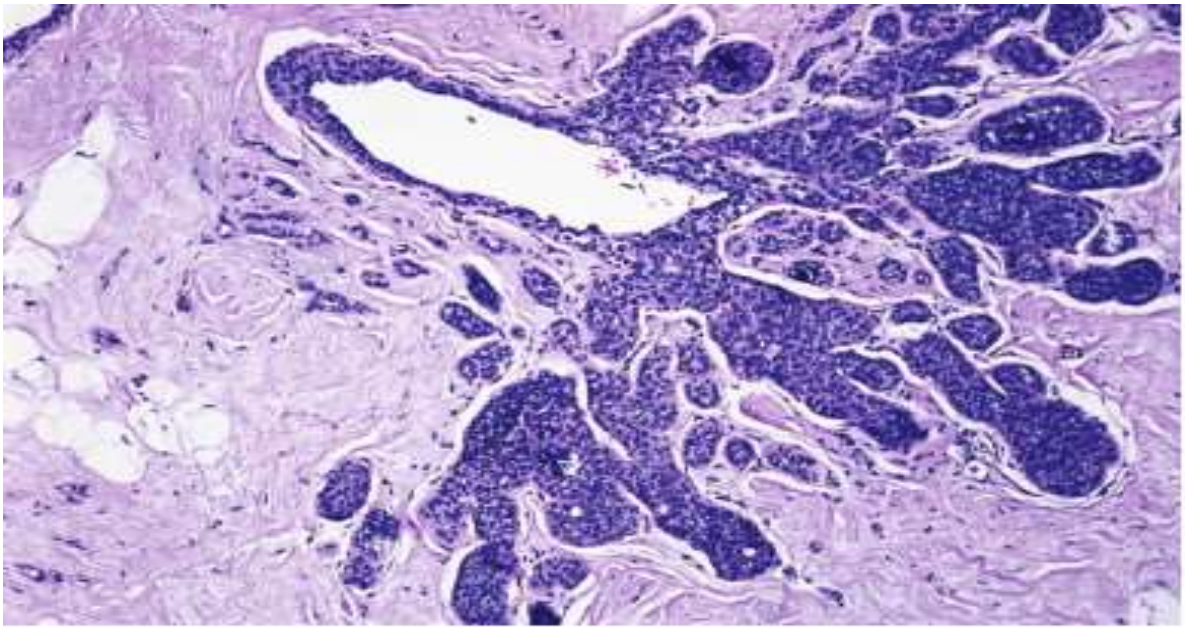

Figure 1.16: Typical pattern of involvement of terminal duct–lobular unit by lobular carcinoma in situ.

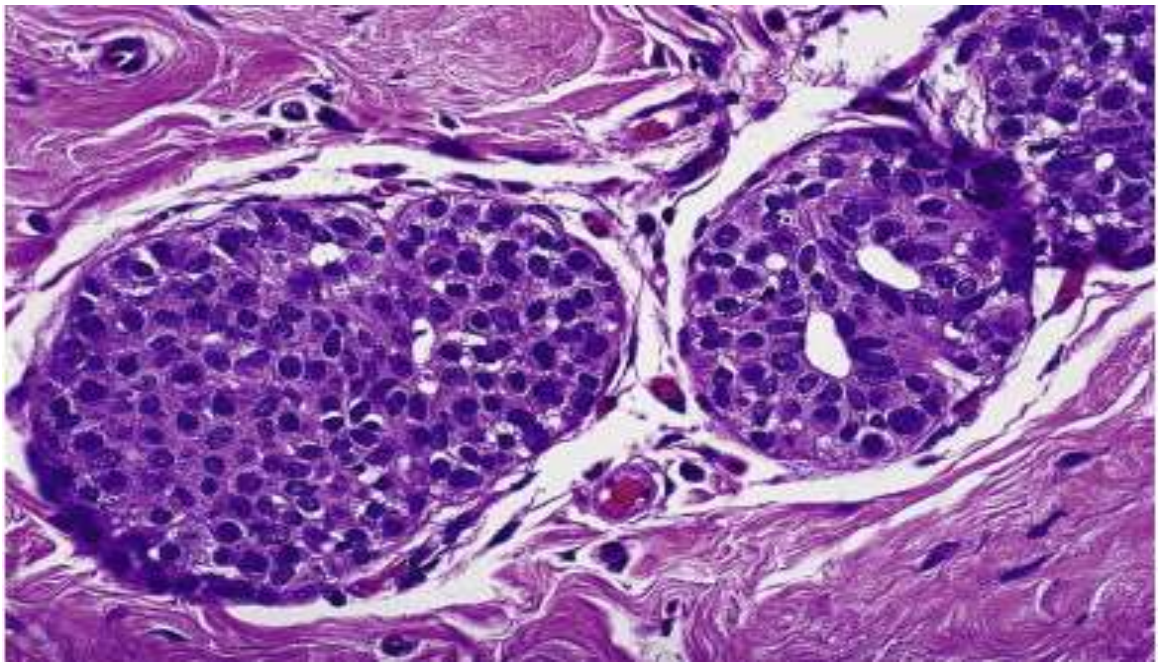

Figure 1.17: Marked expansion of a lobular unit by lobular carcinoma in situ. A few small spaces are still present in the smaller focus.

1.7.2 Invasive (infiltrating)

Tumors included in this category are all those in which stromal invasion is detectable, whether an in situ component is identifiable or not, and regardless of the relative proportion of the two components. In other words, it also includes so-called 'microinvasive carcinoma. Like the in situ lesions, most of the invasive tumors can be divided into two major categories – ductal type and lobular type – acknowledging the existence of mixed and intermediate forms. It should be emphasized that the type of invasive carcinoma should be determined from its own appearance, rather than deduced from the type of in situ component present, if any, since there is not always correspondence between the two.

1.7.2.1 Invasive lobular carcinoma (ILC)

1.7.2.1.1 Classic type

In its most typical form, invasive lobular carcinoma (ILC) is characterized by the presence of small and relatively uniform tumor cells growing singly, in Indian file, and in a concentric ('pagetoid') fashion around lobules involved by in situ lobular neoplasia[72] (Figures 1.18–1.19). Most of the breast tumors designated in the past as small cell carcinomas belong to this category. Gland formation is not a feature of classic ILC. The stroma is usually abundant, of dense fibrous type, and containing foci of periductal and perivenous elastosis in virtually every case. A lymphocytic infiltrate may be present, sometimes so intense as to obscure the neoplastic component.

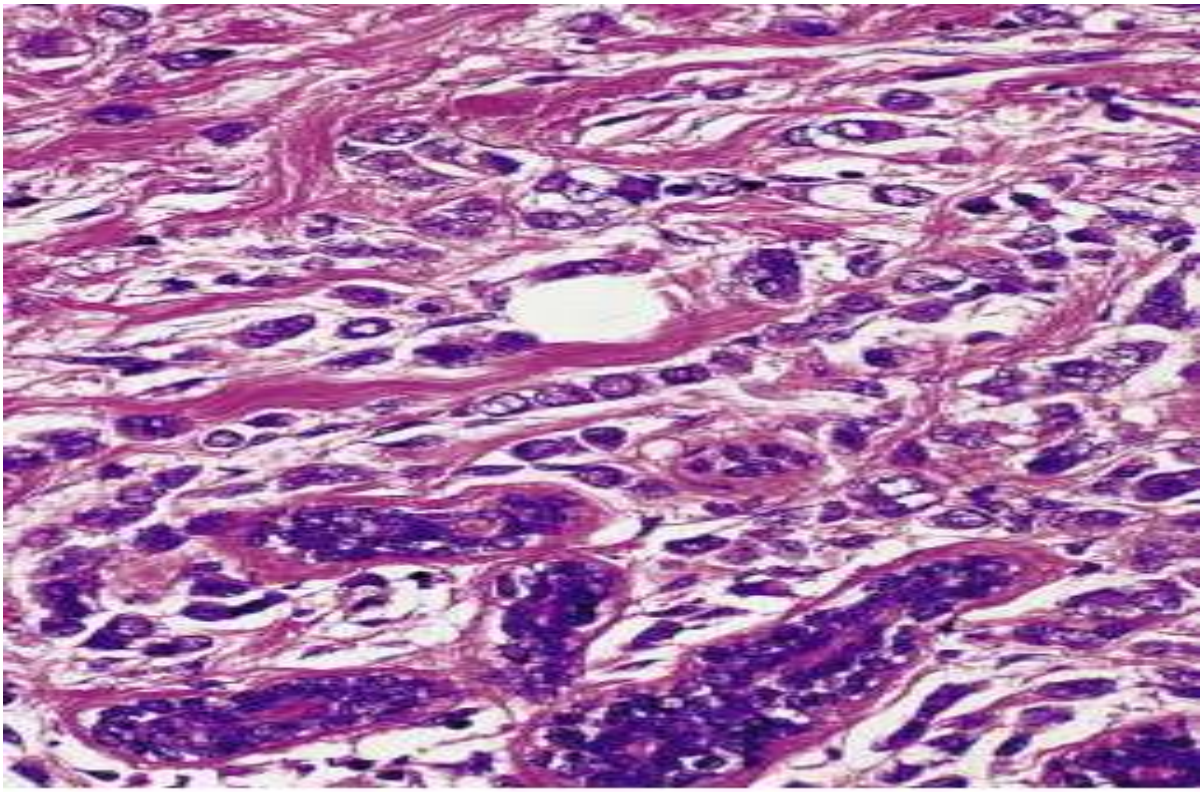

Figure 1.18: Invasive lobular carcinoma. The tumor cells are small and uniform with round nuclei and grow in an Indian file fashion.

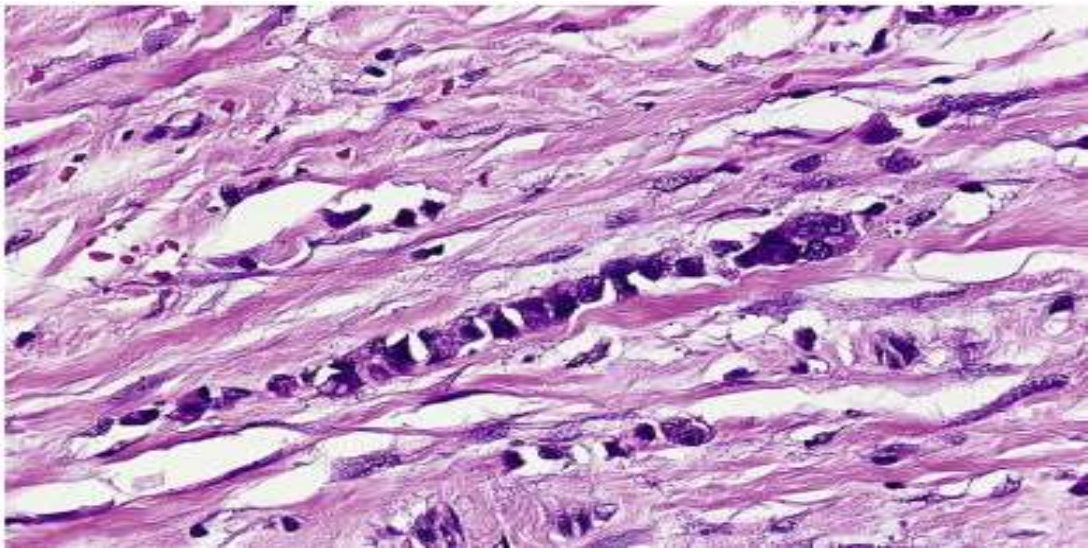

Figure 1.19: Indian file pattern of growth of invasive lobular carcinoma.

1.7.2.1.2 Pleomorphic lobular carcinoma

This form of invasive breast tumor has the pattern of growth of a classic breast carcinoma but exhibits a marked degree of nuclear pleomorphism and abundant cytoplasm (Figure1.20). It also frequently shows apocrine differentiation, focal signet ring morphology, lack of hormone receptors, higher expression of P53 and HER2/*neu*, occasional expression of chromogranin, and lack of E-cadherin staining (the latter in keeping with its lobular nature).[73]

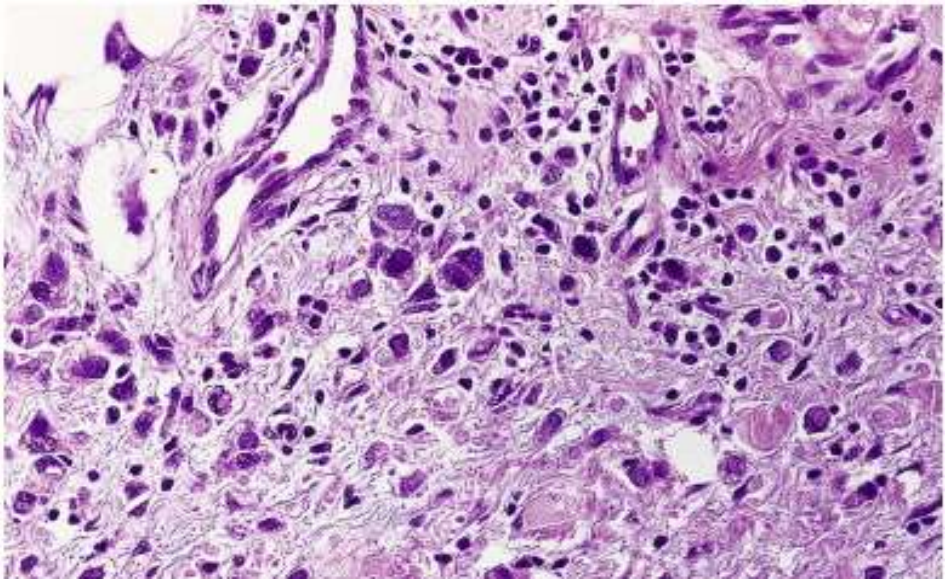

Figure 1.20: Pleomorphic variant of invasive lobular carcinoma.

1.7.2.1.3 Histiocytoid carcinoma

Histiocytoid carcinoma is characterized by a diffuse pattern of growth by tumor cells displaying abundant granular, foamy cytoplasm. It may simulate the appearance of a granular cell tumor (myoblastoma), hence the proposed synonym *myoblastoid carcinoma*. This tumor type is currently viewed as a variant of invasive lobular carcinoma exhibiting apocrine differentiation, as evidenced by immunohistochemical reactivity for GCDFP-15 and the demonstration of mRNA for the related prolactin-inducible protein (PIP) by in situ hybridization. In most cases E-cadherin is absent, as one would expect in a lobular carcinoma-type tumor, but other features are more suggestive of a link with ductal-type tumors. The mucins expressed by this tumor include some 'non-mammary' types, such as MUC2 and MUC5AC.Histiocytoid carcinoma should also be distinguished from *lipid-rich carcinoma*. The latter is simply a form of breast carcinoma showing lipid accumulation in the cytoplasm of the tumor cells (Figure1.21).[47,75]

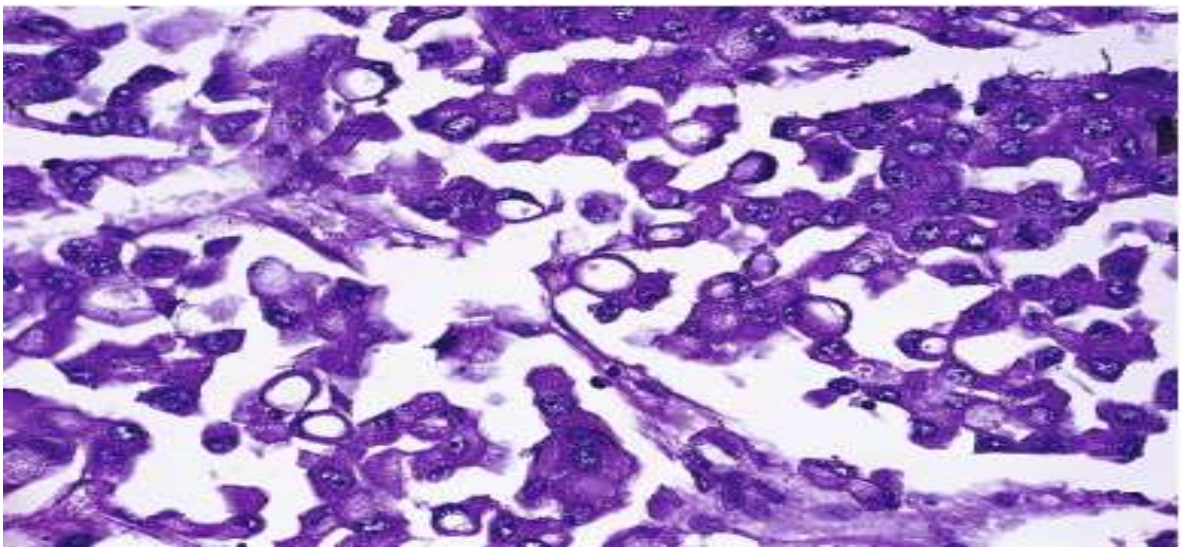

Figure 1.21: Cytoplasmic vacuolization with nuclear displacement in breast carcinoma due to lipid accumulation.

1.7.2.1.4 Signet ring carcinoma

Signet ring carcinoma is a type of breast carcinoma in which a significant number of tumor cells show intracytoplasmic mucin accumulation, resulting in the typical signet ring appearance[76] (Figure1.22). Unfortunately, the term 'significant' is used differently by different people. Some will place a tumor into this category only if the majority of the cells have a signet ring morphology, whereas others would settle for a much smaller number. In any event, it is important to separate this tumor from mucinous carcinoma (in which the mucin is extracellular) because of their vastly different prognoses.

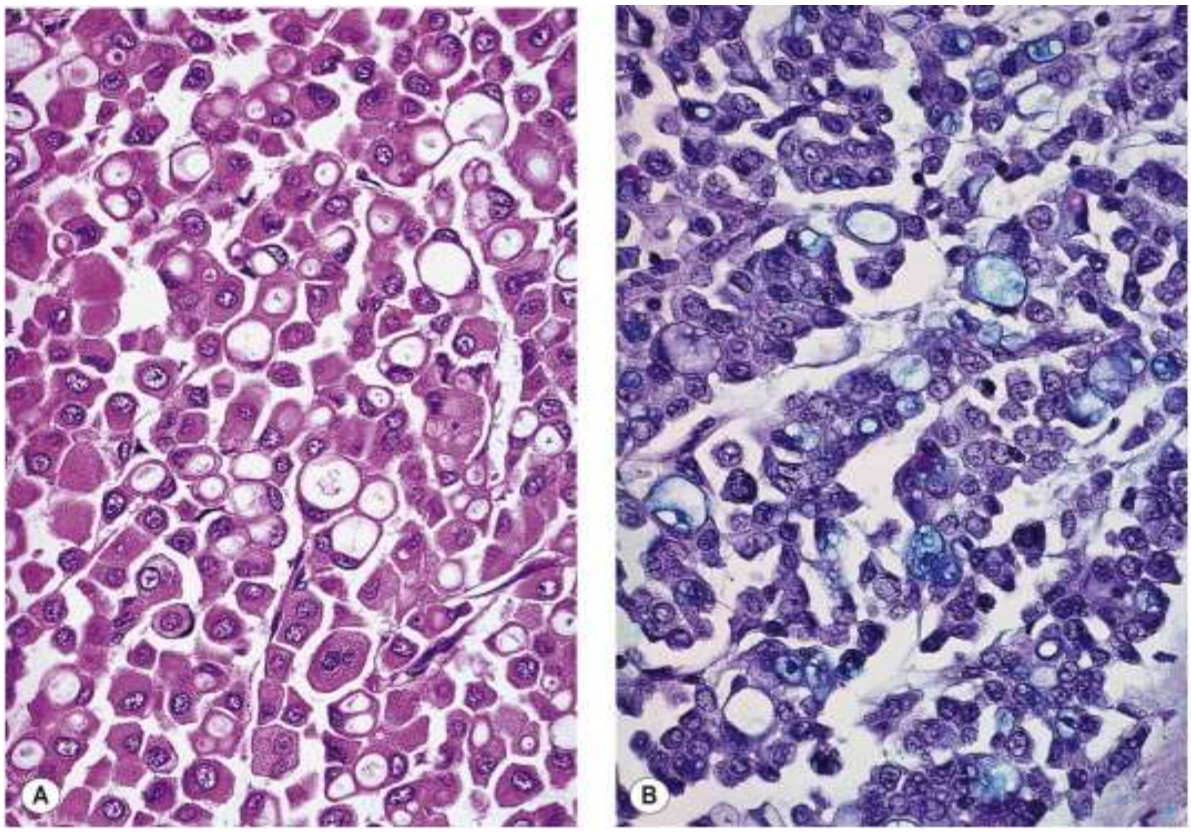

Figure 1.22: A and B, Signet ring carcinoma of the breast. This is regarded as a variant of lobular carcinoma. B, Alcian blue–PAS stain.

1.7.2.1.5 Tubulolobular carcinoma

This variant is characterized by the admixture of small tubular formations having a minute or undetectable lumen (‘closed’ or ‘almost closed’ tubules) with cords of tumor cells growing in a lobular configuration similar to that of invasive lobular carcinoma.The in situ component, if present, may be of lobular, ductal or mixed type. Its immunohistochemical profile is intermediate between those of ductal and lobular carcinoma, in that it shows positivity for both E-cadherin and HMW keratin. It is associated with a higher incidence of multifocality and positive axillary nodes than pure tubular carcinoma.[77,78]

1.7.2.3 Classic (NOS) invasive ductal carcinoma

This lesion represents the prototypic expression of breast carcinoma, and is the tumor type usually implied when the terms ‘breast carcinoma’ or ‘breast cancer’ are used without further qualification. The size, shape, consistency, and type of margins are highly variable; some of these factors depend on the relative amounts of tumor cells and stroma. Grossly, the typical case is firm and poorly circumscribed, cuts with a resistant gritty sensation, and shows a yellowish-gray cut surface, with trabeculae radiating through the surrounding parenchyma into the fat, resulting in the notorious stellate or crab-like configuration, from which the word ‘cancer’ has originated (Figure 1.23). Sometimes these strands are seen connecting with other tumor nodules located at some distance from the primary tumor. Areas of necrosis, hemorrhage, and cystic degeneration may be present, particularly in the larger neoplasms. The tumor may have invaded the overlying skin or the underlying fascia and pectoralis muscle. Tumors that are particularly hard because of the large amounts of stroma were traditionally referred to as ‘scirrhous carcinomas’, a term no longer used. It is common for these neoplasms to

exhibit ‘chalky streaks’ on the cut surface, a feature caused not by necrosis as generally believed, but by duct elastosis[79] .When this occurs, the appearance of the lesion has an uncanny resemblance to an unripe pear, further accentuated by the consistency and the sensation one has while cutting it.

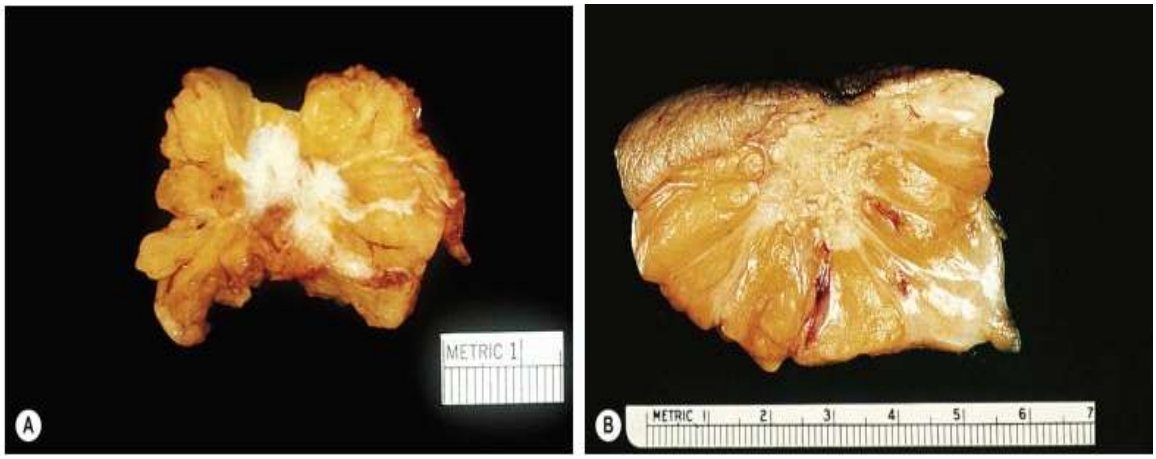

Figure 1:23: A and B, Typical gross appearance of invasive ductal carcinoma. Note the irregular (crab-like) shape of the tumor, white fibrous appearance, and chalky streaks. Retraction of the overlying skin is obvious in the specimen shown in B.

Microscopically, the variations are also legion.[80] The tumor can grow in diffuse sheets, well-defined nests, cords, or as individual cells. Glandular/tubular differentiation may be well developed, barely detectable, or altogether absent. Parenthetically, this is the reason why the term adenocarcinoma is not advisable as a synonym for invasive ductal carcinoma (Figure1.24).

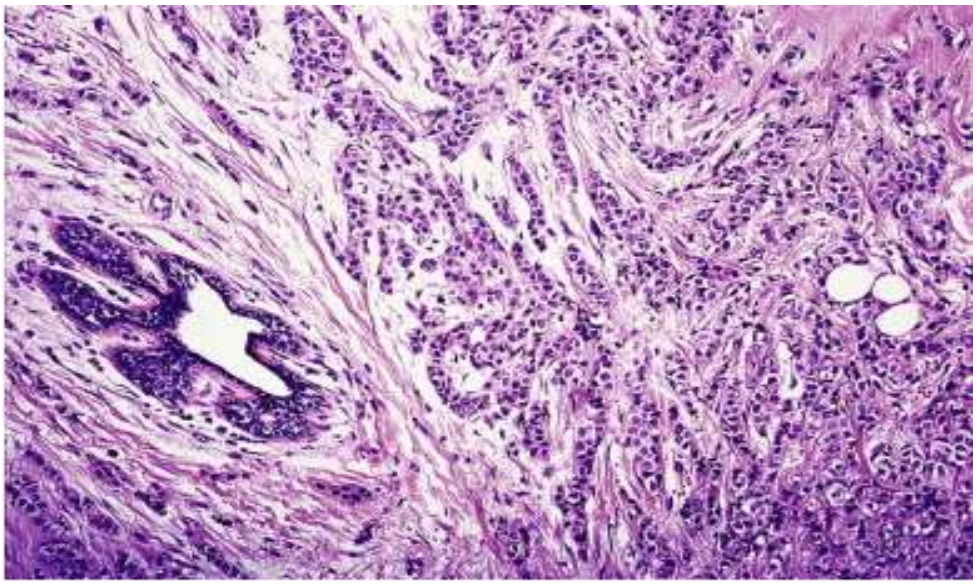

Figure 1.24: Prototypical invasive ductal carcinoma.

1.7.2.4 Tubular carcinoma

Tubular carcinoma has also been designated as well-differentiated carcinoma, but the latter term is not advisable because it has also been used for other well-differentiated tumors with different patterns of growth. The average age of the patients is about 50 years.[81] Grossly, tubular carcinoma suggests malignancy by virtue of its poorly circumscribed margins and hard consistency. It is characteristically small, with a mean diameter of about 1 cm. Microscopically, it simulates a benign condition (particularly radial scar and microglandular adenosis) because of the well-differentiated nature of the glands, absence of necrosis or mitoses, and scanty pleomorphism.[82] The clues to the diagnosis are the haphazard arrangement of the glands in the stroma with absence of any organoid configuration; frequent invasion of fat at the periphery of the lesion; cellular (but often also elastotic) nature of the stroma; irregular and often angulated contours of the glands; open lumina with basophilic secretion; apocrine-type ‘snouts’ in the apical cytoplasm; formation of trabecular bars; lack of a myoepithelial cell

component (well appreciated in immunostained preparations for p63 and CD10); lack of basement membrane (well seen with an immunostain for type IV collagen); and occurrence in two-thirds or more of the cases of typical DCIS in ducts within or outside the lesion, nearly always of low-grade (micropapillary or cribriform) type [83,84,85] (Figure 1.25).

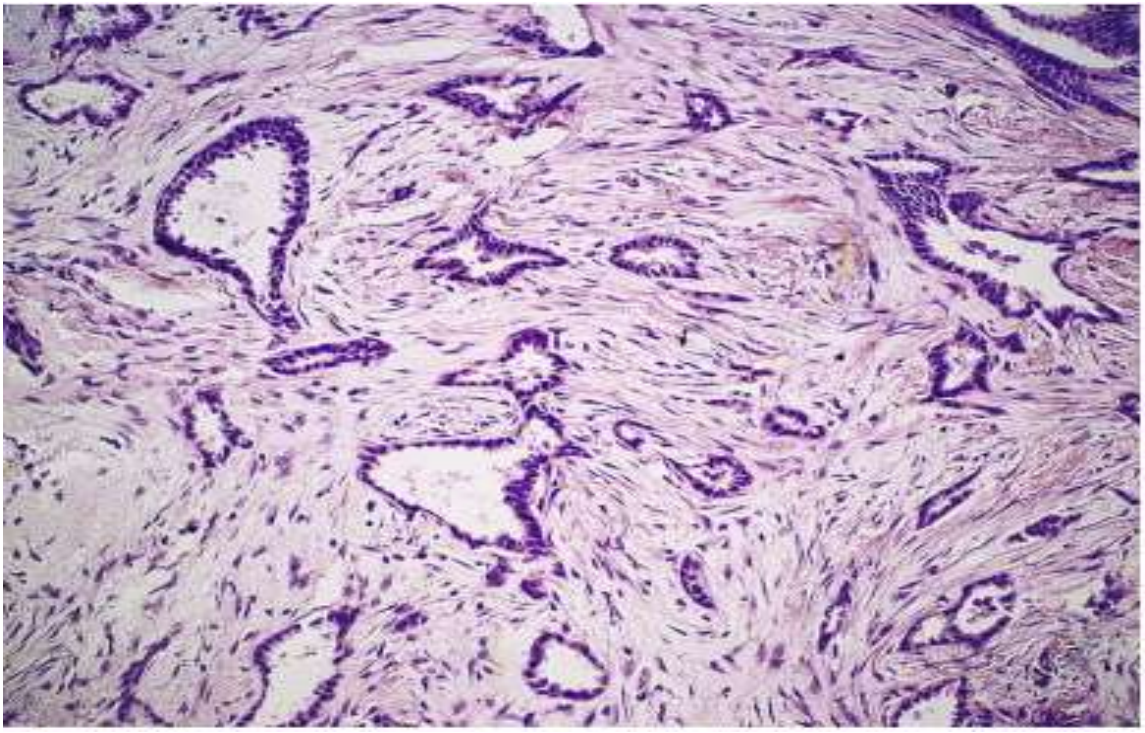

Figure 1.25: Tubular carcinoma of breast. The angulated shape of the glands and the cellular stroma are characteristic of this lesion.

1.7.2.5 Cribriform carcinoma

Invasive cribriform carcinoma is a rare form of breast malignancy closely related to tubular carcinoma and sharing with it an excellent prognosis. As the name indicates, the tumor has a cribriform appearance similar to that seen in the more common in situ counterpart, but it also exhibits stromal invasion (Figure 1.26). This pattern is often seen in association with tubular formations, the relative proportion of the two elements determining the term used, according to the scheme proposed by Page et al.[86] The most

important aspect of this concept is the realization that a breast carcinoma can be cribriform throughout yet invasive; we have seen examples of this tumor extensively invading the breast and beyond and being called in situ tumors simply because they had a cribriform pattern. The proposal has been made for the existence of yet another variation on the theme, in which the tumor has a similar invasive pattern and cytology but a solid configuration (*solid variant* of invasive cribriform carcinoma).[87]

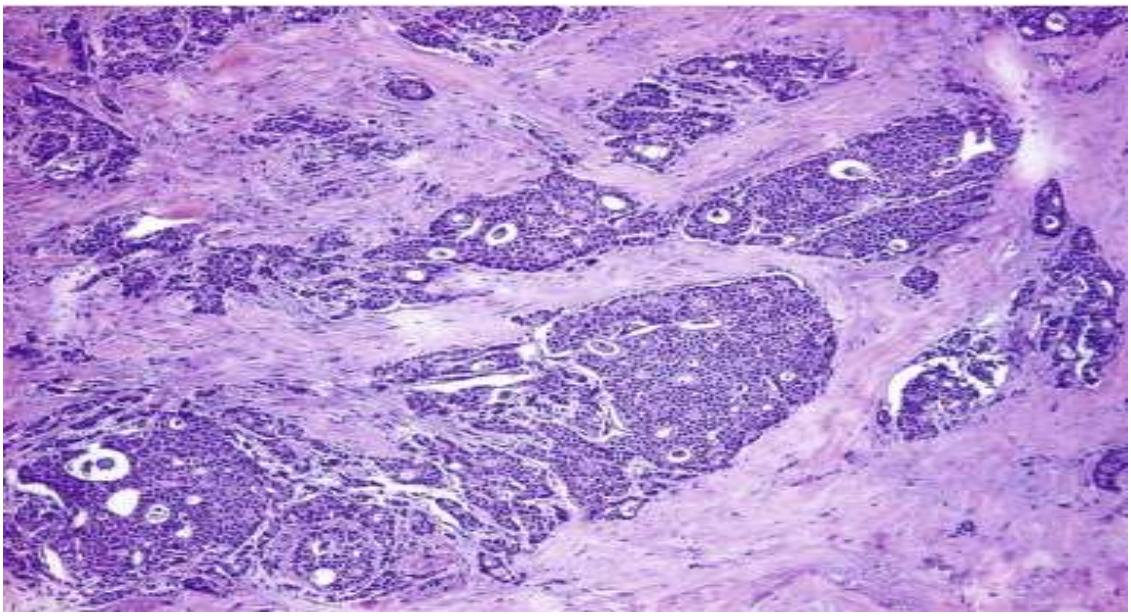

Figure 1.26: Invasive cribriform carcinoma. Some of the nodules have a predominantly solid appearance.

1.7.2.6 Mucinous carcinoma

Mucinous carcinoma, also known as mucoid, colloid, or gelatinous carcinoma, usually occurs in postmenopausal women.[88] Grossly, it is well circumscribed, crepitant to palpation, and formed by a currant jelly-like mass held together by delicate septa (Figure 1.27). Foci of hemorrhage are frequent. Microscopically, the classic and often quoted description is that of small clusters of tumor cells ‘floating in a sea of mucin’ (Figure 1.28).

Figure 1.27: Typical gelatinous gross appearance of pure mucinous carcinoma. Note the sharply circumscribed quality of the tumor.

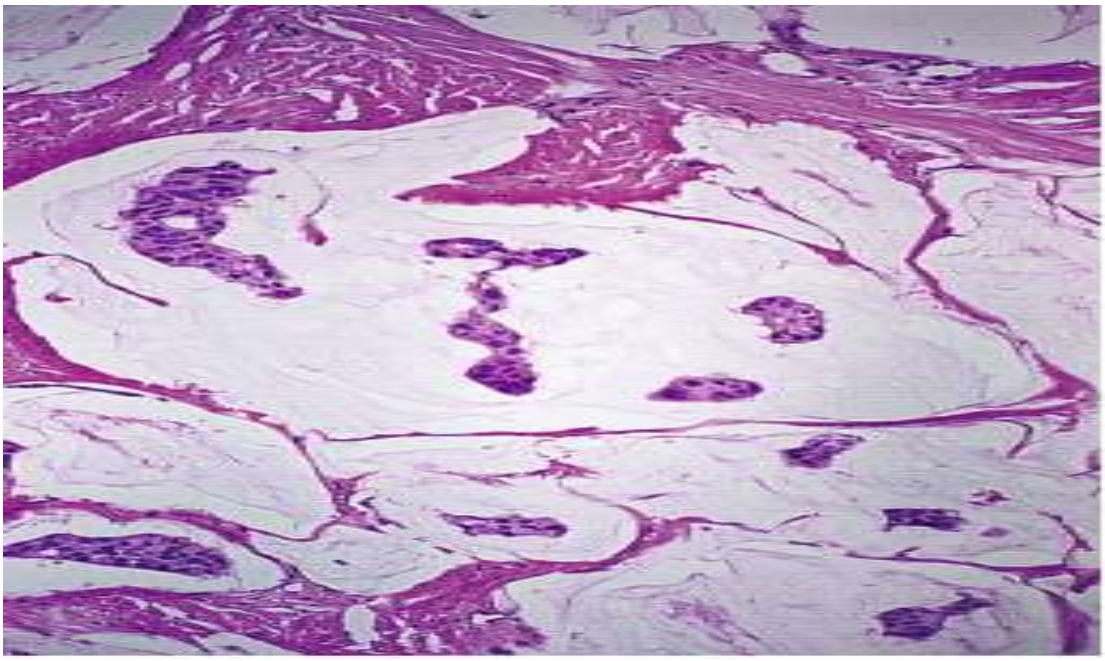

Figure 1.28: Mucinous carcinoma of the breast. Clusters of well-differentiated tumor cells are seen floating in a sea of mucin.

1.7.2.8 Medullary carcinoma

Medullary carcinoma usually appears in patients under 50 years of age and is said to be particularly common in Japanese women. It is also said to be particularly common in carriers of *BRCA1* mutations.[89] Grossly, it is well circumscribed and may become large; it can be mistaken clinically and grossly for a fibroadenoma, but it lacks the trabeculation or whorling of the latter. Its cut surface is solid, homogeneous, and gray, sometimes exhibiting small foci of necrosis (Figure1.29). Rare examples are partially or predominantly cystic.[90] Microscopically, the borders are always of the 'pushing' type. The pattern of growth is diffuse, with minimal or no glandular differentiation or intraductal growth and absence of mucin secretion. The tumor cells are large and pleo-morphic, with large nuclei and prominent nucleoli and numerous mitoses (some of them atypical).

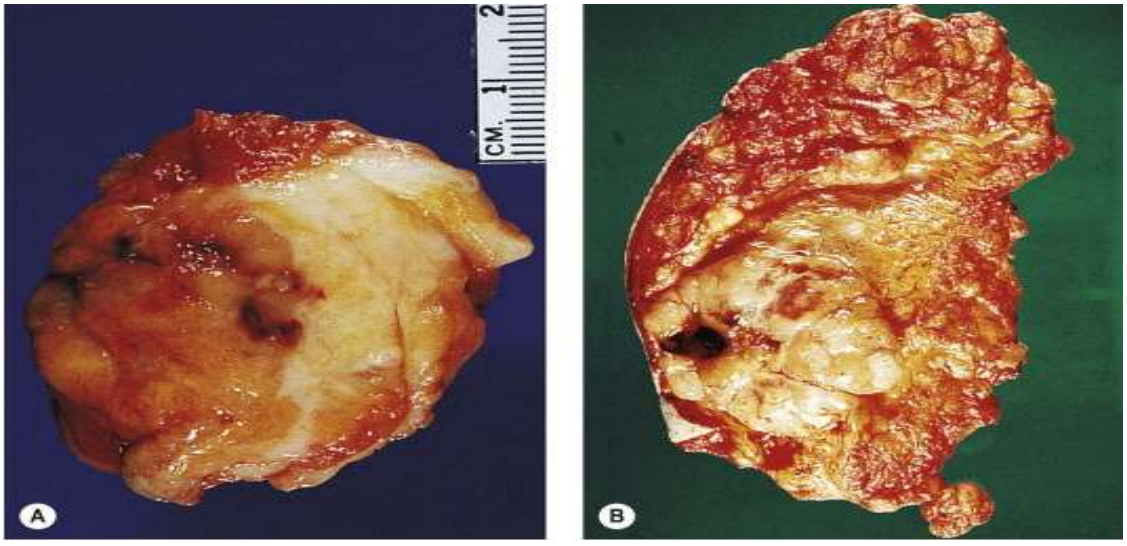

Figure 1.29: A and B, Gross appearance of medullary carcinoma. Note the well-circumscribed character and fleshy appearance.

Microscopically, the borders are always of the 'pushing' type. The pattern of growth is diffuse, with minimal or no glandular differentiation or intraductal growth and absence of mucin secretion. The tumor cells are large and pleomorphic, with large nuclei and

prominent nucleoli and numerous mitoses (some of them atypical). A constant microscopic component is a prominent lymphoplasmacytic infiltrate at the periphery of the tumor, which is thought to represent a reaction of the host tissues to the neoplasm (Figure 1.30).

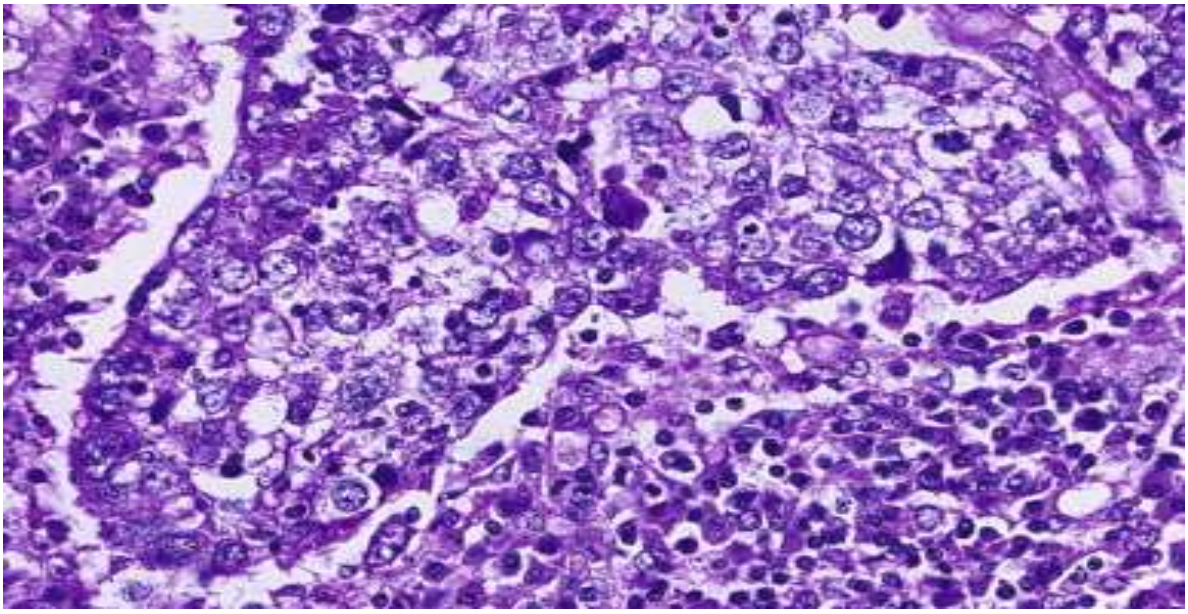

Figure 1.30: Medullary carcinoma. The large tumor cells grow in a 'syncytial' fashion and are sharply separated from the surrounding stroma, which is heavily infiltrated by lymphocytes and plasma cells.

1.7.2.9 Invasive papillary carcinoma

Most papillary carcinomas of the breast are entirely or predominantly in situ lesions; these are discussed on page 1691. The invasive component of a papillary carcinoma may also be papillary or have the features of an ordinary ductal-type carcinoma; the prognosis is substantially better for the former.[91]

1.7.2.10 Invasive micropapillary carcinoma

The recognition of invasive micropapillary carcinoma (IMPCa) as a distinct variant of invasive ductal carcinoma with important prognostic correlates is a relatively recent event, so recent that it was not listed as such in the previous edition of this book. Microscopically, it bears a close similarity with micropapillary carcinoma of other organs, notably endometrium, ovary, and bladder.[92] It is a highly invasive tumor characterized by the formation of pseudopapillary structures lacking a fibrovascular core and by tubular structures free-floating in clear empty spaces.

1.7.2.11 Apocrine carcinoma

Apocrine carcinoma is a very rare form of breast malignancy (ranging from 1% to 4% of all cases), at least when defined as composed entirely or predominantly of apocrine-type epithelium.[93]

1.7.2.12 Secretory (juvenile) carcinoma

This rare form of breast carcinoma is seen primarily in children, but it can also occur in adults.[94] Grossly, it is well circumscribed and usually small (Figure 1.31). The microscopic appearance is distinctive (Figure 1.32). The margins are of the 'pushing' type, and prominent hyalinization is often present in the central portion.

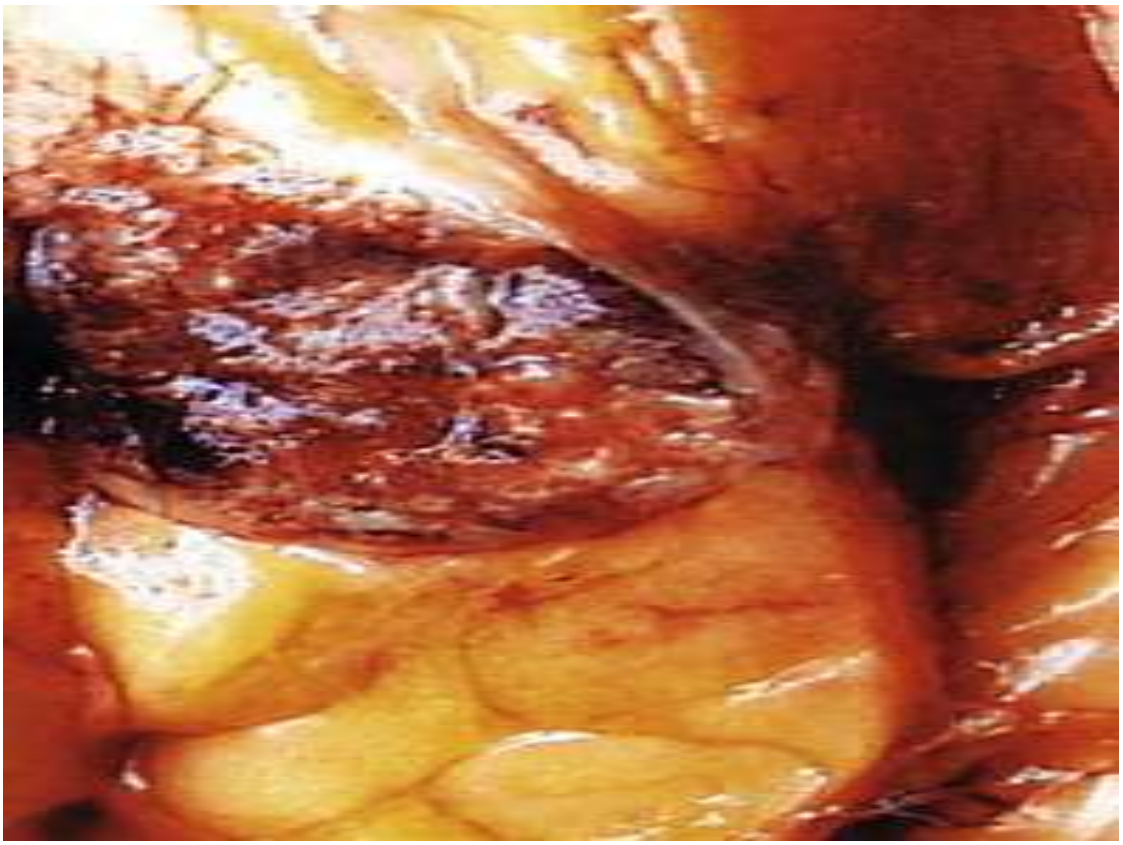

Figure 1.31: Gross appearance of secretory carcinoma. The tumor is well circumscribed and shows a variegated cut surface.

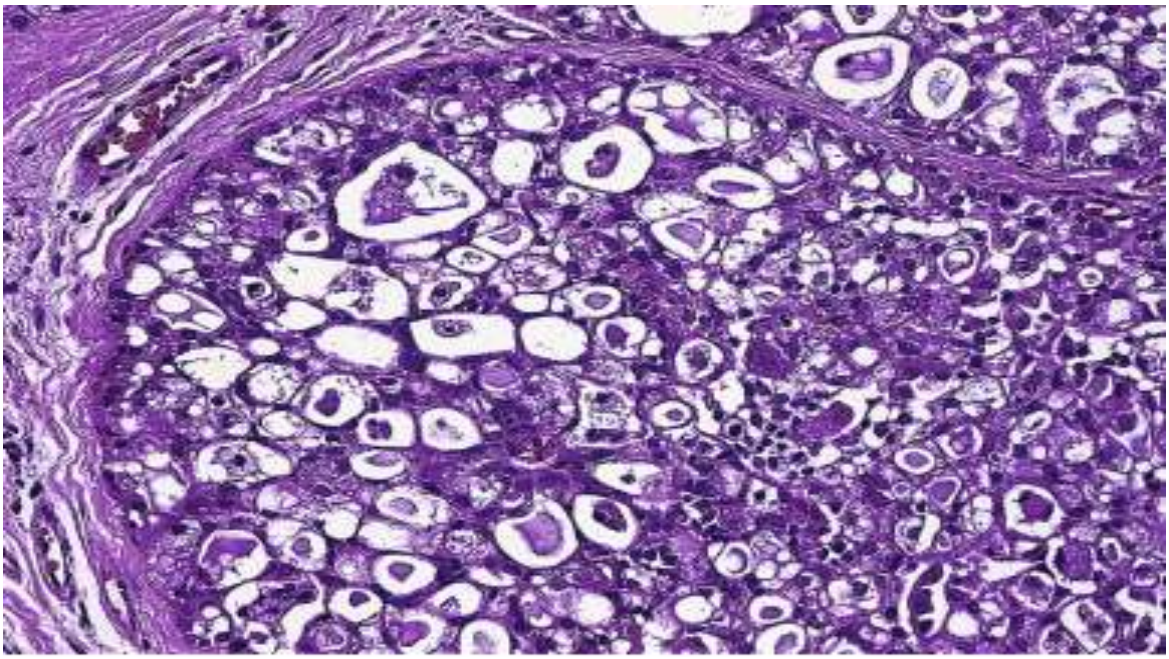

Figure 1.32: Secretory carcinoma. The small uniform glands are filled by a secretory material.

1.7.2.13 Metaplastic carcinoma

Metaplastic carcinoma is a generic term for breast carcinoma of ductal type in which the predominant component of the neoplasm has an appearance other than epithelial and glandular and more in keeping with another cell type.[95]

1.8 Grading system for breast cancer

The grade of a breast cancer is a prognostic factor and is representative of the "aggressive potential" of the tumor. In a broad generalization, "low grade" cancers tend to be less aggressive than "high grade" cancers. Determining the grade is thus very important, and clinicians use this information to help guide treatment options for patients. There are different "scoring systems" available for determining the grade of a breast cancer. One of these systems is the Nottingham Histologic Score system (also termed “the Elston-Ellis modification of Scarff-Bloom-Richardson grading system”). In this scoring system, there are three factors that the pathologists take into consideration: the amount of gland formation (the cell “differentiation,” or how well the tumor cells are trying to recreate normal glands),the nuclear features (the degree of "pleomorphism" or how "ugly" the tumor cells look) and the mitotic activity (how much the tumor cells are dividing, or proliferating) Each of these features is scored from 1-3, and then the scores is added to give a final total score ranging from 3-9.[96] The final total score is used to determine the grade in the following way:

- Grade I tumors have a total score of 3-5
- Grade II tumors have a total score of 6-7
- Grade III tumors have a total score of 8-9

1.9 Staging system for breast cancer

The tumor-node-metastasis (TNM) system is an internationally accepted system used to determine the disease stage was developed by Pierre Denoix starting in 1942 and represented an attempt to classify cancer based on the major morphological attributes of malignant tumors that were thought to influence disease prognosis: size of the primary tumor (T), presence and extent of regional lymph node involvement (N), and presence of distant metastases (M) breast cancer staging provides useful information about the current status of cancer detection and management, and the success of implementing new strategies.[97]

1.10 Prognosis

Prognosis is estimated by looking at what has happened over many years to large groups of people diagnosed with a similar cancer. However, everyone's situation is different so no one can say for certain what will happen to you. Also, treatments and survival rates are constantly improving, which affects the accuracy of estimates for people being treated today is described in different ways it may be put into words (such as excellent, good, poor) or numbers it's often expressed as a five- or ten-year survival rate. This is an estimate of how many people are likely to be alive five or ten years following their diagnosis. A 90% five-year survival rate means that 90 out of 100 people diagnosed with breast cancer are likely to be alive five years after their diagnosis. It doesn't mean these people will only live for five years; it just states how many people are likely to be alive at that point. when determining your prognosis, including the size of the breast cancer the stage of the breast cancer: whether the cancer is only in the breast or has spread to the lymph nodes or other places in the body the type of breast cancer the hormone-receptor status of the cancer HER2 status whether the

cancer is triple-negative (estrogen-receptor-negative, progesterone-receptor-negative, and HER2-negative) the rate of cell growth how likely the cancer is to come back (recurrence) whether the cancer has just been diagnosed or is a recurrence your age your menopausal status your general health**.**[98] The major prognostic factors are used by the American Joint Committee on Cancer to divide breast carcinomas into clinical stages as follows:

- **Stage 0**. DCIS or LCIS (5-year survival rate: 92%).
- **Stage I**. Invasive carcinoma 2 cm or less in diameter (including carcinoma in situ with microinvasion) without nodal involvement (or only metastases < 0.02 cm in diameter) (5-year survival rate: 87%).
- **Stage II**. Invasive carcinoma 5 cm or less in diameter with up to three involved axillary nodes or invasive carcinoma greater than 5 cm without nodal involvement (5-year survival rate: 75%).
- **Stage III**. Invasive carcinoma 5 cm or less in diameter with four or more involved axillary nodes; invasive carcinoma greater than 5 cm in diameter with nodal involvement; invasive carcinoma with 10 or more involved axillary nodes; invasive carcinoma with involvement of the ipsilateral internal mammary lymph nodes; or invasive carcinoma with skin involvement (edema, ulceration, or satellite skin nodules), chest wall fixation, or clinical inflammatory carcinoma (5-year survival rate: 46%).
- **Stage IV**. Any breast cancer with distant metastases (5-year survival rate: 13%).[99]

1.11 Management

The management of breast cancer depends on various factors, including the stage of the cancer. Increasingly aggressive treatments are employed in accordance with the poorer the patient's prognosis and the higher the risk of recurrence of the cancer following treatment. Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. A multidisciplinary approach is preferable. Hormone receptor-positive cancers are often treated with hormone-blocking therapy over courses of several years. Monoclonal antibodies, or other immune-modulating treatments, may be administered in certain cases of metastatic and other advanced stages of breast cancer.[100]

1.11.1 Surgery

Surgery involves the physical removal of the tumor, typically along with some of the surrounding tissue. One or more lymph nodes may be biopsied during the surgery; increasingly the lymph node sampling is performed by a sentinel lymph node biopsy.

Standard surgeries include:

Mastectomy: Removal of the whole breast.

Quadrantectomy : Removal of one quarter of the breast.

Lumpectomy: Removal of a small part of the breast.

Once the tumor has been removed, if the patient desires, breast reconstruction surgery, a type of plastic surgery, may then be performed to improve the aesthetic appearance of the treated site. Alternatively, women use breast prostheses to simulate a breast under clothing, or choose a flat chest. Nipple/areola prostheses can be used at any time following the mastectomy. [101]

1.11.2 Chemotherapy

The process of killing cancer cells by using certain medicines is termed as chemotherapy. It can be given in both situations, before and after surgery, depending upon the condition of the patient. According to the American cancer society the medicines include in chemotherapy are Docetaxel, Paclitaxel, Platinum agents (cisplatin, carboplatin), Vinorelbine (Navelbine), Capecitabine (Xeloda), Liposomal doxorubicin (Doxil), Cyclophosphamide (Cytoxan), Carboplatin (Paraplatin)[102] However it has various side effects. Metastatic or secondary breast can is difficult to treat but it can be controlled and sometime for various years Chemotherapy can be prescribed to manage metastatic breast cancer to minimize or sluggish its development. It can also be administered to decrease some manifestations.[103]

1.11.3 Radiotherapy

is the use of high-energy X-rays to treat cancer. The high-energy rays come from a machine called a linear accelerator and can damage and destroy cancer cells within the area being treated. Radiotherapy also affects normal cells in the area being treated, but these cells can usually recover better than cancer cells. Treatments are usually given regularly over a period of time so that they have the greatest effect on the cancer cells, while limiting the damage to normal cells. Radiotherapy can also be given using radioactive tubes that are put into the area where the cancer is. This is called internal radiotherapy or brachytherapy.[104]

1.11.4 Hormone therapy

is a form of systemic therapy, meaning it reaches cancer cells almost anywhere in the body and not just in the breast. It's recommended for women with hormone receptor-positive (ER-positive and/or PR-positive) breast cancers, and it does not help women whose tumors are hormone receptor-negative (both ER- and PR-negative). is often used after surgery (as adjuvant therapy) to help reduce the risk of the cancer coming back. Sometimes it is started before surgery (as neoadjuvant therapy) as well. It is usually taken for at least 5years. Hormone therapy can also be used to treat cancer that has come back after treatment or that has spread to other parts of the body.[105]

1.12 Aim of this study

To determine the characteristic of breast cancer in female Libyan patient in eastern part of Libya through analysis of several clinco-pathological features and to correlate these features and their immunophenotypical profile with other clinical data to better understand the biological behavior of barest cancer. To phonotypical characterize breast cancer cases according to ER, PR and Her2 status.

CHAPTER 2: Materials and Methods

2.1 Patients and tumor

This study was conducted on a will-characterized consecutive series of cases of female breast cancer cases. Data were collected from archives of Benghazi university clinic and oncology department of Benghazi Medical Center. The patient files were arranged according to the year of case diagnosis. The cases were collected between 2015-2018 and the number of cases was (n=800). Clinicopathological data from patient files were collected which include: patient's name (kept confidential, used only for eliminating repeated samples), age, nationality, address, occupation, family history of breast cancer, social history, staging, grading, hormonal status, Her2 and Ki67 status and mode of treatment. These data collected on Microsoft Excel sheath (Microsoft Excel 2010).

2.2 Tissue preparation

Deparaffinized by running the slides through xylenes and alcohol and then hydrate with water, after that, the slide is put in Mayer's hematoxylin for 15 minutes. Then wash in running tap water for 20 minutes to enhance the hematoxyline staining. Counter stain with eosin from 15 seconds to 2 minutes depending on the age of the eosin, and the depth of the counter stain desired. For even staining results dip slides several times before allowing them to set in the eosin for the desired time. Next, dehydrate in 95% of absolute alcohols, two changes of 2 minutes each or until excess eosin is removed. After that, Check under microscope and clear in xylene, two changes of 2 minutes each. Then, Mount in DPX or Canada Balsam, and apply cover slip. The cover slip not only protects the tissue from damage but also is necessary for viewing the section with the microscope. Last, all the slides are reviewed and re-examined by histopathologist .

2.3 Statistical analysis

The biostatics It is the science of summarizing, collecting, presenting and interpreting data in medical approach and using this data estimate the magnitude of associations and test hypotheses. It has a main role in medical investigations. Biostatistics has played an integral role in modern medicine .Statisticians help researchers design studies, analyze data from medical experiments, decide what data to collect, help interpret the results of the analyses, and collaborate in writing articles to describe the results of medical research. Biostatistics helps researchers make sense of the datas collected to decide whether a treatment is working or to find factors that contribute to diseases.Medical statisticians design and analyses studies to identify the real causes of health issues as distinct from chance variation. There are two main types of statistical analysis: descriptive and inference, also known as modeling.[106]

2.3.1 Descriptive statistics

to analysis of data that helps describe, show or summarize data in a meaningful way such that, intend to describe a big hunk of data with summary charts and tables makes it easier to understand and visualize raw data.[107]

2.3.2 Inference statistic

allows organizations to test a hypothesis and draw conclusions about the data. In these cases, a sample of the entire data is typically examined, with the results applied to the group as a whole.[107]

- **Estimate**

  A particular value that best approximates some parameter of interest

- **Confidence interval**

  an interval constructed using a data set drawn from a population so that, under repeated sampling of such data sets, such intervals would contain the true parameter value with the probability at the stated confidence level.[107]

2.3.3 Test of hypothesis

is a statistical method that is used in making statistical decisions using experimental data. Hypothesis Testing is basically an assumption that we make about the population parameter.[108]

2.3.4 Chi-Squared test

is a statistical hypothesis test that assumes (the null hypothesis) that the observed frequencies for a categorical variable match the expected frequencies for the categorical variable.[108]

CHAPTER 3: Results

In a total of 800 only 233 cases with full data were available in our study. The most common age affected were between (40-50years) while the least age affected were between {(20-30 years, Figure 3.1)}. According to the breast cancer grade; most cases were grade II 311 {(56.8%,Figure 3.2)}, and in terms of staging; most of them were stage III 233{(39.1%,Figure3.3)}. With regards to histopathological type; most of the cases were invasive ductal carcinoma 642 {(88.6%, Figures 3.4)}, other types of breast cancer include comedocarcinoma, invasive lobular carcinoma, invasive ductal carcinoma and mucions carcinoma (Figure3.5). The breast cancer were more common in left side 365 {(49.9%,Figure 3.6). In addition; the most common tumor size were between 2-5cm 321{(66.6%,Figure3.7)}. Furthermore most of the cases were diagnosed using core biopsy 277{(53.0%,Figure3.8)}. With regards to surgical procedures; The majority of cases were treated by simple mastectomy with axillary clearance 269{(48.3%,Figure3.9)}.

Based on the results of immunehistochemistry test; 446 (69.9%) of patients were ER positive while 197{(30.6%,Figure 3.10)} ER negative; and 410 (63.6%) of patients were PR positive and 235 {(36.4%,Figure 3.11)} were PR negative. On the other hand, 365 (58.6%) of patients were HER 2 negative and HER 2 equivocal 15 (2.4%) and HER2 positive were 243{(39.0%,Figure 3.12)}. Furthermore results clarified the high percentage of patients with no metastasis 489 {(84.3% , Figure 3.13)}. In addition, patients with positive lymph node metastasis were 336 {(63.6% , Figure 3.14)} As per the studies made 691{(93.9%, Figure 3.15)} patients were Libyan and patients from Benghazi covered the higher percentage with 408{(55.7% , Figure 3.16)}. Family history was positive in 179 of patients {(74.0% , Figure 3.17)}. Married patients where 229{(80.1% ,Figure 3.18)}. In terms of social life, patients that were not working were 186 (80.2%,) , while working 46{(19.8%,Figure 3.19).There were significant

correlation between staging and grading of tumor p.value (0.022) while there is no other significant correlation among staging with other variables (Table 3.1). In addition; a significant correlation between the grading and the metastasis was found p.value (0.042) unlike other variables (Table 3.2). In accordance with immunehistochemistry table results; no significant correlations were found (Table 3.3. 3.4 & 3.5). The majority of patients that received chemotherapy were 654 (85.0%) while only small number of patients received palliative treatment 9 (1.1%) (Table 3.6)

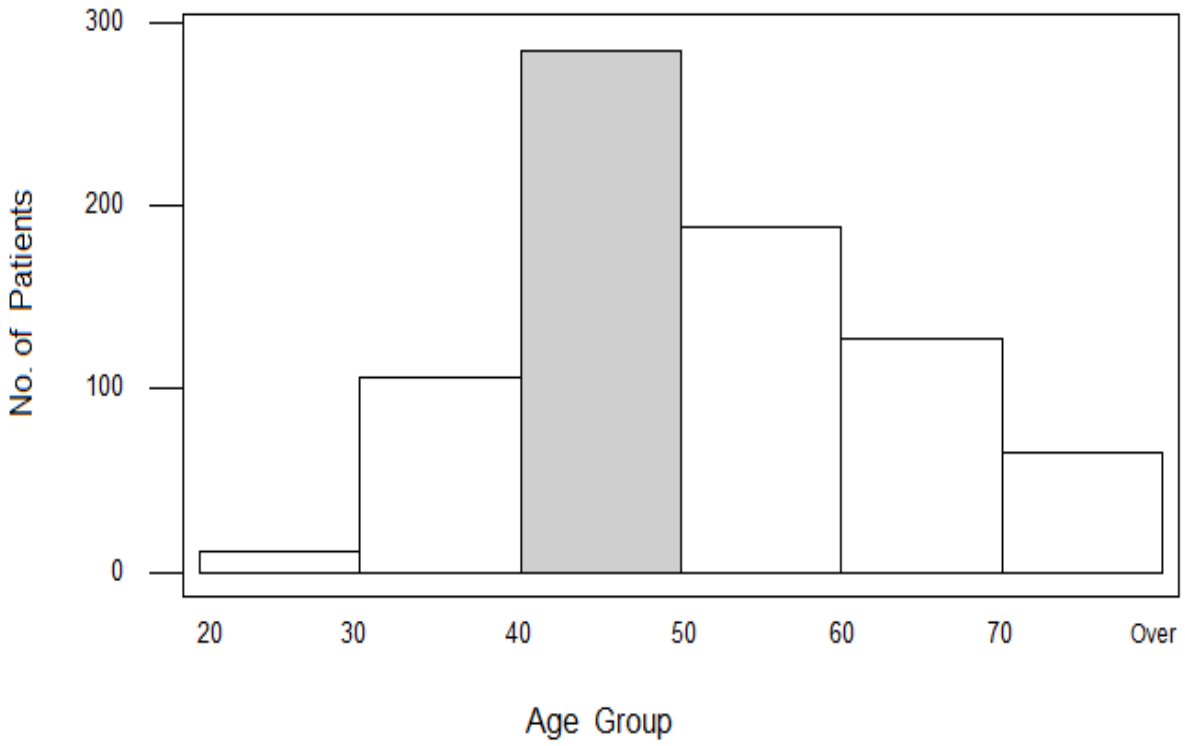

Figure 3.1: Age distribution in breast cancer case.

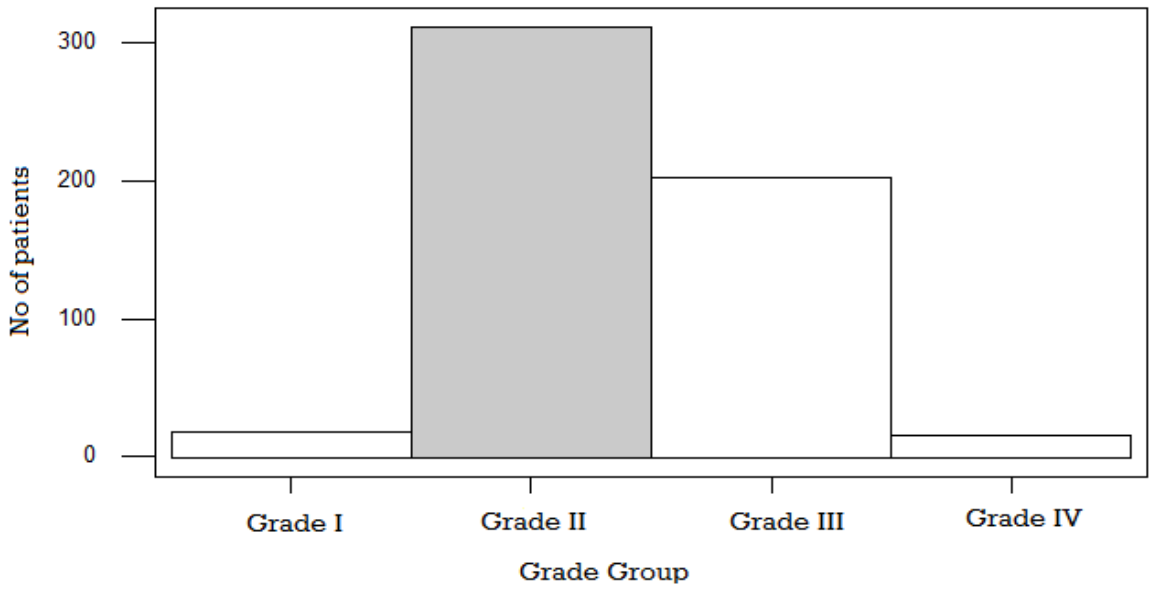

Figure 3.2: Grade distribution in breast cancer cases.

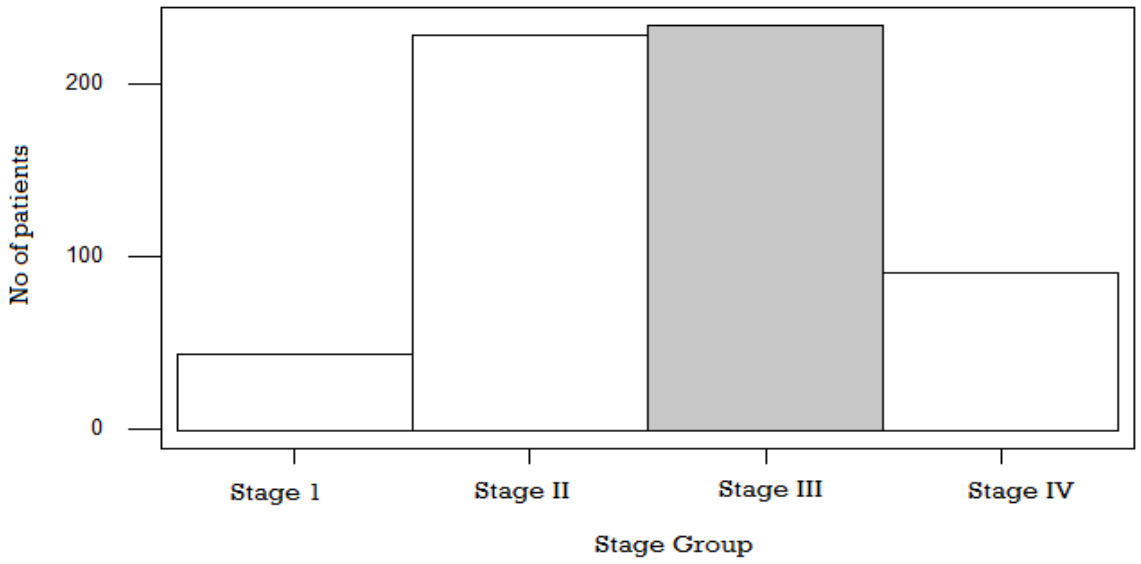

Figure 3.3: Stage distribution in breast cancer cases.

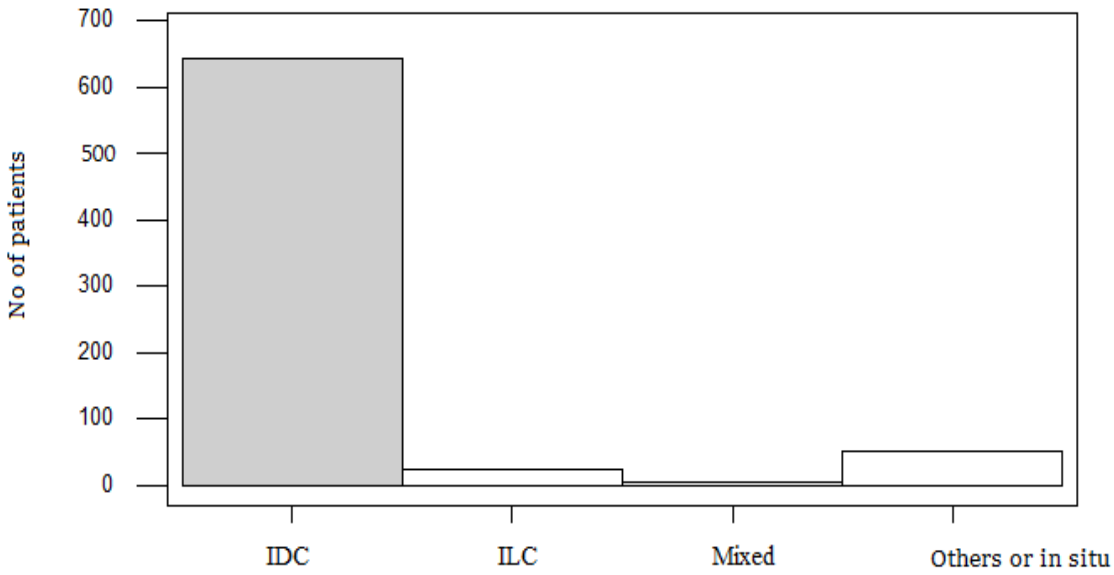

Figure 3.4: Histopathological type in breast cancer.

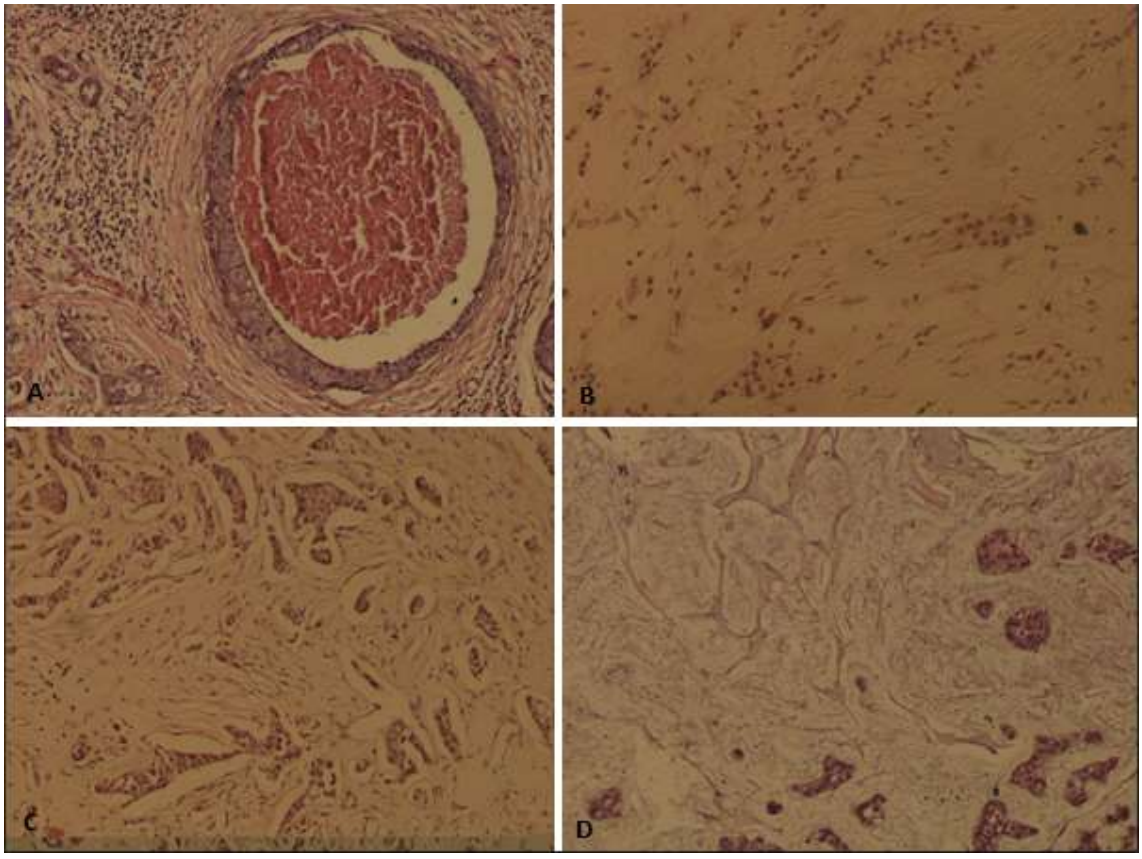

Figure 3.5: A: In situ ductal carcinoma with comedo carcinoma. B: invasive lobular carcinoma. C: invasive ductal carcinoma. D: mucinus carcinoma.

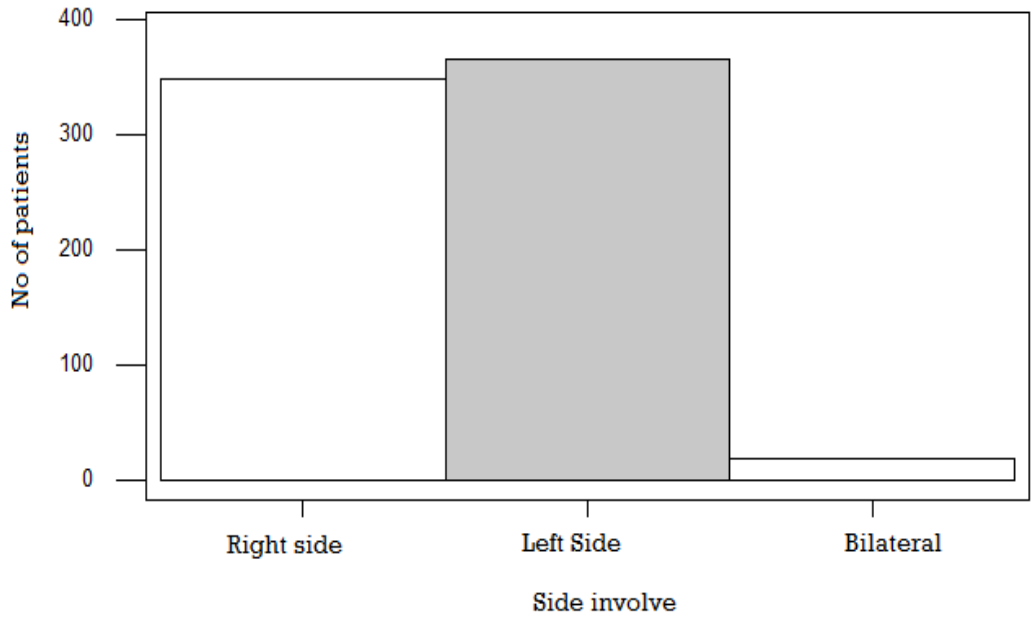

Figure 3.6: Sides involved in breast cancer cases.

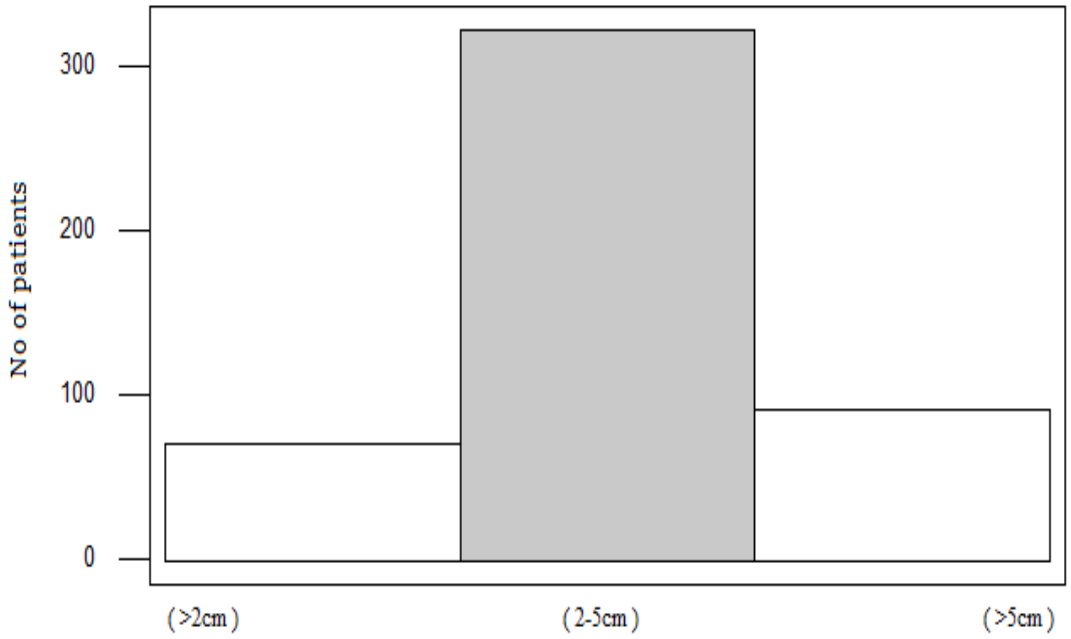

Figure 3.7: Distribution of the size of breast cancer cases.

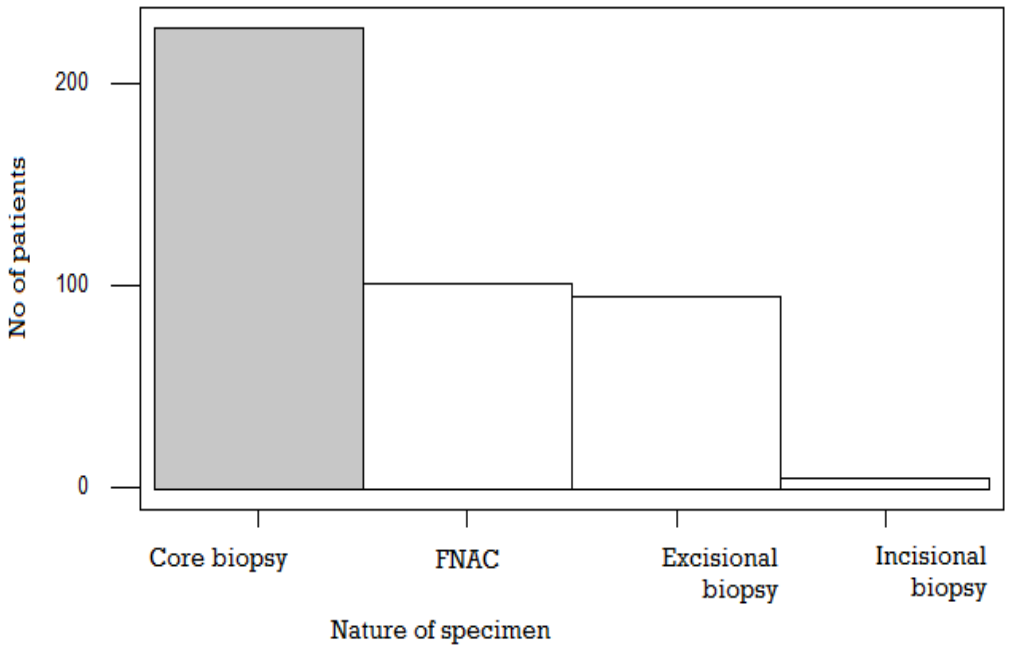

Figure 3.8: Distribution of nature of specimens in breast cancer cases.

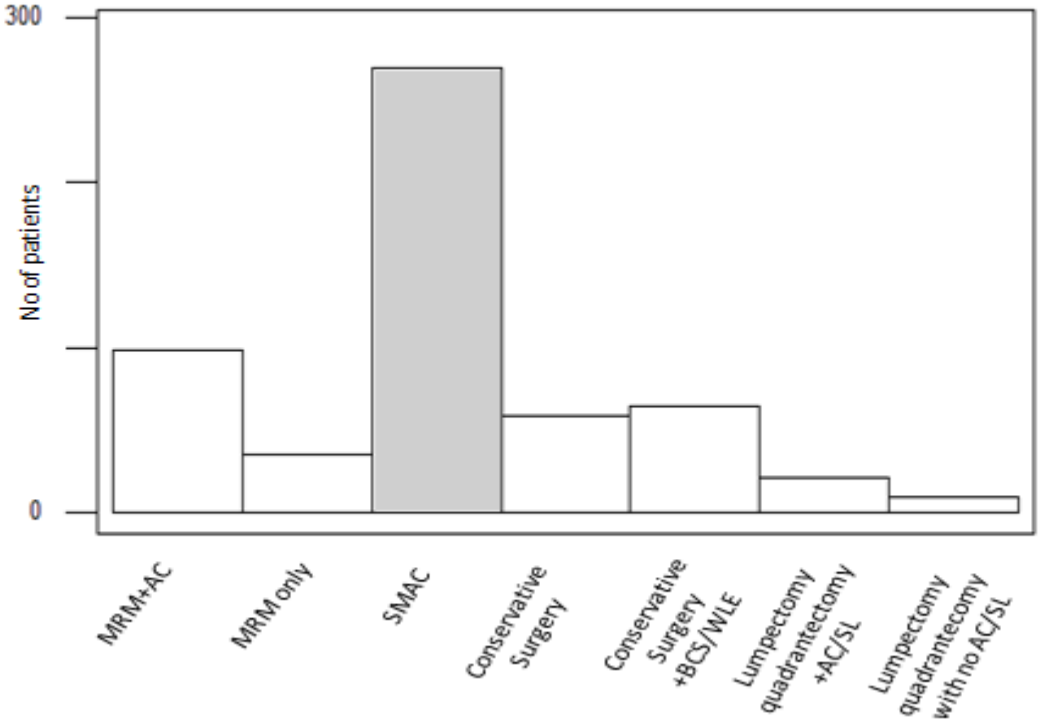

Figure 3.9: Mode of surgical treatment in breast cancer cases.

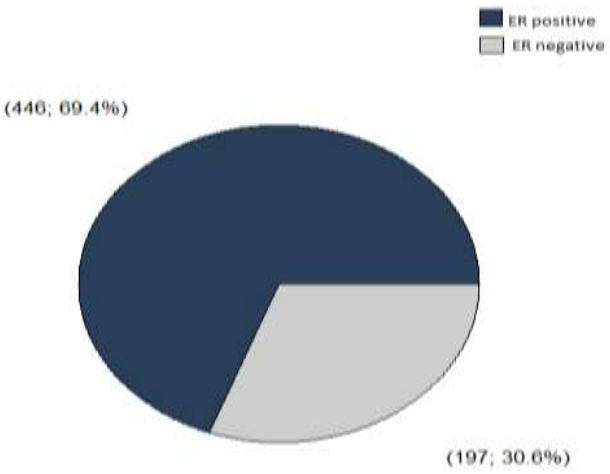

Figure 3.10: Distribution of ER status.

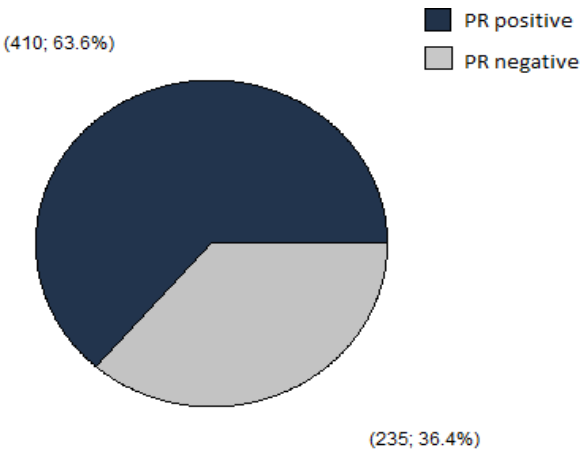

Figure 3.11: Distribution of PR status.

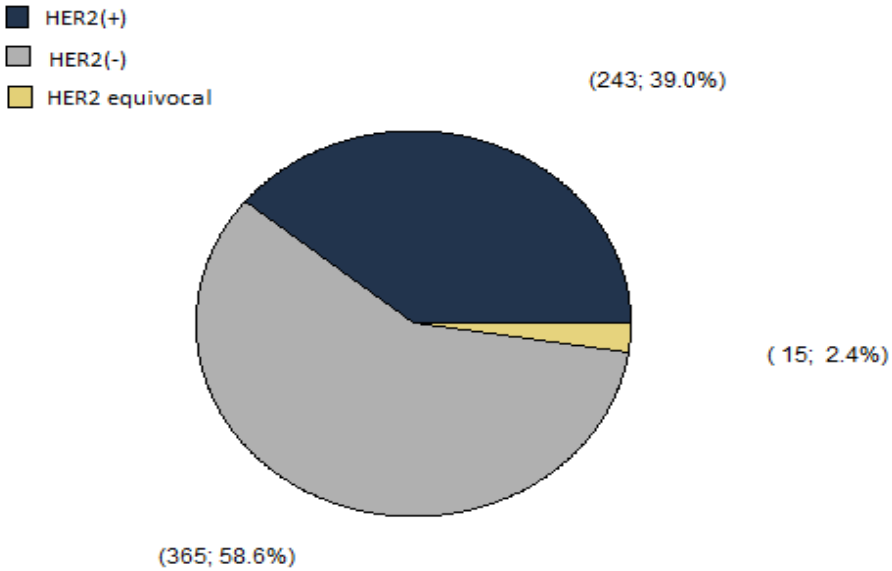

Figure 3.12: Distribution of HER2 status in breast cancer.

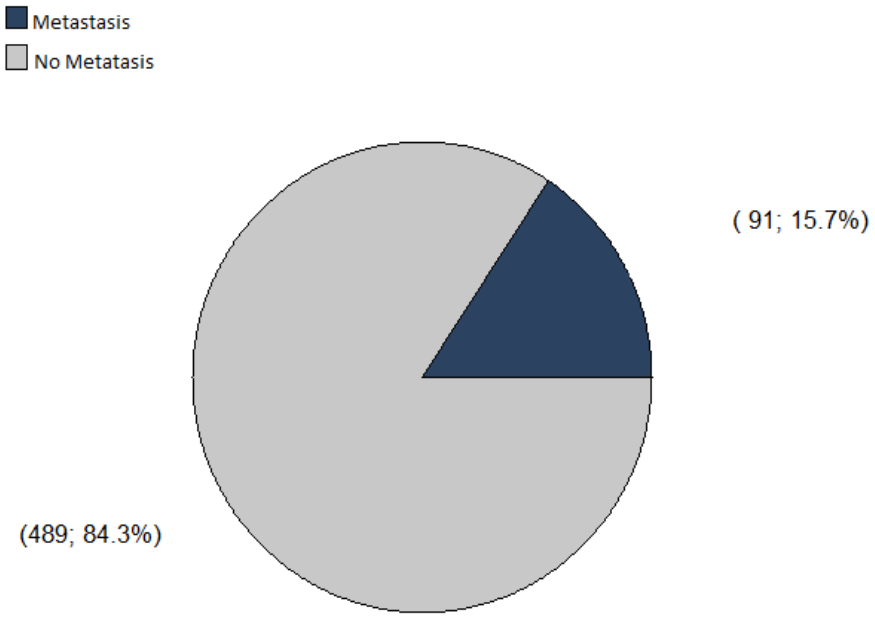

Figure 3.13: Distribution of status of metastasis in breast cancer.

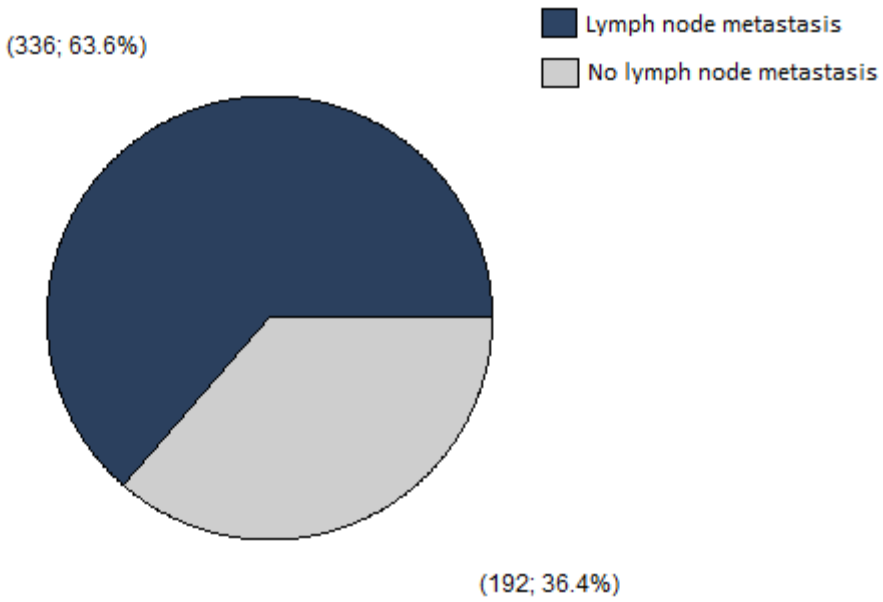

Figure 3.14: Distribution of lymph node status in breast cancer.

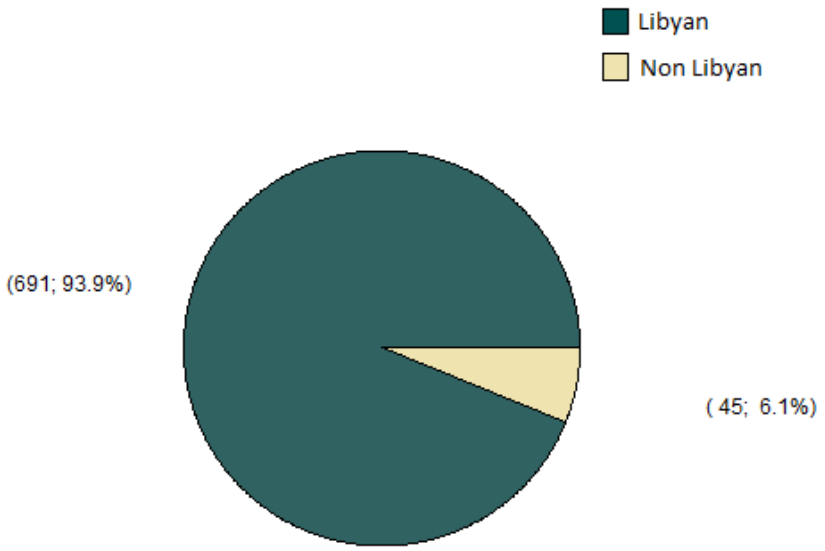

Figure 3.15: Distribution of nationality in breast cancer.

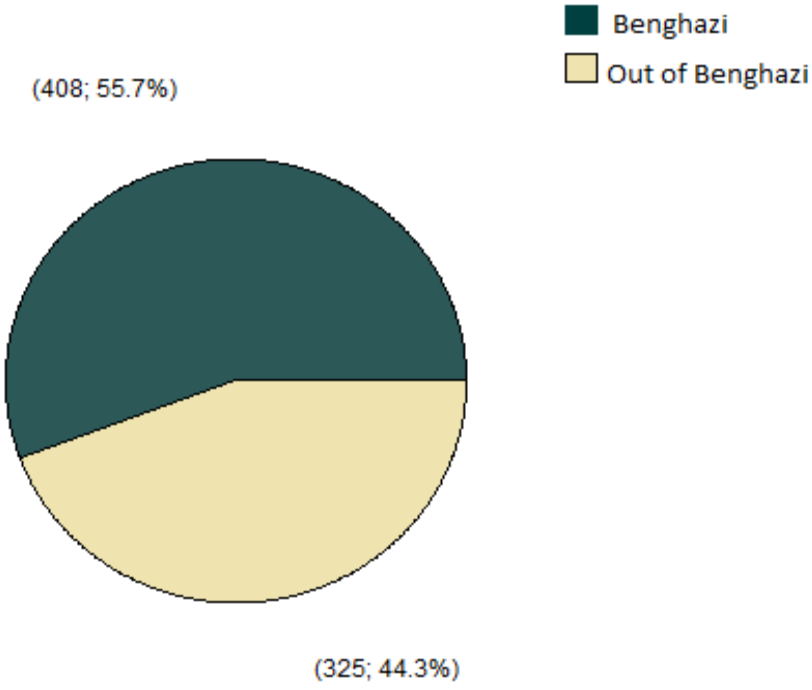

Figure 3.16: Distribution of resident in breast cancer.

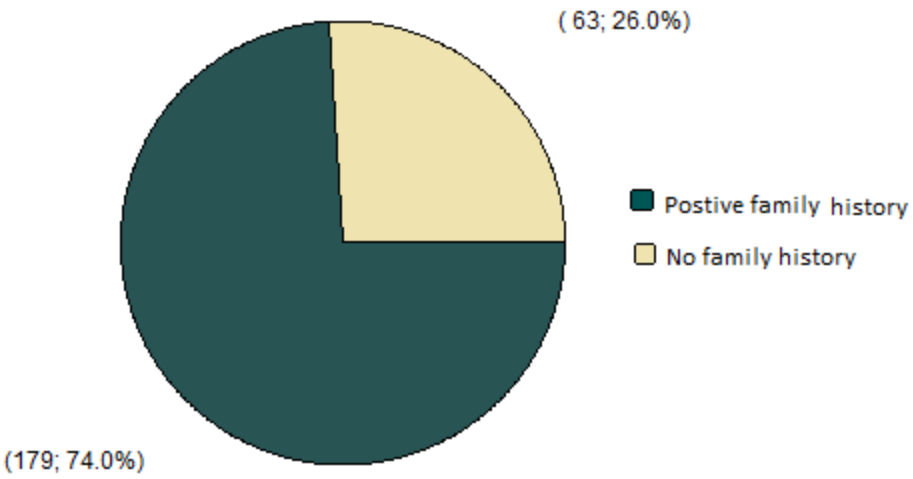

Figure 3.17: Distribution of family history in breast cancer.

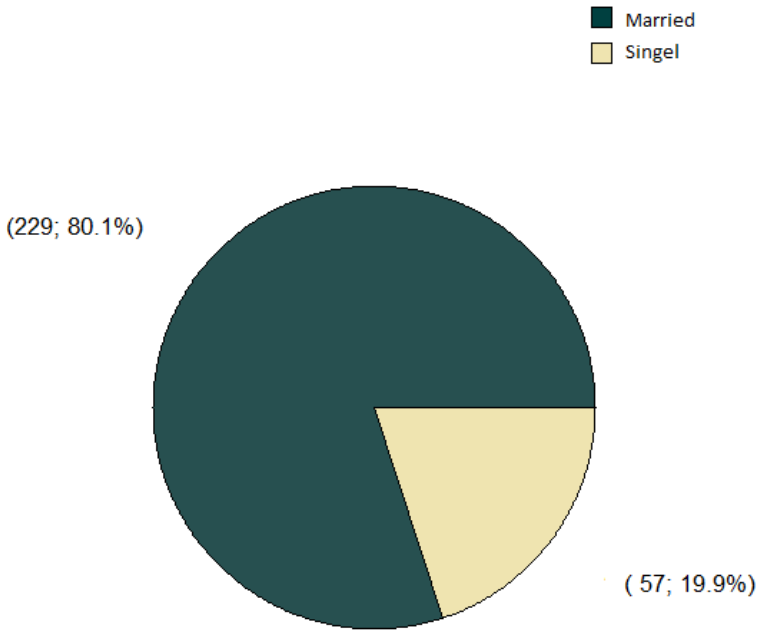

Figure 3.18: Distribution of status of marriage in breast cancer.

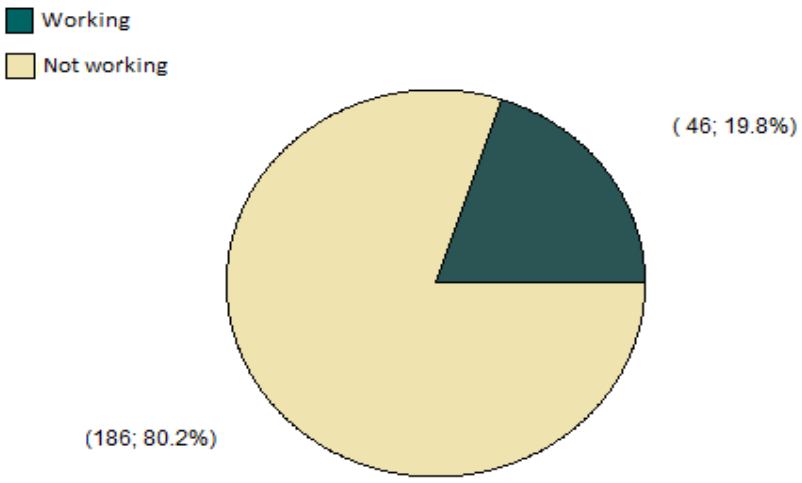

Figure 3.19: Distribution of occupation in breast cancer.

Table 3.1: Bivariate distribution between staging and other clinicopathological data.

Characteristics	Whole series NO(%)	Stage I	Stage II	Stage III	Stage IV	p.Value
Age						0.175
<50%	596	18 (3.02%)	126 (21.1%)	116 (19.4%)	53 (8.89%)	
≥50%		26 (4.36%)	102 (17.1%)	117 (19.6%)	38 (6.37%)	
Tumor Type						
		40 (6.71%)	199 (33.3%)	210 (35.2%)	80 (13.4%)	0.751
Ductal no special type	596	4 (0.67%)	29 (4.86%)	23 (3.85%)	11 (1.84%)	
Other Histological type						
Tumor Grade						
I		5 (0.91%)	3 (0.54%)	5 (0.91%)	5 (0.91%)	0.022
II	548	21 (3.83%)	126 (22.9%)	118 (0.21%)	46 (8.39%)	
III		14 (2.55%)	79 (0.14%)	79 (0.14%)	31 (5.65%)	
IV		3 (0.54%)	4 (0.72%)	8 (1.45%)	1 (0.18%)	
Tumor Size						
<2cm		5 (1.03%)	24 (4.97)	9 (1.86%)	70 (14.5%)	
2-5cm	482	24 (4.97%)	125 (25.9%)	47 (9.75%)	321 (66.5%)	0.891
>5cm		6 (1.24%)	32 (6.63%)	17 (3.52%)	91 (18.8%)	
Lymph node						
Positive		27 (5.11%)	130 (24.6%)	132 (23.2%)	47 (0.08%)	0.730
Negative	528	14 (2.65%)	76 (14.3%)	69 (13.0%)	33 (6.25%)	
Metastasis						
Yes		6 (1.03%)	34 (5.86%)	30 (5.17%)	21 (3.62%)	0.175
NO	580	38 (6.55%)	190 (0.32%)	129 (22.2%)	69 (11.8%)	

Table 3.2: Bivariate distribution between grading and other clincopathological data.

Characteristics	Whole series NO(%)	Grade I	Grade II	Grade III	Grade IV	p.Value
Age <50% ≥50%	548	8 (1.45%) 10 (1.82%)	164 (29.9%) 147 (26.8%)	105 (19.1%) 98 (17.8%)	9 (1.64%) 7 (1.27%)	0.898
Tumor Type Ductal no special type Other Histological type	548	16 (2.91%) 2 (0.36%)	274 (50%) 37 (6.7%)	180 (32.8%) 23 (4.19%)	15(2.73%) 1 (0.18%)	0.921
Tumor Size <2cm 2-5cm >5cm	482	1 (0.20%) 13 (2.69%) 2 (0.41%)	39 (8.09%) 179 (37.13%) 51(10.5%)	26 (5.39%) 120 (24.8%) 35 (7.26%)	4 (0.82%) 9 (1.86) 3 (0.62%)	0.795
Lymph node Positive Negative	528	10 (1.89%) 7 (1.32%)	179 (33.9%) 114 (21.59%)	133 (25.18%) 69 (13.06%)	14 (2.65%) 2 (0.37%)	0.148
Metastasis Yes NO	548	6 (1.09%) 12 (2.18%)	54 (8.21%) 257 (46.8%)	28 (5.10%) 175 (31.93%)	0 16 (2.91%)	0.042

Table 3.3: Bivariate distribution between ER status and other clincopathological data.

Characteristics	Whole series NO(%)	ER (+) NO(%)	ER (-) NO(%)	P-value
Age <50% ≥50%	643	228 (35.4%) 218 (33.9%)	107 (16.6%) 90 (13.9%)	(0.455)
Tumor Type Ductal no special type Other Histological type	643	397 (61.7%) 173 (26.9%)	49 (7.62%) 24 (3.73%)	(0.659)
Tumor Grade I II III IV	548	14 (2.5%) 209 (38.13%) 151 (27.5%) 12 (2.18%)	4 (0.72%) 102 (18.6%) 52 (9.4%) 4 (0.72%)	(0.295)
Tumor Stage I II III IV	596	30 (5.03%) 159 (26.6%) 162 (27.18%) 58 (9.73%)	14 (2.34%) 69 (11.5%) 71 (0.11%) 33 (5.53)	(0.745)
Tumor Size <2cm 2-5cm >5cm	482	47 (9.75%) 227 (47.0%) 60 (12.4%)	23 (4.7%) 94 (19.5%) 31 (6.43%)	(0.625)
Lymph node Positive Negative	528	234 (44.3%) 136 (25.7%)	102 (19.3%) 56 (10.6%)	(0.774)
Metastasis Yes NO	580	59 (10.1%) 344 (59.3%)	32 (5.5%) 145 (25%)	(0.294)

Table 3.4: Bivariate distribution between PR status and other clincopathological data.

Characteristics	Whole series NO(%)	PR (+) NO(%)	PR (-) NO(%)	P-value
Age <50% ≥50%	645	215 (0.33%) 195 (30.2%)	121 (18.7%) 114 (17.6%)	0.816
TumorType Ductal no special type Other Histological type	645	357 (55.3%) 53 (0.08%)	215 (33.3%) 20 (3.10%)	0.088
Tumor Grade I II III IV	548	10 (1.82%) 207 (37.7%) 129 (23.5%) 8 (1.4%)	8 (1.4%) 104 (18.9%) 74 (13.5%) 8 (1.4%)	0.431
Tumor Stage I III II IV	596	31 (5.20%) 148 (24.8%) 140 (23.4%) 57 (9.5%)	13 (2.18%) 80 (13.4%) 93 (15.6%) 34 (5.70%)	0.520
Tumor Size <2cm 2-5cm >5cm	482	43 (8.92%) 206 (42.7%) 53 (10.9%)	27 (5.60%) 115 (23.8%) 28 (5.8%)	0.571
Lymph node Positive Negative	528	212 (40.1%) 124 (23.4%)	124 (23.4%) 86 (12.8%)	0.732
Metastasis Yes NO	580	61 (10.5%) 309 (53.2%)	30 (5.17%) 180 (31.0%)	0.484

Table 3.5: Bivariate distribution between HER2 status and other clincopathological data.

Characteristic	Whole series NO(%)	HER2(+) NO(%)	HER2(-) NO(%)	HER2 equivocal	P.Value
Age <50% ≥50%	623	133 (21.3%) 110 (17.6%)	189 (30.3%) 176 (28.2%)	5 (0.80%) 10 (1.60%)	0.250
Tumor Type Ductal no special type Other Histological type	623	209 (33.5%) 34 (10.4%)	330 (52.9%) 35 (5.61%)	13 (2.08%) 2 (0.32%)	0.240
Tumor Grade I II III IV	548	7 (1.27%) 122 (22.2%) 82 (14.9%) 3 (0.54%)	11 (2.00%) 138 (0.25%) 114 (20.8%) 12 (2.18%)	0 6 (0.010%) 7 (1.27%) 1 (0.18%)	-
Tumor Stage I II III IV	596	10 (1.67%) 89 (14.9%) 95 (15.9%) 36 (0.06%)	34 (5.70%) 135 (0.22%) 129 (21.6%) 53 (8.89%)	0 4 (0.67%) 9 (1.51%) 2 (0.33%)	0.144
Tumor Size <2cm 2-5cm >5cm	482	27 (5.60%) 122 (25.3%) 38 (7.88%)	42 (8.71%) 194 (20.4%) 47 (9.7%)	1 (0.20%) 5 (1.03%) 6 (0.71%)	0.066
Lymph node Positive Negative	528	131 (24.8%) 72 (13.6%)	196 (37.12%) 116 (21.9%)	9 (1.70%) 4 (0.75%)	0.845
Metastasis Yes NO	580	40 (9.89%) 148 (25.5%)	196 (29.1%) 292 (50.3%)	1 (0.17%) 13 (2.24%)	0.394

Table 3.6: Mode of treatment in breast cancer.

Type of Treatment	NO of patient	Percentage
Surgical	269	(48.3%)
Chemotherapy	654	(85.0%)
Radiotherapy	472	(61.3%)
Hormonal therapy	426	(55.5%)
Adjuvant therapy	67	(8.7%)
Neoadjuvant therapy	145	(18.9%)
Palliative	9	(1.1%)

CHAPTER 4: Discussion

in the current study, 800 cases of breast cancer were collected from histopathology lab in Benghazi University clinic and oncology department in Benghazi medical center (BMC) from 2015-2018,wich shows that the most affected age were 40-50 years. Therefore breast cancer in eastern part of Libya is more common in premenopausal period. This study is supported by another study done in eastern part of Libya where they have found that the mean age of breast cancer patients in Libya was 46 years which was almost identical to our study.[109] Furthermore, this is consistent with Arab nations, the average age at diagnosis of breast cancer was available in 18 articles from 11 countries, it was 48 years old, which is shown to be decade earlier than western countries.[110] This is also supported by WHO statistics, which show that the median age of breast cancer in Arab nations is almost one decade younger than western countries. On the contrary, the majority of women present at postmenopausal in Europe. In the United Kingdom, the median age at presentation is 67 years.[111]While the median age at the time of breast cancer diagnosis in US was 62 years.[112] The factors responsible for the difference in age at presentation between the Arab nation, United Kingdom and United state are not fully understood, although it could be due to the breast cancer genes (BRCA 1 and 2) and their variants.[113] Regarding the histological types of breast cancer, the most common type in this study was the invasive ductal carcinoma IDC (88.6%), followed by invasive lobular carcinoma ILC (7.2%), which is approximately resemble to the study done in Saudi Arabia[114] in which most of cases, 85% (n=305) were IDC, and 11.1% (n=40) were ILC. In addition Samina Khokher et al[115] study shown that vast majority of patients around (91%) had invasive ductal carcinoma and only 2% had invasive lobular carcinoma. Our study shows that breast tumor in the left breast is slightly more common (49.9%) than the right breast (52.17%), and that results matches with same result in study done by Mohammad Naeem.[116] It is well established that the

highest cure rates are achieved in women with small size tumor and lymph node-negative breast cancers.[117] However, in this study we found the majority of the patient presented with large tumor size 2.5cm (66.6%), and lymph node positive in (63.6%) of the patient. This indicates that greater the size of tumor, the more probability of positive lymph nodes. In terms of grade of breast cancer in this study, it was found that the most common grade was grade II (56.8%) of patients, which is close to in percentage to Gulam Nabi Sofi et al[118] that also showed the most common grade was grade II with a (52.1%). Considering the staging system that is currently in use for breast cancer is based on the size of the primary tumor, and degree of spread to lymph nodes and the presence of systemic metastasis, it was found that the most common stage was stage III covering a percentage of (39.1%) of patients, which is consistent with the study done in Iran where the most common stage in their study were stage III (54.0%).[119] A connection was found between the histological grade and stage of disease at time of diagnosis. As the stage of disease increases the percent distribution of cases assigned Grade III or Grade IV also increases, whereas the percent assigned Grade I or Grade II decreases according to Donald Earl Henson et al[120]study which is also similar to our study in which there is a significant correlation between staging and grading of tumor; p.value (0.022). Following the data of immunohistochemical results of our study; most of the cases were ER positive (69.4%) and PR positive (63.6%) which are matching with the results of the study that was done in Saudi Arabia[114] the ER immunostain was positive in (70.8%) and the PR in (63.8%). in our study more than half of the cases (58.6%) were immunostain negative for HER2 and this is disagreeing to the results of Prati, Raquel, et al[121] study where they found that less than one-third of the cases (31.15%) were HER2 positive. More than half of our cases (53.0%) were diagnosed using core biopsy procedure and this is consistent with the study that carried out by

Hatada, Takuya, et al[122] in which they found that most of their cases(95%) were diagnosed using the same procedure.

5. Conclusion

In the current study, most of the patient in eastern Libya presented with advanced stage of breast cancer due to daley in the diagnosis. Diagnosis daley was associated with complex interactions between social, medical and other patient associated factors. Furthermore, there was a high recurrence rate among breast cancer cases with invasive ductal carcinoma as well as cases with high tumor grade. Most patients were middle aged, for the immunohistochemical data results; nearly two-third of the cases were ER and PR positive and that was beneficial to patients as hormonal therapy is one of the breast cancer treatment options. We also concluded that there was a significant correlation between the grade of the cancer and metastasis, as well as a significant correlation between stage and grade.

6. Limitation and recommendation

Although material of this study originally accounts 800 breast cancer cases, there was a problem related to the accessibility of the selective and relevant medical information and the medical files of the patients. Some of these information were missed in patients files. Therefore, there is a need to build resources through high-quality, consistent, multicentre collection of clinical data from female Libyan patients with breast cancer. This includes data before and during treatment which is covering primary tumors cases as well as cases of metastatic deposits. Additionally, this study emphasizes the importance of national cancer registry and presence of electronic archives in Libya. Furthermore, it is essential to improve breast cancer awareness as well as to train general practitioners to reduce breast cancer mortality by promoting early detection. Moreover, this study suggested that the screening programme of breast cancer has to start earlier in Libya compared with the rest of the world. Since the results of HER2 immunostain of some cases were equivocal; FISH test should be available and easy accessible to know the HER2 status of the tumor and accordingly to decide which treatment plan should we follow. The study support the establishment of National Cancer Institute in the city of Benghazi, which will accommodate all the clinical and pathological information regarding patients to facilitate the research and studies on cancer, to result in better understanding of cancer indicators in Libya.

7. References

1. Drake R, Vogl W, Mitchell A et al. *Gray's Anatomy For Students*. 1st edition,2005.

2. Netter F, Machado C, Hansen J, Benninger B, Brueckner J, Netter F. *Atlas Of Human Anatomy*.38th ed.,2005.

3. Richard S.Snell,Clinical Anatomy by Regions ,8th Eition,Philadelphia USA ,Wolters Kluwer,2006.

4. Netter F, Machado C, Hansen J, Benninger B, Brueckner J, Netter F. *Atlas Of Human Anatomy*.,20th edition.

5.Color Text Book for; 3rd Edition Leslie P. Gartner James L. Hiatt.

6. Ross & Pawlina, Histology: A Test and Atlas 5th ed.

7. MONTAGNA,WILLIAM."Histology and cytochemistry of human skin XXXV. the nipple and areola. *"British journal of Dermatology* 83.1(197);2-13.

8. J. Sariego, “Breast Cancer in the Young Patient,” The American Surgeon, Vol. 76, No. 12, 2010, pp. 1397-1401.

9. Florescu A.; Amir E.; Bouganim N. and Clemons M. (2011): "Immune therapy for breast cancer in 2010—hype or hope?". *Cu*rrent Oncology; 18: e9–e18.

10. Buchholz T.A. (2009): "Radiation therapy for early-stage breast cancer after breast-conserving surgery". N. Engl. J. Med.; 360: 63–70.

11. Omran AR. The epidemiologic transition. A theory of the epidemiology of population change. Milbank Mem Fund Q. 1971;49:509-538. 3. Gersten O, Wilmoth JR. The cancer transition in Japan since 1951. Demogr Res. 2002;7:271-306.

12. Benson, John R., and Ismail Jatoi. "The global breast cancer burden." *Future oncology* 8.6 (2012): 697-702.

13. Zheng, Rongshou, et al. "National estimates of cancer prevalence in China, 2011." *Cancer letters* 370.1 (2016): 33-38.

14. Jeremy L (2006): "Breast cancer cases rise 80% since Seventies". The Independent (London). Retrieved 24-3-2014.

15. WebMD (2009): "Breast Cancer: Breast Cancer in Young Women". Retrieved on 24-2-2014.

16. Stewart B. W. and Kleihues P. (2003): World Cancer Report. Inernational agency of research of cancer.WHO.

17. Ermiah E.; Abdalla F.; Buhmeida A.; larbesh E.; Pyrhonen S. And Collan Y. (2012): "Diagnosis delay in libyan female breast cancer" . BMC Rec Note; 5:452.

18. Yager J.D. (2006): "Estrogen carcinogenesis in breast cancer". New Engl J Med; 354: 270–282.

19. Skolnick MH, Cannon-Albright LA: Genetic predisposition to breast cancer. *Cancer* 1992; 70:1747-1754.

20. Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. Epidemiol Rev 1993; 15:36-47.

21.Wang DY, Rubens RD, Allen DS, Millis RR, Bulbrook RD, Chaudary MA, Haywar d JL: Influence of reproductive history of age at diagnosis of breast cancer and prognosis. *Int J Cancer* 1985; 36:427-432.

22.Newcomb PA, Storer BE, Longnecker MP, Mittendorf R, Greenberg ER, Clapp RW, Burke KP, Willett WC, MacMahon B: Lactation and a reduced risk of premenopausal breast cancer. *N Engl J Med* 1994; 330:81-87

23. IARC : Hormonal contraception and post-menopausal hormonal therapy. IARC monographs on the evaluation of carcinogenic risks to humans, vol. 72. Lyon, IARC Press, 1998.

24. Kelsey JL, Gammon MD: The epidemiology of breast cancer. *CA Cancer J Clin* 1991; 41:146-165.

25. Hoover R, Gray Sr LA, Cole P, MacMahon B: Menopausal estrogens and breast cancer. *N Engl J Med* 1976; 295:401-405.

26. Ross RK, Paganini-Hill A, Wan PC, Pike MC: Effect of hormone replacement therapy on breast cancer risk estrogen versus estrogen plus progestin. *J Natl Cancer*

Inst 2000; 92:328-32. Fechner RE: Breast cancer during oral contraceptive therapy. *Cancer* 1970; 26:1204-1211.

27. Chen CL, Weiss NS, Newcomb P, Barlow W, White E: Hormone replacement therapy in relation to breast cancer. *JAMA* 2002; 287:734-741.

28. Endogenous Hormones and Breast Cancer Collaborative Group Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst. 2002;94:606–616.

29. Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S, Boyle P. Overview of the main outcomes in breast-cancer prevention trials. Lancet. 2003;361:296–300.

30. Fechner RE: The surgical pathology of the reproductive system and breast during oral contraceptive therapy. *Pathol Annu* 1971; 6:299-319.

31. Goss PE, Sierra S: Current perspectives on radiation-induced breast cancer. *J Clin Oncol* 1998; 16:338-347.

32. Clark CP, Peters GN, O'Brien KM: Cancer in the augmented breast. Diagnosis and prognosis. *Cancer* 1993; 72:2170-2174.

33. Berkel H, Birdsell DC, Jenkins H: Breast augmentation. A risk factor for breast cancer?. *N Engl J Med* 1992; 326:1649-1653.

34. Abbas A, Fausto N, Mitchell R, Kumar V. *Robbins, Patologia Básica*. Rio de Janeiro: Elsevier; 2008.

35. Cortan Pathologic Basis of & Aster. Robbins & Kumar, Abbas, Fausto . Disease. 8th edition. Philadelphia: Saunders Elsevier; 2010.

36. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406:747–52.

37. Perou CM, Børresen-Dale AL. Systems biology and genomics of breast cancer. Cold Spring Harb Perspect Biol. 2011;3:pii: a003293.

38. Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F, et al. Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. Breast Cancer Res Treat. 2016;157:363–71.

39. Inic Z, Zegarac M, Inic M, Markovic I, Kozomara Z, Djurisic I, et al. Difference between luminal A and luminal B subtypes according to Ki-67, tumor size, and progesterone receptor negativity providing prognostic information. Clin Med Insights Oncol. 2014;8:107–11.

40. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, et al. Tailoring therapies--improving the management of early breast cancer: St gallen international expert consensus on the primary therapy of early breast cancer 2015. Ann Oncol. 2015;26:1533–46.

41. Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat. 2008;109:123–39.

42. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011;103:1656–64.

43. Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Exp Ther Med. 2010;1:747–54.

44. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351:2817–26.

45. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol. 2006;24:3726–34.

46. Bellamy CD, McDonald C, Salter DM, Chetty U, Anderson TJ: Noninvasive ductal carcinoma of the breast. The relevance of histologic categorization. *Hum Pathol* 1993; 24:16-23.

47.Holland R, Peterse JL, Millis RR, Eusebi V, Faverly D, vandeVijver MJ, Zafrani B: Ductal carcinoma in situ. A proposal for a new classification. *Semin Diagn Pathol* 1994; 11:167-180.

48.Holland R, Hendriks JH: Microcalcifications associated with ductal carcinoma in situ. Mammographic–pathologic correlation. *Semin Diagn Pathol* 1994; 11:181-192.

49.Collins LC, Achacoso N, Nekhlyudov L, Fletcher SW, Haque R, Quesenberry Jr CP, Puligandla B, Alshak NS, Goldstein LC, Gown AM, Schnitt SJ, Habel LA: Relationshi p between clinical and pathologic features of ductal carcinoma in situ and patient age: an analysis of 657 patients. *Am J Surg Pathol* 2009; 33:1802-1808.

50. Mai KT, Yazdi HM, Burns BF, Perkins DG: Pattern of distribution of intraductal and infiltrating ductal carcinoma: a three-dimensional study using serial coronal giant sections of the breast. *Hum Pathol* 2000; 31:464-474.

51. Brown PW, Silverman J, Owens E, Tabor DC, Terz JJ, Lawrence Jr W: Intraductal 'noninfiltrating' carcinoma of the breast. *Arch Surg* 1976; 111:1063-1067.

52. Mayr NA, Staples JJ, Robinson RA, Vanmetre JE, Hussey DH: Morphometric studies in intraductal breast carcinoma using computerized image analysis. *Cancer* 1991; 67:2805-2812.

53.Damiani S, Ludvikova M, Tomasic G, Bianchi S, Gown AM, Eusebi V: Myoepitheli al cells and basal lamina in poorly differentiated in situ duct carcinoma of the breast. An immunocytochemical study. *Virchows Arch* 1999; 434:227-234.

54. Chivukula M, Domfeh A, Carter G, Tseng G, Dabbs DJ: Characterization of high-grade ductal carcinoma in situ with and without regressive changes: diagnostic and biologic implications. *Appl Immunohistochem Mol Morphol* 2009; 17:495-499.

55. Moriya T, Silverberg SG: Intraductal carcinoma (ductal carcinoma in situ) of the breast. A comparison of pure noninvasive tumors with those including different proportions of infiltrating carcinoma. *Cancer* 1994; 74:2972-2978.

56. Silverberg SG, Chitale AR: Assessment of significance of proportions of intraductal and infiltrating tumor growth in ductal carcinoma of the breast. *Cancer* 1973; 32:830-837.

57. Carter D, Orr SL, Merino MJ: Intracystic papillary carcinoma of the breast. After mastectomy, radiotherapy or excisional biopsy alone. *Cancer* 1983; 52:14-19.

58.Azzopardi JG: *Problems in breast pathology*. (consulting ed.) In: Bennington JL, ed. Major problems in pathology, vol. 11. Philadelphia: W.B. Saunders; 1979.

59.Maluf HM: Differential diagnosis of solid carcinoma in situ. *Semin Diagn Pathol* 2004; 21:25-31.

60. Bellamy CO, McDonald C, Salter DM, Chetty U, Anderson TJ: Noninvasive ductal carcinoma of the breast. The relevance of histologic categorization. *Hum Pathol* 1993; 24:16-23.

61.Schnitt SJ: Clinging carcinoma: an American perspective. *Semin Diagn Pathol* 2010; 27:31-36.

62. Andersen JA: Invasive breast carcinoma with lobular involvement. Frequency and location of lobular carcinoma in situ. *Acta Pathol Microbiol Scand (A)* 1974; 82:719-729.

63. Cross AS, Azzopardi JG, Krausz T, Van Noorden S, Polak JM: A morphological and immunocytochemical study of a distinctive variant of ductal carcinoma in situ of the breast. *Histopathology* 1985; 9:21-37.

64.Kawasaki T, Nakamura S, Sakamoto G, Murata S, TsunodaShimizu H, Suzuki K, Takahashi O, Nakazawa T, Kondo T, Katoh R: Neuroendocrine ductal carcinoma in situ (NE-DCIS) of the breast – comparative clinicopathological study of 20 NE-DCIS cases and 274 non-NE-DCIS cases. *Histopathology* 2008; 53:288-298.

65. Tsang WY, Chan JK: Endocrine ductal carcinoma in situ (E-DCIS) of the breast: a form of low-grade DCIS with distinctive clinicopathologic and biologic characteristics. *Am J Surg Pathol* 1996; 20:921-943.

66. Andersen JA: Lobular carcinoma in situ. A long-term follow-up in 52 cases. *Acta Pathol Microbiol Scand (A)* 1974; 82:519-533.

67. Oberman HA: Invasive carcinoma of the breast with granulomatous response. *Am J Clin Pathol* 1987; 88:718-721.

68. Breslow A, Brancaccio ME: Intracellular mucin production by lobular breast carcinoma cells. *Arch Pathol Lab Med* 1976; 100:620-621.

69.Eusebi V, Betts C, Haagensen Jr DE, Gugliotta P, Bussolati G, Azzopardi JG: Apocr ine differentiation in lobular carcinoma of the breast. A morphologic, immunologic, and ultrastructural study. *Hum Pathol* 1984; 15:134-140.

70. Haagensen CD, Lane N, Bodian C: Coexisting lobular neoplasia and carcinoma of the breast. *Cancer* 1983; 51:1468-1482.

71.Sneige N, Wang J, Baker BA, Krishnamurthy S, Middleton LP: Clinical, histopathologic, and biologic features of pleomorphic lobular (ductal–lobular) carcinoma in situ of the breast: a report of 24 cases. *Mod Pathol* 2002; 15:1044-1050.

72. Rakha EA, Ellis IO: Lobular breast carcinoma and its variants. *Semin Diagn Pathol* 2010; 27:49-61.

73. Wahed A, Connelly J, Reese T: E-cadherin expression in pleomorphic lobular carcinoma: an aid to differentiation from ductal carcinoma. *Ann Diagn Pathol* 2002; 6:349-351.

74. Fisher ER, Gregorio R, Kim WS, Redmond C: Lipid in invasive cancer of the breast. *Am J Clin Pathol* 1977; 68:558-561.

75. van Bogaert LJ, Maldague P: Histologic variants of lipid-secreting carcinoma of the breast. *Virchows Arch [A]* 1977; 375:345-353.

76. Frost AR, Terahata S, Yeh IT, Siegel RS, Overmoyer B, Silverberg SG: The significance of signet ring cells in infiltrating lobular carcinoma of the breast. *Arch Pathol Lab Med* 1995; 119:64-68.

77. Esposito NN, Chivukula M, Dabbs DJ: The ductal phenotypic expression of the E-cadherin/catenin complex in tubulolobular carcinoma of the breast: an immunohistochemical and clinicopathologic study. *Mod Pathol* 2007; 20:130-138.

78. Green I, McCormick B, Cranor M, Rosen PP: A comparative study of pure tubular and tubulolobular carcinoma of the breast. *Am J Surg Pathol* 1997; 21:653-657.

79. Jackson JG, Orr JW: The ducts of carcinomatous breasts, with particular reference to connective-tissue changes. *J Pathol Bacteriol* 1957; 74:265-273.

80. Fisher ER, Gregorio RM: Fisher B, with the assistance of Redmond C, Vellios F, Sommers SC, and cooperating investigators. The pathology of invasive breast cancer. A syllabus derived from findings of the National Surgical Adjuvant Breast Project (Protocol No.4). *Cancer* 1975; 36:185.

81.van Bogaert L-J: Clinicopathologic hallmarks of mammary tubular carcinoma. *Hum Pathol* 1982; 13:558-562.

82. Oberman HA, Fidler Jr WJ: Tubular carcinoma of the breast. *Am J Surg Pathol* 1979; 3:387-395.

83.Tavassoli FA, Jones MW, Majeste RM, Bratthauer GL, O'Leary TJ: Immunohistoch emical staining with monoclonal Ab B72.3 in benign and malignant breast disease. *Am J Surg Pathol* 1990; 14:128-133.

84. Flotte TJ, Bell DA, Greco MA: Tubular carcinoma and sclerosing adenosis. The use of basal lamina as a differential feature. *Am J Surg Pathol* 1980; 4:75-77.

85. Lele SM, Graves K, Galatica Z: Immunohistochemical detection of maspin is a useful adjunct in distinguishing radial sclerosing lesion from tubular carcinoma of the breast. *Appl Immunohistochem Mol Morphol* 2000; 8:32-36.

86. Page DL, Dixon JM, Anderson TJ, Lee D, Stewart HJ: Invasive cribriform carcinoma of the breast. *Histopathology* 1983; 7:525-536.

87. Sanders ME, Page DL, Simpson JF, Edgerton ME, Jensen RA: Solid variant of cribriform carcinoma: a study of 24 cases [abstract]. *Mod Pathol* 2003; 16:45a.

88. Clayton F: Pure mucinous carcinomas of breast. Morphologic features and prognostic correlates. *Hum Pathol* 1986; 17:34-38.

89. Armes JE, Venter DJ: The pathology of inherited breast cancer. *Pathology* 2002; 34:309-314.

90. Howell LP, Kline TS: Medullary carcinoma of the breast. An unusual cytologic finding in cyst fluid aspirates. *Cancer* 1990; 65:277-282.

91. Nassar H, Qureshi H, Volkanadsay N, Visscher D: Clinicopathologic analysis of solid papillary carcinoma of the breast and associated invasive carcinomas. *Am J Surg Pathol* 2006; 30:501-507.

92. Wellings SR, Jensen HM, Marcum RG: An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. *J Natl Cancer Inst* 1975; 55:231-273.

93. Abati AD, Kimmel M, Rosen PP: Apocrine mammary carcinoma. A clinicopathologic study of 72 cases. *Am J Clin Pathol* 1990; 94:371-377.

94. Krausz T, Jenkins D, Grontoft O, Pollock DJ, Azzopardi JG: Secretory carcinoma of the breast in adults. Emphasis on late recurrence and metastasis. *Histopathology* 1989; 14:25-36.

95.Okada N, Hasebe T, Iwasaki M, Tamura N, AkashiTanaka S, Hojo T, Shibata T, Sas ajima Y, Kanai Y, Kinoshita T: Metaplastic carcinoma of the breast. *Hum Pathol* 2010; 41:960-970.

96. Byman D. *Keeping The Peace*. Baltimore: Johans Hopkins University Press; 2002.

97. OH Beahrs, DT Carr, P Rubin, eds. *Manual for Staging of Cancer*. Philadelphia: Lippincott; 1977.

98.Tandon, Atul K., et al. "HER-2/neu oncogene protein and prognosis in breast cancer." *Journal of Clinical Oncology* 7.8 (1989): 1120-1128.

99. Singletary, S. Eva, et al. "Revision of the American Joint Committee on Cancer staging system for breast cancer." *Journal of clinical oncology* 20.17 (2002): 3628-3636.

100.Saini K.S.; Taylor C.; Ramirez A.J.; Palmieri C.; Gunnarsson U.; Schmoll H.J, Dolci S.M.; Ghenne C.; Metzger-Filho O.; Skrzypski M.; Paesmans M.; Ameye L.; Piccart-Gebhart M.J and de Azambuja E. (2011): "Role of the multidisciplinary team in breast cancer management: results from a large international survey involving 39 countries".Annals of Oncology; 23: 853–859.

101. Mariani, Giuliano, et al. "Radioguided sentinel lymph node biopsy in breast cancer surgery." *Journal of Nuclear Medicine*42.8 (2001): 1198-1215.

102. Benson I, Schrag D, Somerfield M, Cohen A, Figueredo A, Flynn P. American society of clinical oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol. 2004;22(16):3408–3419.

103. Muller A, Homey B, Soto H, Ge N. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410:50–55.

104. American Society for Therapeutic Radiology and Oncology. Answers to Your Radiation Therapy Questions. Accessed at www.rtanswers.org on October 23, 2014.

105. Allred, D. Craig, Powel Brown, and Daniel Medina. "The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer." *Breast cancer research* 6.6 (2004): 240.

106. Heagerty.p.J and Lumley , T [2004] . Biostatistics: A methodology for the health science. /nd ed . Hoboken , NJ : Wiley-Interscience.

107. Evans ,M.J [2005] minitab manual for introduction to the practice of statistics, 5th ed New york W.H.Freeman .

108.Tukery, J. w [19771. Exploratory Data Analysis,MA: addison-wesley

109. Boder, Jamela Mostafa E., et al. "Breast cancer patients in Libya: comparison with European and central African patients." *Oncology letters* 2.2 (2011): 323-330.

110. Najjar, Hesahm, and Alexandra Easson. "Age at diagnosis of breast cancer in Arab nations." *International journal of surgery* 8.6 (2010): 448-452.

111. Abdulrahman, Ganiy Opeyemi, and Ganiyu Adebisi Rahman. "Epidemiology of breast cancer in Europe and Africa." *Journal of cancer epidemiology* 2012 (2012).

112. American Cancer Society. "Breast cancer facts & figures 2017–2018." (2017)..

113. Gao, Qing, et al. "Prevalence of BRCA1 and BRCA2 mutations among clinic-based African American families with breast cancer." *Human genetics* 107.2 (2000): 186-191

114. Alnegheimish, Norah A., et al. "Molecular subtypes of breast carcinoma in Saudi Arabia: a retrospective study." *Saudi medical journal* 37.5 (2016): 506.

115. Khokher, Samina, et al. "Clinicopathologic profile of breast cancer patients in Pakistan: ten years data of a local cancer hospital." *Asian Pacific Journal of Cancer Prevention* 13.2 (2012): 693-698.

116. Naeem, Muhammad, et al. "Pattern of breast cancer: experience at Lady Reading Hospital, Peshawar." *J Ayub Med Coll Abbottabad* 20.4 (2008): 22-5.

117. Narod, S. A. "Tumour size predicts long-term survival among women with lymph node-positive breast cancer." *Current Oncology* 19.5 (2012): 249.

118. Sofi, Gulam Nabi, et al. "Estrogen receptor and progesterone receptor status in breast cancer in relation to age, histological grade, size of lesion and lymph node involvement." *Asian pacific journal of cancer prevention* 13.10 (2012): 5047-5052.

119. Harirchi, Iraj, et al. "Breast cancer in Iran: results of a multi-center study." *Asian pacific journal of cancer prevention* 5.1 (2004): 24-27

120. Henson, Donald Earl, et al. "Relationship among outcome, stage of disease, and histologic grade for 22,616 cases of breast cancer. The basis for a prognostic index." *Cancer* 68.10 (1991): 2142-2149.

121. Prati, Raquel, et al. "Histopathologic characteristics predicting HER-2/neu amplification in breast cancer." *The breast journal* 11.6 (2005): 433-439

122. Hatada, Takuya, et al. "Diagnostic value of ultrasound-guided fine-needle aspiration biopsy, core-needle biopsy, and evaluation of combined use in the diagnosis of breast lesions." *Journal of the American College of Surgeons* 190.3 (2000): 299-303.