تقييم دور كلا من معامل نمو البطانة الوعائية
و
متوسط الكثافة الوعائية
كعوامل مؤثرة لتكوين الأوعية الدموية
فى مرضى سرطان الثدى

رسالة علمية
مقدمة إلى
معهد البحوث الطبية-جامعة الإسكندرية
إيفاءً جزئياً لشروط الحصول على

درجة الماجستير
في
الكيمياء الطبية التطبيقية

مقدمة من
سلوى عبد الرحمن محمد الصباغ
بكالوريوس علوم- كيمياء حيوية
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جامعة الأسكندرية-١٩٨٨

قسم الكيمياء الطبية التطبيقية
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٢٠٠٩

السـادة المشرفـون

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EVALUATION OF THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND MEAN VASCULAR DENSITY (MVD) AS FACTORS AFFECTING ANGIOGENISIS IN PATIENTS WITH BREAST CANCER

Thesis submitted to
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بسم الله الرحمن الرحیم

" و قل رب زدنی علما "

صدق الله العظیم

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Aim of The Work

The current study is directed to explore the relationship between the expression level of the vascular endothelial growth factors (VEGF) and the lymphangio/ vascular characteristics of breast cancer .

In order to approach the aim of the present study:

1- The concentration level of serum VEGF of breast cancer patients is measured.

2- The tumour vascular characteristics is examined by counting blood vessels via the Chalkley method[147] to assess MVD value.

3- The correlation between expression level of VEGF and clinicopathological data, are explored.

Breast cancer

1-Epidemiology

Breast cancer is the most prevalent form of cancer in the world[1]. Where its incidence is rising throughout the world. It is slowly becoming more prevalent in countries which previously had low rates of cancer as well as becoming a leading cause of cancer death in some countries[2]. In United States and West Europe it is the most common cancer in women [3] and the second leading cause of cancer death in American women[4]

In the last decade there were a significant changes in breast cancer mortality that is declining since the 90 s in several countries, because of early detection, screening programme and proper treatment in the early 90 s[5].In low- and mid-level resource countries of the world, breast cancer is an increasingly urgent problem where patients commonly present with more advanced disease at diagnosis[6].

In Arab countries, breast cancer is the most common cancer among women with a young age of around 50 years at presentation. It has been reported that advanced disease is very common and total mastectomy is the most commonly performed surgery. Also,awareness campaigns and value of clinical breast examination were validated in the Cairo Breast Cancer Screening Trial. Furthermore, it was concluded that radiation centers and early detection would optimize care and reduce the currently high rate of total mastectomies. Population-based screening in those countries with affluent resources and accessible care should be implemented[7].

In Egypt ,it has been reported that breast cancer is the leading tumor in females in all cancer accounting for 37.6% of all reported tumors On the other hand , age – specific rates in females 25-34 years were high revealed that the age- specific rates are in the very young age groups in Egyptians reflects the younger demographic profile [8]. Moreover, in a preliminary study conducted by The Cairo Breast Screening Trial (CBST) was designed to evaluate the role of clinical breast examination as a primary screening modality in the context of primary care, as in Egypt breast cancer is usually diagnosed at an advanced stage. A specialized medical centre in Cairo (the Italian Hospital) was selected as the headquarters of the study. The initial target group was women age 35-64 living in a geographically defined area around the Italian Hospital, 4116 being contacted by social workers and invited to attend a Primary Health Centre for clinical breast examination. High rates of breast cancer were observed; 8 per 1000 at the first examination and approximately 2 per thousand among those who attended for re-screening. The initial prevalence suggests that many women in the community with early

but palpable breast cancer fail to seek medical attention until their cancer is advanced. The detection rate on re-screening, and after follow-up of those who only received one or no screens,(approximately3/1000)is similar to expectation [9].

2- Etiologic Factors of Breast Cancer

Causes, Incidence and Risk factor: [10-14]

Risk:

1-Age and gender : The risk of developing breast cancer increases as woman get older. The majority of advanced breast cancer cases are found in women over age 50. Women are 100 times more likely to get breast cancer than men.

2-Family history of breast cancer: woman have a higher risk for breast cancer if she has a close relative having breast, uterine, ovarian, or colon cancer. About 20-30% of women with breast cancer have a family history of the disease.

3-Genes: Some people have genes that make them more prone to developing breast cancer. The most common gene defects are found in the BRCA1 and BRCA2 stand for breast cancer susceptibility gene 1 and breast cancer susceptibility gene 2, respectively..BRCA1 and BRCA2 genes are human genes that belong to a class of genes known as tumor suppressors. These genes normally produce proteins that protect a woman from cancer. But if a parent passes her a defective gene, she has an increased risk for breast cancer. Women with one of these defects have up to an 80% chance of getting breast cancer sometime during their life. Other genetic defects have been linked to breast cancer, including those found in the ataxia-telangiectasia mutated (ATM) gene, the checkpoint kinase 2 (CHEK-2) gene, and the p53 tumor suppressor gene, but these are very rare. Several gene sets, mainly developed in high-risk cancers, predict metastasis from low-malignant cancer [15].

4-Menstrual cycle: Women who get their periods early (before age 12) or went through menopause late (after age 55) have an increased risk for breast cancer.

Other Risk factors include:

1-Alcohol use: Drinking more than 1-2 glasses of alcohol a day may increase the risk for breast cancer.

2-Childbirth: Women who have never had children or who had them only after age 30 have an increased risk for breast cancer. Being pregnant more than once or becoming pregnant at an early age reduces the risk of breast cancer.

3- Diethylstilbestrol (DES): Women who took (DES) to prevent miscarriage may have an increased risk of breast cancer after age 40. This drug was given to the women in the 1940s-1960s.

4-Hormone replacement therapy (HRT) :The higher the risk for breast cancer the received hormone replacement therapy for several years or more. Many women take HRT to reduce the symptoms of menopause. The association between breast cancer and prolonged exposure to estrogens suggests that this hormone also may have a role in such process [16].

5-Obesity : Breast cancer and obesity represent an important public health issue in different countries where a link between them has been suggested , however, this link is controversial. Theory of this suggestion is based on the observation that obese women produce more estrogen which can fuel the development of breast cancer. In a large cohort study subjected to investigating several prognosis events, the prognosis role of obesity was confirmed .Also, it has been stressed that obesity prognosis effect was related to breast cancer presentation at diagnosis time while independent obesity effect linked to hormonal disorders appeared consistent as obesity's mechanism [17].

6-Radiation: Woman received radiation therapy as a child or young adult to treat cancer of the chest area, has a significantly higher risk for developing breast cancer. The younger she started such radiation, the higher her risk -- especially if the radiation was given when a female was developing breasts. Although few women 50 years of age or older have risks from mammography that outweigh the benefits, the evidence suggests that more women 40 to 49 years of age have such risks[18].

7-Environmental compounds: seem to be involved in the etiology of this disease. Many studies have found an association between human cancer and exposure to agricultural pesticides and among them parathion, it was suggested that parathion has the potency to cause malignant transformation of breast epithelial cells through modulation of expressionof cell cycle regulated genes [16].

8-Physical Activity:Stronger risk decreases were observed for recreational activity, lifetime or later life activity, vigorous activity, among postmenopausal women, women with normal body mass index BMI, non-white racial groups, hormone receptor negative tumours, women without a family history of breast cancer and parous women. The effect of physical activity on breast cancer risk is stronger in specific population sub-groups and for certain parameters of activity that can be further explored in future intervention trials[19].

3- Pathological Aspects

Classification and Grading:

The main reasons for applying a classification system to breast carcinoma are to obtain a correlation with prognosis and tumour biology. Invasive carcinomas may be sub-divided morphologically according to their degree of differentiation.This is achieved in two ways, by assessing histological type and histological grade [(20)].

A-Pathological type:

Breast cancer is an abnormal growth of the cells that normally line the ducts and the lobules. Breast cancer is classified by whether the cancer started in the ducts or lobules, whether the cells have "invaded" (grown or spread) through the duct or lobule, and by the way the cancer cells look under a microscope. Breast cancers are broadly grouped into those that are still in the breast lobules or ducts (referred to as "noninvasive" or "carcinoma in situ") and those that have started to grow and spread beyond the walls of the ducts or lobules (referred to as "infiltrating" or "invasive"). It is not unusual for a single breast tumor to have combinations of these types, and to have a mixture of invasive and non-invasive cancer[(21)].

I-Ductal carcinoma in situ

Ductal carcinoma starts in the tubes (ducts) that move milk from the breast to the nipple. Most breast cancers are of this type[(10-14)]. Ductal carcinoma in situ (DCIS; intraductal carcinoma) is pathologically defined by the presence of carcinoma cells in well-defined ductal structures without penetration of the duct wall as seen by conventional light microscopic evaluation. Ductal carcinoma in situ can be subdivided many different ways from a clinical and pathologic perspective: palpable versus mammographic detection, architectural pattern (comedo, papillary, micropapillary, cribriform, and solid subtypes), presence or absence of comedo necrosis, nuclear grade, size, and margin status. Frequently mixed histologic subtypes may be seen. The variations in clinical and pathologic presentations and the differences in their natural histories suggest that intraductal carcinoma includes multiple subsets of disease, which in turn may require different treatments. The propensity for local recurrence is significantly greater after breast conservation treatment for comedo histologies, high-grade lesions, close or positive surgical margins, and younger patients[(22)].

Flat epithelial atypia is an alteration of mammary terminal duct lobular units that is considered to be a precursor to, or early stage in, the development of some forms of ductal carcinoma in situ. In multivariable analysis, features of ductal carcinoma in situ independently associated with flat epithelial atypia were micropapillary and cribriform patterns and absence of comedo necrosis. . Additionally, flat epithelial atypia was significantly associated with the presence of atypical ductal hyperplasia, lobular neoplasia, and columnar cell lesions in both univariate and multivariable analyses. These observations provide support for a precursor-product relationship between flat epithelial

atypia and ductal carcinoma in situ lesions that exhibit particular features such as micropapillary and cribriform patterns and absence of comedo necrosis[23].

II-Lobular carcinoma in situ

Lobular carcinoma starts in parts of the breast, called lobules, that produce milk[10-14]. Each breast contains hundreds of tiny lobules where milk is produced before and after childbirth. With lobular carcinoma in situ (LCIS), changes are found in the cells in the lining of the lobules or lobes of the breast. It is often present in both breasts. LCIS is more common in women who have not reached their menopause (change of life). However,it is not a cancer, but its presence means that there is a small increase in the risk of developing breast cancer later in life. Even so, most women with LCIS do not develop breast cancer[24].

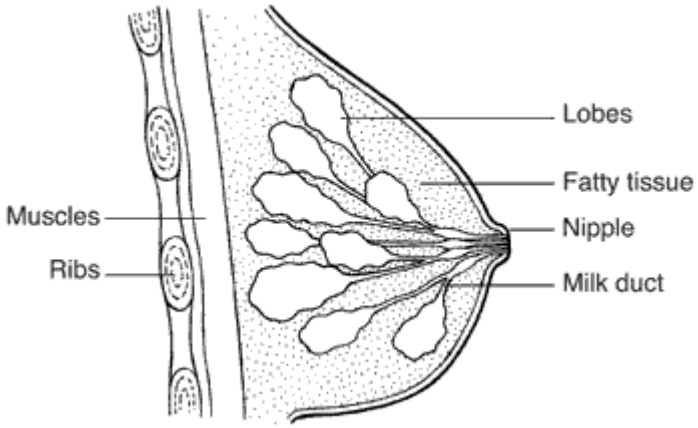

Fig (1): Internal structure of the breast

(LCIS) of the breast is generally considered an indicator for a bilaterally increased risk of invasive breast cancer (IBC). , LCIS represents both an indicator lesion for an increased risk of subsequent invasive breast cancer and in some cases a precursor of ILC[25].

Recent reports indicate that the incidence of lobular breast cancer is increasing at a faster rate than ductal breast cancer, which may be due to the differential effects of exogenous hormones by histology [26].

III-Invasive carcinoma:

A wide range of histological patterns is recognised in invasive carcinoma of the breast and four broad prognostic groups are recognised: the excellent prognosis group comprises tubular, cribriform, mucinous carcinomas; the good group tubular mixed, mixed ductal NST(non-specified tumor) /special type and classical lobular carcinoma; the average group mixed lobular, medullary and atypical medullary carcinoma and the poor group is composed of ductal NST, mixed ductal and solid lobular carcinoma understanding of the biology of breast cancer[20].

1-Invasive ductal carcinoma:

Invasive duct carcinoma may be associated with large irregular, rod or V shaped, pleomorphic or branching type calcifications that follow the distribution of the duct. Furthermore, analysis of the characteristics of the calcifications may help to predict the tumour size and grade, and presence of invasion. [27].

2-Invasive lobular carcinoma:

Invasive lobular carcinoma of the breast (ILC) is known to be substantially underestimated by mammography, which makes correct planning of treatment difficult. Magnetic resonance imaging (MRI)is a more accurate modality for determining tumour size in patients with ILC than mammography. The typical underestimation of lesion size by mammography can be prevented with the aid of MRI, without increasing the risk of lesion overestimation[28].

3-Tubular carcinoma:

Tubular carcinoma of the breast is a special type of breast carcinoma with a particularly favorable prognosis and this tumor is composed of distinct, well-differentiated tubular structures with open lumens that are lined by a single layer of epithelial cells. Tubular carcinoma constitutes less than 2% of all breast carcinomas , but because they tend to manifest as small lesions, they are found at a higher frequency, i.e., up to 7% in a series of small T1 breast cancers. Tubular carcinomas are often readily detectable mammographically because of their spiculate nature and the associated cellular stroma, and they were seen at higher frequencies of 9-19% in a mammographic screening series . . According to the new WHO classification, the diagnosis of pure tubular carcinoma (PT) is made when more than 90% of the tumor exhibits the tubular growth pattern. Tumors that are composed of between 50 to 90% of the tubular growth pattern and the other histologic subtypes should be regarded as a mixed type of tubular carcinoma (MT)[29].

4-Paget disease (PD):

Paget disease (PD) patients with Paget disease of the nipple, preoperative imaging to detect and evaluate the extent of an underlying malignancy can facilitate appropriate treatment planning. Negative preoperative imaging did not reliably exclude an underlying cancer, but the increased sensitivity of MRI detected otherwise occult disease. In the setting of negative mammography, MRI can facilitate treatment planning for patients with PD [30].

5-Basaloid carcinoma:

Basaloid carcinoma of the breast (BCB) is an unusual neoplasm composed of basal-type neoplastic cells similar to those found in adenoid cystic carcinoma (ACC), although lacking distinctive features such as a cribriform pattern, a dual neoplastic population (epithelial-myoepithelial/basaloid), and stromal deposits of basement membrane-like material . Microscopically, they featured sheets, nests, and cords of proliferating basaloid tumor cells with ovoid, hyperchromatic nuclei with inconspicuous nucleoli and scant

cytoplasm. No foci with characteristics of ACC were found in any of the tumors. Compared with ACC, BCB appears to be more aggressive and may entail a more guarded prognosis [(31)].

B-Tumor grade:

Histological grading refers to the semi-quantitative evaluation of the morphological structure of breast carcinomas. In the Nottingham method three characteristics of the tumour are evaluated, glandular differentiation, nuclear pleomorphism and mitotic counts. A numerical scoring system on a scale of 1-3 is used to ensure that each factor is assessed individually. Overall grade is assigned as follows: Grade 1: 3-5 points, Grade 2: 6-7 points, Grade 3: 8-9 points. There is a highly significant relationship between histological grade and prognosis; survival worsens with increasing grade. Histological grading has been shown to have good reproducibility and has been adopted for use in Europe, Australasia and the United States. When combined with pathological tumour size and lymph node stage into the Nottingham Prognostic Index there is excellent stratification for patient management [(20)].

Tumor grade is a system used to classify cancer cells in terms of how abnormal they look under a microscope and how quickly the tumor is likely to grow and spread. Many factors are considered when determining tumor grade, including the structure and growth pattern of the cells. The specific factors used to determine tumor grade vary with each type of cancer[(32)].

Histologic grade, also called differentiation, refers to how much the tumor cells resemble normal cells of the same tissue type. Nuclear grade refers to the size and shape of the nucleus in tumor cells and the percentage of tumor cells that are dividing.Tumor grade should not be confused with the stage of a cancer. Cancer stage refers to the extent or severity of the cancer, based on factors such as the location of the primary tumor, tumor size, number of tumors, and lymph node involvement (spread of cancer into lymph nodes) [(32)]. Histologic grading in breast cancer is based on the evaluation of 3 morphologic features (tubule formation, nuclear pleomorphism and mitotic count), is essentially describing proliferation and differentiation in breast cancer, and is considered an important prognostic factor for this disease [(33)].

Breast carcinomas are graded according to the "Nottingham modification of the Bloom-Richardson system" (SBR). Its objective was to evaluate a new grading system [the nuclear grade plus proliferation (N+P) system] for subjectivity, ease, and better representation of tumor biology. Its components are nuclear grade and automated proliferation index.The two systems were compared with each other and correlated with patients' overall survival, tumor size, angiolymphatic invasion, lymph node status, and biomarker status including estrogen receptor, progesterone receptor, p53, epidermal growth factor receptor, B-cell lymphoma 2 (BCL-2), and human epidermal growth factor receptor **2**(Her-2). Although there was an agreement between the two systems with histologic and prognostic parameters studied.Distinction among the different histologic grades for overall survival curves was better indicated by the N+P than the SBR system [(34)].