A CLINICOPATHOLOGIC STUDY ON TRIPLE-NEGATIVE BREAST CANCER PATIENTS: HUSM EXPERIENCE

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Introduction : Breast cancer is the most common cancer among Malaysian women. There are many prognostic factors contributing to the disease and the outcome of the patients. Triple negative breast cancers are defined by a lack of expression of oestrogen, progesterone, and c erbB-2 receptors. They tend to have a higher grade with a poorer outcome compared to non-triple negative breast cancers.

Objectives : The study was carried out aiming to observe the association of triple negative (oestrogen receptor (ER), progesterone receptor (PR) and c erbB-2) breast cancer patients to the pathological (histological subtype, tumour grade, tumour size and lymph node involvement) and non pathological parameters (patient's age and ethnicity).

Methodology : Retrospective review of histopathology reports in Hospital Universiti Sains Malaysia from 1st January, 2002 to 31st December, 2004. Twenty three cases of triple negative breast cancer among 115 cases of breast cancer diagnosed in three years (2002 to 2004) were reviewed. They represented 20.0% of total breast cancer patients.

Results : There were significant association between triple negative breast cancer with tumour size, lymph node involvement and lymphovascular invasion. However, age, race, histological subtype and histological grade did not show significant association.

Conclusion : From these findings, we conclude that tumour size is the strongest factor associated with the triple negative breast cancer. Besides that, lymph node involvement is also associated with triple negative breast cancer. However, lymph vascular invasion is not associated with triple negative vascular breast cancer.

Dr. Venkatesh R Naik : Supervisor

ABSTRACT

Breast cancer is the most common cancer among Malaysian women. There are many prognostic factors contributing to the disease and the outcome of the patients. Triple negative breast cancers are defined by a lack of expression of oestrogen, progesterone, and c erbB-2 receptors. They tend to have a higher grade with a poorer outcome compared to non-triple negative breast cancers. Hence, a retrospective study was carried out aiming to observe the association of triple negative (oestrogen receptor (ER), progesterone receptor (PR) and c erbB-2) breast cancer patients to the pathological (histological subtype, tumour grade, tumour size and lymph node involvement) and non pathological parameters (patient's age and ethnicity).

Twenty three cases of triple negative breast cancer among 115 cases of breast cancer diagnosed in three years (2002 to 2004) were reviewed. They represented 20.0% of total breast cancer patients.

There were significant association between triple negative breast cancer with tumour size, lymph node involvement and lymphovascular invasion. However, age, race, histological subtype and histological grade did not show significant association. From these findings, we conclude that tumour size is the strongest factor associated with the triple negative breast cancer. Besides that, lymph node involvement is also associated with triple negative breast cancer. However, lymph vascular invasion is not associated with triple negative vascular breast cancer.

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1. INTRODUCTION.

The most common tumors in men are prostate, lung, and colorectal cancers. In women, cancers of the breast, lung, and colon and rectum are the most frequent. Cancers of the lung, female breast, prostate, and colon/rectum constitute more than 50% of cancer diagnoses and cancer deaths in the U.S. population. Breast carcinoma is the most common malignancy in women worldwide with more than one million cases and nearly 600 000 death annually (WHO, 2003).

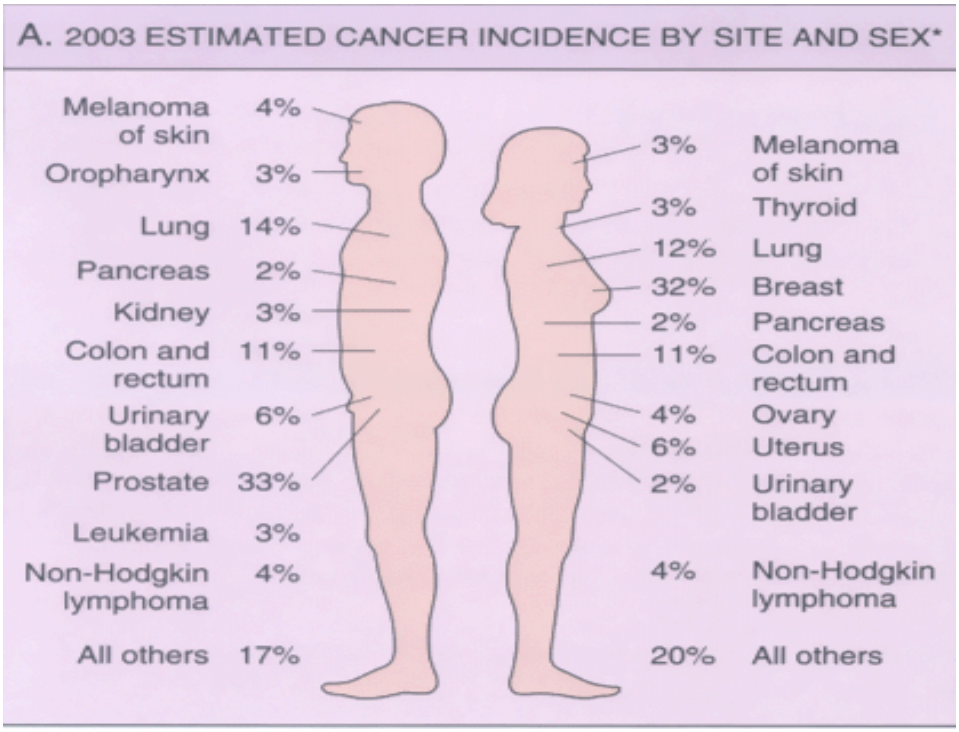

Figure 1: Estimated cancer incidence by site and sex (2003).

The age-adjusted death rates over 50 years of the twentieth century are significantly increased in both men and women. However, since 1995 the cancer incidence rate in men has stabilized and since 1990 the cancer death rate in men has decreased to 18.4%. In women the cancer incidence rate stabilized in 1995 and the cancer death rate has decreased to 10.4% since 1991. Nearly 40% cancer death rates are decreases are due to reduction in lung cancer deaths in men and breast cancer deaths in women.

Reviewed data from Second Report of National Cancer Registry: In year 2003, cancer incidences in Malaysia showed there were 3738 female breast cancer cases reported, which accounted for 31.0 % of total newly diagnosed cancer in female. Breast cancer was the commonest cancer in all ethnic groups and all age groups in females from the age of 15 years. The overall age-standardized incidence (ASR) was 46.2 per 100,000 populations. The age pattern in 2003 showed a peak age specific incidence rate at the 50-59 age groups in Malays, Chinese, and Indians, and the rates then declined in the older age groups. Of the cases diagnosed in 2003, 64.1 % were women in between 40 and 60 years of age. Chinese had the highest incidence with an ASR of 59.7 per 100,000 population followed by Indian women with an ASR of 55.8 per 100,000 population and Malay women with an ASR of 33.9 per 100,000 population.

According to Kelantan Cancer Registry Report 1999-2003, the commonest cancer among females was breast carcinoma with 370 cases or 19.6% out of 1887 cancer cased

among the women. The youngest age with breast cancer in the registry was 19 years. The peak age group was between 50-54 years.

Breast cancer is a heterogeneous disease leading clinicians to discover better prognostic parameters, and optimize the treatment options (Fernandopulle *et al.*, 2006). Pathological prognostic factors are used in clinical practice for a variety of reasons, for example, the identification of patients who would benefit from neoadjuvant treatment (predominantly patients with grade 3 tumors); those in whom conservation surgery is inadvisable (patients with definite vascular channel invasion); and those who may benefit from specific treatment modalities (for example: tamoxifen, herceptin) (Denley *et al.*, 2001).

Early detection of breast cancer and the use of aggressive multimodal treatment have successfully resulted in a decrease in the mortality due to the disease. Prognostic and predictive factors have been widely used in treatment decisions. These factors include: the extent of axillaries lymph node involvement, histological grade, age of the patient, status of hormone receptors (HRs) and human epidermal growth factor receptor 2 (HER2), and involvement of lymphatic or micro vascular spaces. Recent studies suggest that breast cancer is a heterogeneous disease and patients with the same diagnostic and clinical prognostic profile can have markedly different clinical outcomes. Therefore, further understanding of the biology of the disease is needed to improve treatment outcome and reduce mortality (Jiyoung Rhee *et al*., 2008), (Quenel *et al.*, 1995), (Osin and Lakhani, 1999).

Although one study showed that similar risk factors identified in Western populations were responsible for the occurrence of breast cancer in Kelantan (Norsa'adah *et al.*, 2005), a few studies had shown that breast cancer behaved differently in different population and the trends of this malignancy differed with race and ethnicity (Ghafoor *et al.*, 2003), (Shavers and Brown, 2002). Since this malignancy is the most common cancer in women in our population, it is essential to have more data regarding the behavior of breast cancer in our population.

Triple negative breast cancer is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and HER2/neu. It is noted to be a clinicopathologic entity with aggressive behavior and poorer prognosis. Standard therapy is associated with high relapse rates and the most appropriate treatment is as yet unknown (Cleator et al., 2007).

The objective of this study is to determine the incidence and associations with pathologic and non pathologic parameters of triple negative breast cancer patients in HUSM.

2. LITERATURE REVIEW.

2.1 General.

Globally, carcinoma of the breast is the most common malignancy and the leading cause of cancer death in women (Parkin *et al.*, 2000). In 2001, almost 240,000 women were diagnosed with breast cancer, and over 40,000 died of the disease. As the demographic bulge of the "baby boomers" continues to grow older, the absolute number of women with breast cancer is expected to increase by about a third over the next 20 years, just because of the effect of the aging of the population. It is both ironic and tragic that a neoplasm arising in an exposed organ, readily accessible to self-examination and clinical diagnosis, continues to exact such a heavy toll.

Breast cancer is characterized by its molecular and clinical heterogeneity. Studies using cDNA microarrays and immunohistochemical (IHC) markers (Carey *et al.*, 2006; Sorlie *et al.*, 2001) have classified breast cancers into five distinct subtypes: luminal A (oestrogen receptor (ER) positive and/or progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (Her2) negative), luminal B (ER positive and/or PR positive, Her2 positive), Her2 over expressing (ER negative, PR negative, Her2 positive), basal-like (ER negative, PR negative, Her2 negative, cytokeratin (CK) 5/6 positive and/or epidermal growth factor receptor (EGFR) positive) and normal breast-like tumours. Approximately 70% of triple negative breast cancers (ER negative, PR negative, Her2 negative) express basal markers resulting in the triple-negative subtype commonly being used as a surrogate marker for the basal-like subtype .

Luminal tumours have been associated with the most favourable prognoses, while Her2-overexpressing and basal-like tumours, or their surrogate triple negative tumours, have been associated with the worst prognoses (Rakha *et al*., 2006). For triple-negative tumours, the peak risk of recurrence occurs within three years of diagnosis, and mortality rates are increased for five years after diagnosis (Dent *et al.*, 2007). The subtype accounts for approximately 15% of invasive breast cancers (Bauer *et al.*, 2007) and is commonly associated with African American race (Lund *et al.*, 2009), younger age at diagnosis, more advanced stage (Bauer *et al*., 2007), higher grade, high mitotic indices (Carey *et al.*, 2006), family history of breast cancer (Marilyn *et al*., 2009) and BRCA1 mutations (Sorlie *et al*., 2006). Although many studies have examined associations between common breast cancer risk factors such as race and hormone receptor, few studies have explored the relationship between common breast cancer risk factors and the molecular subtypes of breast cancer (Marilyn *et al*., 2009).

Previous studies in Western countries show that triple negative breast cancer has aggressive clinical and pathologic features, including onset at a young age, advanced stage at diagnosis, high histologic and nuclear grade, high mitotic index, higher frequency of unfavourable histologies, and more distant recurrence (Bauer *et al.*, 2007; Dent *et al.*, 2007). In addition, evidence indicates that the prevalence and clinical outcome of triple negative breast cancer differs among races (Carey *et al.*, 2006). Bauer *et al.* have reported that triple negative breast cancer is more prevalent among non-Hispanic black compared with other ethnic group, who, when affected with this subtype had the worst survival (Bauer *et al*., 2007). Carey *et al.* also reported that basal-like

breast tumours occurred at a higher prevalence among African-American women compared with other racial group (Carey *et al.,* 2006). However, there are limited studies of the prevalence, characteristics, and prognosis of triple negative breast cancer in Asian populations.

A recent study of Korean patients indicated that the basal-like subtype, which is positive for one or more of the basal markers and negative for hormone receptors and Her2/neu, was not associated with a poor prognosis. This study also showed that the survival rate associated with the basal-like subtype does not differ from that of other subtypes, with the exception of the Her2/neu over expressing subtype, which has the worst survival rate (Kim *et al*., 2006). In contrast, a recent study of breast cancer patients receiving neoadjuvant chemotherapy showed that triple negative breast cancer was associated with shorter survival than other subtypes, even though it was associated with a higher response rate (Keam *et al*., 2007).

International comparisons involving developing countries are few in number. Where done, survival differences have been largely attributed to differences in patient's age, stage of disease at diagnosis, and the presence of metastasis. Socioeconomic factors, differential access to health care, insurance status, co morbidities and tolerance to prescribed treatment have also been suggested to determine survival (Alrieza Sadjadi *et al.,* 2009). Immigration status and ethnicity may also play a role.

A study of breast cancer among ethnic Chinese women reported that those born in East Asia had lower survival than those born in the US (Alireza Sadjadi *et al*., 2009). A recent study in British Columbia (BC) compared survival for three cancer sites in Chinese, South Asians and the predominantly Caucasian general population and found that Chinese women had the highest survival rates for both breast and cervical cancer, whereas South Asian women had the highest rate for colorectal cancer and the lowest rate for cervical cancer (Alireza Sadjadi *et al.,* 2009). Patterns in cancer incidence can provide important insight into the impact of lifestyle upon cancer development whereas patterns in survival can provide information about the burden and severity of cancer.

While the risk of breast cancer in Asian countries is lower than in the West, increasing numbers of women are being diagnosed with this disease in parts of Asia, including Singapore and Japan. In Singapore, breast cancer is the most common female malignancy, accounting for 28% of all malignancies in women. The age standardized rate is 54.9 per 100 000 per year (Seow A *et al.,* 2004). There is a large variation in the rates of breast cancer among countries (Western countries versus Eastern countries, especially Japan), over time within countries, and large increase in the incidence of breast cancer among populations migrating from nations with low incidence to those with high incidence. These observations indicate the existence of both genetic and environmental factors associated with an increased risk of breast cancer development (Beckmann *et al.*, 1997).

2.2 Female breast.

The class Mammalian is remarkable for the evolution of modified skin appendages that provide complete nourishment and immunologic protection for the young. In humans, paired mammary glands rest on the pectoralis muscle on the upper chest wall.

The breast is composed of specialized epithelium and stroma that give rise to both benign and malignant lesions specific to the organ. Six to ten major ductal systems originate at the nipple. The keratinizing squamous epithelium of the overlying skin continues into the ducts and then abruptly changes to a double-layered cuboidal epithelium. A small keratin plug is often found at the duct orifice. The surrounding areolar skin is pigmented and supported by smooth muscle. Successive branching of the large ducts eventually leads to the terminal duct lobular unit (TDLU).

In the adult woman, the terminal duct branches into a grapelike cluster of small acini to form a lobule. Each ductal system typically occupies over a quarter of the breast, and the systems extensively overlap each other. In some women, ducts extend into the subcutaneous tissue of the chest wall and into the axilla.

2.3 Risk factors.

The major risk factors for the development of breast cancer are hormonal and genetic (family history). Breast carcinomas can, therefore, be divided into sporadic cases, possibly related to hormonal exposure, and hereditary cases, associated with family history or germ-line mutations. Hereditary carcinoma has received intense scrutiny in the hopes that the specific genetic mutations can be identified and that these alterations will illuminate the causes of all breast cancer.

The structure and function of the normal breast require complex interactions between luminal cells, myoepithelial cells, and stromal cells. The same functions that allow for normal formation of new ductal branch points and lobules during puberty and pregnancy abrogation of the basement membrane, increased proliferation, escape from growth inhibition, angiogenesis, and invasion of stroma can be co-opted during carcinogenesis by abnormal epithelial cells, stromal cells, or both (Wiseman *et al*., 2002). While the changes described above are accumulating in the luminal cells (or, less commonly, myoepithelial cells), parallel changes also occur due to mutation or epigenetic changes (e.g., DNA methylation) or via abnormal signaling pathways in these other cell types, resulting in the loss of normal cellular interactions and tissue structure. Loss of these normal functions also occurs with age, and this loss might contribute to the increased risk of breast cancer in older women.

The most established risk factors for breast cancer like early menarche, late age at first childbirth and menopause, history of benign breast disease, exposure to ionizing radiation are generally associated with only weak or moderate elevations in risk. Markedly increased risk occurs in patients with a family history of breast cancer, especially at a young age or with various affected family members, or with bilateral disease (Beckmann *et al.*, 1997). Other known risk factors include race, length of the life time exposure to oestrogen, carcinoma of the contra lateral breast or endometrial, high fat diet, cigarette smoking, less exercise, exposure to environmental toxin such as organochlorine pesticides and breast feeding which reduce risk of breast cancer (Cotran *et al.*, 2009). However, two-thirds of women developing breast cancer have none of these factors (Beckmann *et al.*, 1997).

2.4 Classification of Breast Carcinoma.

Benign lesions of the breast can be divided into non-proliferative and proliferative lesions. Non-proliferative lesions include cysts, apocrine metaplasia, duct ectasia, and mild hyperplasia of the simple or usual type. Proliferative lesions are subdivided into those without atypia and those with atypia. Proliferative lesions without atypia encompass moderate or florid hyperplasia which have more than four layers of epithelium but are cytologically benign. Intraductal papillomas and sclerosing adenosis are also grouped into this category. Atypical hyperplasia is a hyperplastic lesion with some but not all characteristics of the in situ carcinoma. Atypical hyperplasias are subdivided into the lobular and ductal types; the anatomical origin of these lesions is similar to that of in situ carcinoma (Beckmann *et al.*, 1997).

Carcinomas are divided into in situ carcinomas and invasive carcinomas. Carcinoma in situ refers to a neoplastic population of cells limited to ducts and lobules by the basement membrane. In some cases, the cells can extend to the overlying skin without crossing the basement membrane and appear clinically as Paget disease. However, carcinoma in situ does not invade into lymphatics and blood vessels and cannot metastasize. Invasive carcinoma (synonymous with "infiltrating" carcinoma) has invaded beyond the basement membrane into the stroma. Here, the cells might also invade into the vasculature and thereby reach regional lymph nodes and distant sites. Even the smallest invasive breast carcinomas have some capacity to metastasize.

Carcinoma in situ was originally classified as ductal or lobular on the basis of the resemblance of the involved spaces to ducts and lobules. Invasive ductal and lobular carcinomas were named by their association with the characteristic in situ component. Although these descriptive terms are still used, all carcinomas are thought to arise from the terminal duct lobular unit, and the terms "ductal" and "lobular" do not imply a site or cell type of origin.

2.4.1 Carcinoma in Situ.

Proliferations of the glandular epithelium combined with cellular features of malignancy but without infiltration are called carcinoma in situ, distinguished by microscopy as the ductal (DCIS) or the lobular (LCIS) form. Both lesions arise in the terminal duct lobular unit, and in both; the cells of origin are unknown.

2.4.1.1 Ductal Carcinoma in Situ (DCIS).

DCIS consists of a malignant population of cells limited to ducts and lobules by the basement membrane. The myoepithelial cells are preserved, although they may be diminished in number. DCIS is a clonal proliferation and usually involves only a single ductal system. However, the cells can spread throughout ducts and lobules and produce extensive lesions involving an entire sector of a breast. When DCIS involves lobules, the acini are often distorted and unfolded and take on the appearance of small ducts.

With increasing screening activities by mammography the number of these preinvasive entities in breast biopsies has considerably increased as microcalcifications

detected on X-ray are classical features of the common comedo type of DCIS. Various subtypes of DCIS have been described: comedo, cribriform, micropapillary, papillary, solid, clinging, signet-ring cell, and cystic hypersecretory. As the major issue in the management of DCIS is the risk of progression to invasive carcinoma, several classification systems have been suggested according to cellular differentiation, cytonuclear malignancy grade, and degree of necrosis. Multicentricity of DCIS has been described in a high proportion of histological specimens, but in nearly all cases systematic pathological investigations reveal a continuum of DCIS growth in a glandular segment. True multicentricity seems therefore to be a rare event (Beckmann *et al.*, 1997).

2.4.1.2 Lobular Carcinoma in Situ (LCIS).

LCIS is always an incidental finding in a biopsy performed for another reason, as LCIS is not associated with calcifications or a stromal reaction that would form a density. Therefore, it remains infrequent (1% to 6% of all carcinomas) with or without mammographic screening. LCIS is bilateral in 20% to 40% of women when both breasts are biopsied, compared to 10% to 20% of cases of DCIS. LCIS is more common in young women, 80% to 90% of cases occurring prior to menopause.

Because LCIS is frequently multicentric and bilateral and subsequent carcinomas occur at equal frequency in both breasts, it has been suggested that LCIS is not a true neoplasm but rather is a marker of breast cancer risk. However, the cells of LCIS and invasive lobular carcinoma are identical in appearance, and both lack expression of e-cadherin, the transmembrane protein that is responsible for epithelial cell adhesion. The

loss of expression correlates with the histologic appearance of lobular carcinomas as single detached cells.

Recent molecular studies, however, indicate that at various genetic loci DCIS and LCIS are indistinguishable. This is of particular interest as LCIS does seem to be equally liable to go on to invasive ductal and lobular carcinoma (Beckmann *et al.*, 1997).

2.4.2 Invasive (Infiltrating) Carcinoma.

Anatomical histopathology describes the type, grade, and growth parameters of each individual breast carcinoma. Concerning the histological type the basic classification is made according to the supposed origin within the glandule: ductal or lobular type. It has subsequently been shown that this classification is based on a false premise, and that the only carcinomas which truly arise within the ducts are the papillary carcinomas, the others arising in the functional units of the breast called the lobules. The majority of invasive carcinomas are referred to as ductal (85–95%). Within this group there are some tumours with characteristic features and an individual name. These encompass about 15–20% of all breast cancers, for example, tubular, medullary, mucinous, papillary, adenoid cystic, metaplastic, apocrine, squamous, secretory, lipid-rich, and cystic hypersecretory.

The remaining ductal carcinomas (65–80%) are classified as ‘not otherwise specified’ (NOS) or more recently as ‘no special type’ (NST). The abundance of the infiltrating lobular carcinoma varies from 3% to 14%. In addition to the classical ‘Indian

file' linear growth pattern, some structural variants are recognized as lobular carcinomas: tubular, solid, trabecular and alveolar forms. One-third of all lobular types are mixtures of the classical and the variant types. Grade is a semi quantitative description of the nuclear and/or architectural dedifferentiation of the tumours. It is of interest that breast carcinomas arising in *BRCA1* carriers have high-grade tumours with a very high mitotic rate but historically are recorded as having a good prognosis. This anomaly requires further investigation (Rosen, 2006).

Additional parameters such as lymph or blood vessel invasion, perineural invasion, angiogenic capacity, and mode of growth at the tumour front have all been reported as important prognostic factors, but there is currently no consensus on their application in general practice (Beckmann *et al.*, 1997).

2.4.2.1 Invasive Ductal Carcinoma, No Special Type (NST).

Invasive ductal carcinomas of no special type include the majority of carcinomas (70% to 80%). On gross examination, most carcinomas are firm to hard and have an irregular border. Less frequently, carcinomas have a well-circumscribed border and may be soft to firm in consistency. These carcinomas display a wide spectrum of appearances. Most carcinomas induce a marked increase in dense, fibrous desmoplastic stroma replacing fat, giving the tumour a hard consistency on palpation, resulting in a mammographic density (scirrhous carcinoma) (Cotran *et al.*, 2009) .

The microscopic appearance of invasive ductal carcinoma is highly heterogenous with regards to growth pattern, cytologic features, stromal desmoplasia, extent of the associated DCIS and contour. Variability in histologic features may even be seen within a single case. The tumour cells may be arranged as glandular structures, as nest, cords, or trabeculae of various sizes, or as solid sheet. Cytologically, the tumour cells range from those that show little deviation from normal breast epithelial cells to those exhibiting marked cellular pleomorphism and nuclear atypia (Connolly *et al.*, 2006).

2.4.2.2 Invasive Lobular Carcinoma.

Invasive lobular carcinomas usually present like carcinomas of NST as a palpable mass or mammographic density. However, about one-fourth of cases has a diffuse pattern of invasion without prominent desmoplasia and might produce only a vaguely thickened area of the breast or subtle architectural changes on mammography. Metastases can also be difficult to detect clinically and radiologically owing to this type of invasion (Cotran *et al.*, 2009).

Grossly, most tumours are firm to hard with an irregular margin. Occasionally, the tissue may feel diffusely thickened and a discrete tumour mass cannot be defined. The histological hallmark of lobular carcinomas is the pattern of single infiltrating tumour cells, often only one cell in width (in the form of a single file) or in loose clusters or sheets. The desmoplastic response may be minimal or absent. The cells have the same cytologic features as LCIS and lack cohesion, without formation of tubules or papillae. Signet-ring cells are common. Tumour cells are frequently arranged in concentric rings

surrounding normal ducts. Several variants, including tumours with large nests of cells and a high degree of pleomorphism, have also been described (Cotran *et al.*, 2009).

2.4.2.3 Medullary Carcinoma.

Medullary carcinoma presents as a well-circumscribed mass and may be mistaken clinically and radiologically for a fibroadenoma. There is sometimes a history of rapid and almost explosive growth.

These tumours do not have the striking desmoplasia of the usual carcinoma and therefore are distinctly more yielding on external palpation and on cut section. The tumour has a soft, fleshy consistency (*medulla* is Latin for "marrow") and is well circumscribed. The carcinoma is characterized by (1) solid, syncytium-like sheets (occupying more than 75% of the tumour) of large cells with vesicular, pleomorphic nuclei, containing prominent nucleoli and frequent mitoses; (2) a moderate to marked lymphoplasmacytic infiltrate surrounding and within the tumour; (3) grade 2 or 3 nuclei, (4) absence of glandular differentiation and (5) a pushing (non infiltrative) border. All medullary carcinomas are poorly differentiated. DCIS is minimal or absent (Connolly *et al.*, 2006).

2.4.2.4 Mucinous (Colloid) Carcinoma.

This unusual type (1% to 6% of all breast carcinomas) also commonly presents as a circumscribed mass. It tends to occur in older women and may grow slowly during the course of many years. The tumour is extremely soft and has the consistency and

appearance of pale gray-blue gelatin. The tumour cells are seen as clusters and small islands of cells within large lakes of mucin that push into the adjacent stroma (Cotran *et al.*, 2009).

Mucinous carcinoma is characterized by the accumulation of abundant extracellular mucin around invasive tumour cell. The relative proportion of secretion and neoplastic epithelium vary from one case to the next, but the distribution in any one tumour is fairly constant. The proportion of extracellular mucin in the tumour classified as pure carcinomas varied from slightly less than 40% to 99.8% (Rosen, 2006).

2.4.2.5 Tubular Carcinoma.

Tubular carcinomas accounts for only 2% of all breast carcinomas before mammographic screening but have increased in frequency representing up to 10% of carcinomas less than 1 cm in diameter. Tubular carcinomas are typically detected as irregular mammographic densities. Women usually present in their late forties. Tumours are multifocal within one breast in 10% to 56% of cases and bilateral in 9% to 38%. These tumours consist exclusively of well-formed tubules and are sometimes mistaken for benign sclerosing lesions. However, a myoepithelial cell layer is absent, and tumour cells are in direct contact with stroma. Cribriform spaces may also be present. Apocrine snouts are typical, and calcifications may be present within the lumens. LCIS is frequently present, but this association has not been explained (Rosen, 2006).

More than 95% of all tubular carcinomas are diploid and express hormone receptors. By definition, all are well differentiated. Axillaries metastases occur in fewer than 10% of cases unless multiple foci of invasion are present. This subtype is important to recognize because of its excellent prognosis.

2.5 Mechanisms of Carcinogenesis.

Carcinogenesis is a multistep process involving alterations in at least two distinct classes of genes. Protooncogenes are activated qualitatively or quantitatively in certain tumours, and they appear to act as positive proliferative signals for neoplastic growth. In contrast, tumour suppressor genes are normal genes that must be inactivated or lost for tumour development. When active, tumour suppressor genes control neoplastic growth in a negative manner (Barrett and Wiseman, 1987).

Genes that promote autonomous cell growth in cancer cells are called oncogenes, and their normal cellular counterparts are called protooncogenes. Protooncogenes are physiologic regulators of cell proliferation and differentiation; oncogenes are characterized by the ability to promote cell growth in the absence of normal mitogenic signals. Their products, called oncoproteins, resemble the normal products of protooncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals cell proliferation can be readily resolved into the following steps:

i. The binding of a growth factor to its specific receptor generally located on the cell membrane.

ii. Transient and limited activation of the growth factor receptor, which, in turn, activates several signal-transducing proteins on the inner leaflet of the plasma membrane.

iii. Transmission of the transduced signal across the cytosol to the nucleus via second messengers or by signal transduction molecules that directly activate transcription.

iv. Induction and activation of nuclear regulatory factors that initiate DNA transcription.

v. Entry and progression of the cell into the cell cycle, ultimately resulting in cell division.

With this background, we can readily identify the strategies used by cancer cells to acquire self-sufficiency in growth signals. They can be grouped on the basis of their role in growth factor-mediated signal transduction cascades and cell-cycle regulation.

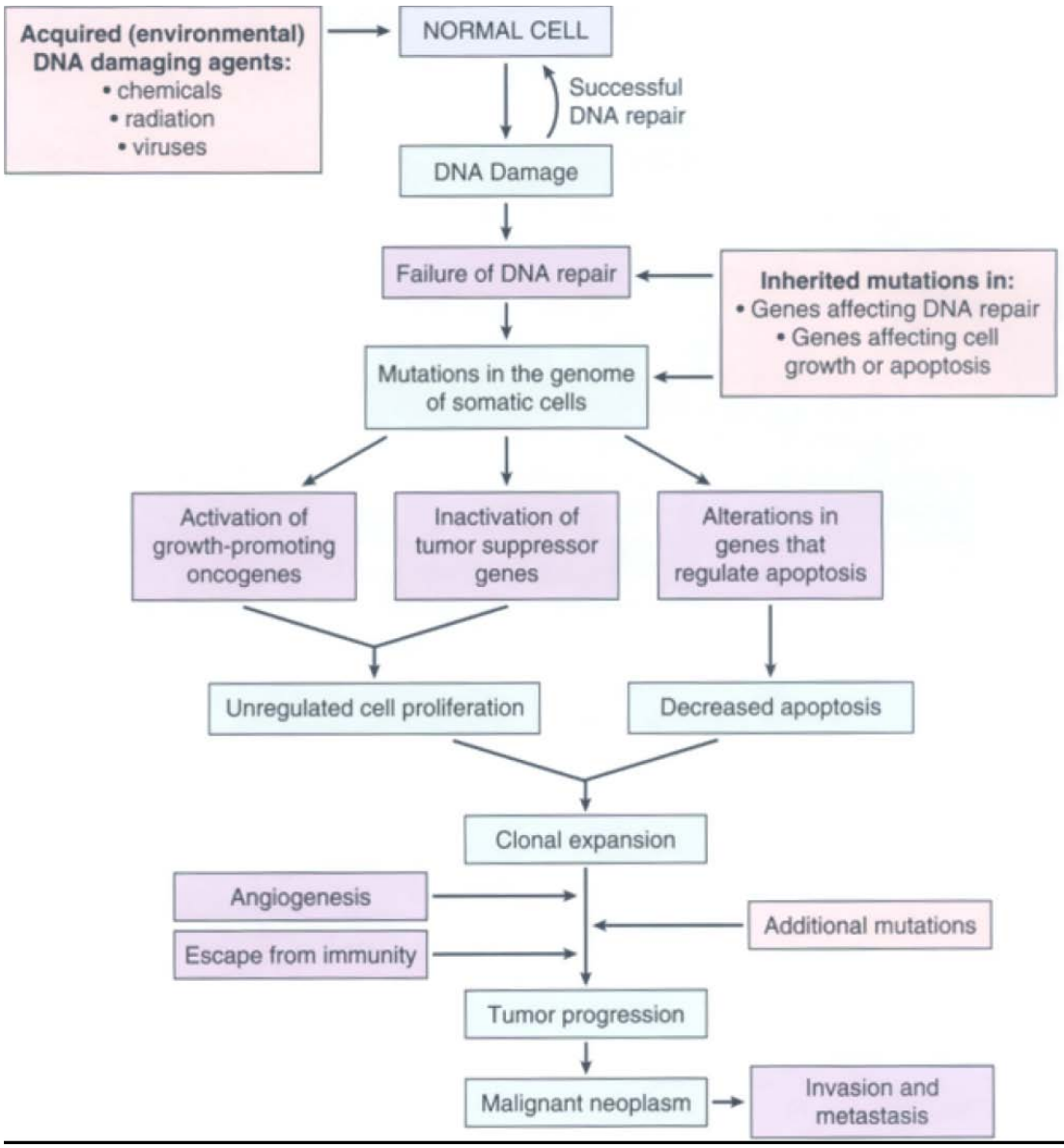

Figure 2.1: Flow chart depicting a simplified scheme of the molecular basis of cancer

Breast cancer emerges by a multistep process which can be broadly equated to transformation of normal cells via the steps of hyperplasia, premalignant change and in situ carcinoma. The elucidation of molecular interdependencies, which lead to development of primary breast cancer, its progression, and its formation of metastases, is the main focus for new strategies targeted at prevention and treatment.

Cytogenetic and molecular genetic analysis of breast cancer samples demonstrates that tumour development involves the accumulation of various genetic alterations including amplification of oncogenes and mutation or loss of tumour suppressor genes. Amplification of certain oncogenes with concomitant over expression of the oncoprotein seems to be specific for certain histological types. Loss of normal tumour suppressor protein function can occur through sequential gene mutation events (somatic alteration) or through a single mutational event of a remaining normal copy, when a germline mutation is present.

The second event is usually chromosome loss, mitotic recombination, or partial chromosome deletion. Chromosome loci 16q and 17p harbour tumour suppressor genes, which seem to be pathognomonic for the development or progression of a specific histological subtype. There are an overwhelming number of abnormalities that have been identified at the molecular level which fit the model of multistep carcinogenesis of breast cancer. When the functions of all of these genes are known and how they participate in malignant progression, we will have the tools for a more rational approach to diagnosis, prevention and treatment (Beckmann *et al*., 1997).