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A Study of Some Coagulation Parameters in Stages of Breast Cancer

A Thesis Submitted to the Council of the College of Medicine and the Committee of Postgraduate Studies of the University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master in Pathology

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بسم الله الرحمن الرحيم

((قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُو

الْأَلْبَابِ))

صدَقَ الله العلي العظيم

الزمر- اية 9

Certification of Supervision

We certify that this thesis entitled " Coagulation defects among stages of breast cancer " was prepared under our supervision at the College of Medicine/ Babylon University as a partial requirement for the master degree in pathology.

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Dedication

To my family for their eternal love and support…

To the souls of my beloved mother and father…

This thesis is dedicated to you…

Hawra

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My heart felt gratitude and thanks must be firstly offered to Allah for his merciful support and guidance to me to complete this study.

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Hawra Shakir Hadi

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List of Abbreviations

Ab	Antibody
ANOVA	Analysis of Variance
aPT	Activated partial thromboplastin
aPTT	Activated partial thromboplastin time
bFGF	Basic fibroblast growth factor
CBC	Complete blood count
CP	Cancer procoagulant
CT	Computed tomography
CVC	Central venous catheter
DCIS	Ductal Carcinoma in-Situ
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
ECM	Extracellular matrix
EDTA	Ethylene Diamine Tetraacetic Acid
ELISA	Enzyme-linked immunosorbent assay
EP tube	Eppendorf Tube
EPO	Erythropoietin
ER	Estrogen receptor
FDP	Fibrin degradation products
HRP	Horseradish peroxidase
IL-1/3	Interleukin 1/3
LCIS	Lobular Carcinoma in-Situ
min	Minute
mL	Milliliter
µL	Microliter
MMP	Metalloproteinase

MP	Plasma microparticles
MRI	Magnetic resonance imaging
N	Number
ng/mL	Nanogram per milliliter
nm	Nanomiter
NST	No Special Type
OD	Optical density
PA	Plasminogen activator
PAI	Plasminogen activator inhibitor
PAR	Protease-activated receptor
pg/mL	Picograms per milliliter
PL	Phospholipids
PR	Progesterone receptor
PT	Prothrombin time
SD	Standard deviation
SD card	Secure digital card
SPSS	Statistical Package for the Social Sciences
TAT	Thrombin-antithrombin complex
TF	Tissue factor
TILs	Tumor-infiltrating lymphocytes
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator
tPAI	Tissue plasminogen activator inhibitor
uPA	Urokinase plasminogen activator
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VTE	Venous thrombo-embolism

ABSTRACT

Background:

Breast cancer is most commonly diagnosed cancer and considered leading cause of cancer mortality among women. Breast cancers show considerable variation in the sense of their histological features and molecular alterations and such factors are known to influence the patient's outcome and clinical behavior.

Alterations of hemostasis commonly accompany the progression of malignant disease and every known component of the hemostatic mechanism may be affected by this disease process. Various laboratory signs of coagulation activation in cancer patients are caused by tumor growth, neoangiogenesis and affected organ dysfunction. Anticancer chemotherapeutic agents may further aggravate patient's general condition and precipitate coagulation disorders.

So, many efforts focus on finding reliable indicators that can help to predict the outcome and complications of breast cancer according to stage.

Aim of study:

- To assess the levels of tissue factor, tissue plasminogen activator, plasminogen activator inhibitor, D-dimer as well as coagulation profile in patients with breast cancer.
- Correlate the results with different stages of breast cancer to review them as possible prognostic factors.

Patients and Methods:

This is a case control study that included sixty- two Iraqi newly diagnosed breast cancer female patients who haven't started treatment yet. The patients are divided according to the stage of breast cancer (Stage I, II, III and IV). They were staged according to TNM staging system. From each patient blood samples were collected, and following investigations were done: D- dimer, prothrombin time (PT), activated partial thromboplastin time (aPTT) and ELISA assay for (tissue factor, tissue plasminogen activator and plasminogen activator inhibitor).

Results:

The patients' ages ranged from 28 to 84 years with the highest percentage in the (50- 59 years) age group. According to molecular subtypes, luminal- like breast cancer represent majority of patients (82.3%), basal-like breast cancer represent only (3.2%) and Her2 positive represent (14.5%) of total patients. According to histological subtypes, ductal histological subtype represents majority of patients (80.6%), lobular histological subtype represents (11.3%) and mixed histological subtype represent only (8.1%). According to stages of disease, stage I represents (11.3%), stage II represents (41.9%), stage III represents (24.2%) and stage IV represents (22.6%) of total cases.

The mean age (years) according to stages of breast cancer were: stage I (52.29 ± 11.61), stage II (54.42 ± 12.31), stage III (55.00 ± 12.83) and stage IV (58.50 ± 14.91). There were significant differences between medians of tissue factor (pg/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of tissue factor (pg/ml) in comparison to other stages.

The mean values of plasminogen activator inhibitor (ng/ml) according to stages of breast cancer were: stage I (0.39 ± 0.30), stage II (0.26 ± 0.27), stage III (0.23 ± 0.19) and stage IV (0.18 ± 0.22). There were no significant differences between means of Plasminogen activator inhibitor (ng/ml) according to stages of breast cancer.

The mean values of tissue plasminogen activator (ng/ml) according to stages of breast cancer were: stage I (21.12 ± 18.37), stage II (20.84 ± 15.54), stage III (16.00 ± 13.48) and stage IV (24.99 ± 16.62). There were no significant differences between means of Tissue plasminogen activator (ng/ml) according to stages of breast cancer.

The mean differences of prothrombin time (seconds) according to stages of breast cancer were: Stage I (15.54 ± 1.19), Stage II (14.71 ± 1.42), Stage III (14.53 ± 1.39), Stage IV (15.44 ± 1.18). No significant differences were found between prothrombin time means (seconds) regarding breast cancer stages.

The mean differences of partial thromboplastin time (seconds) according to stages of breast cancer were: Stage I (31.39 ± 4.43), Stage II (31.68 ± 4.41), Stage III (32.25 ± 4.23), Stage IV (34.54 ± 2.31). No significant differences were found between partial thromboplastin time means (seconds) regarding breast cancer stages.

The mean differences of D-dimer (ng/ml) according to stages of breast cancer were: Stage I (501.43 ± 228.65), Stage II (455.38 ± 135.48), Stage III (466.67 ± 154.86), Stage IV (1181.43 ± 595.44). There were significant differences between means of D-dimer (ng/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of D-dimer (ng/ml) in comparison to other stages of breast cancer.

Conclusions:

From the current study we can conclude the following:

1. Patients with breast cancer had higher serum tissue factor and D-dimer levels than normal ranges.
2. Tissue factor and D- dimer levels are significantly associated with more advanced breast cancer stage (stage IV).
3. No correlation was found between tissue plasminogen activator, plasminogen activator inhibitor, prothrombin time and partial thromboplastin time with stages of breast cancer.
4. Tissue factor and D- dimer are considered as prognostic factors in breast cancer regarding coagulation defects when linked to clinical stage and disease progression. Hence it can be used to predict outcome of coagulation defects in breast cancer and may be future therapeutic targets.

Chapter One

Introduction

1.1 Introduction

Breast cancer is the most frequently diagnosed cancer and the main cause of death from cancer in women. With a projected 2.3 million new cases, or 11.7% of all cancer cases worldwide in 2020, female breast cancer has surpassed lung cancer as the main cause of global cancer incidence in both sexes combined. As the sixth most common cause of cancer-related death worldwide, breast cancer affects 1 in 4 women and results in 1 in 6 cancer fatalities.[1]

In Iraq, Breast cancer established 22.2% of all cancer cases in 2020 and accounted for 37.9% of all new cancer cases among females of all ages. It is also the first malignancy to rank among all demographics. Breast cancer accounts for 15.3% of all cancer-related deaths, placing it at the first place.[2]

Breast cancer had the highest mortality rate among female cancer patients (4.79/100,000 female populations).[3]

The histological characteristics and molecular modifications of breast cancers vary widely, and these aspects are known to affect the patient's prognosis and clinical behavior.[4]

The two primary components of breast cancer treatment are locoregional therapy and systemic therapy; histological and molecular characteristics significantly influence treatment choices.[5]

Every known element of the hemostatic mechanism may be impacted by this disease process, and changes in hemostasis frequently accompany the development of malignant disease. Many patients with an active neoplasm will also experience clinical thrombosis or hemorrhage, but the majority of patients will show at least mild biochemical abnormalities in hemostasis.[6]

Malignancy-related hemostasis changes are undoubtedly complex, and the onset of clinical bleeding or thrombosis is the clinical culmination of a number of different hemostatic changes. The tumors that cause thrombi most frequently are mucinous adenocarcinomas. The sialic acid moiety of the released mucin has been demonstrated to be capable of starting coagulation in this malignancy by the apparent nonenzymatic activation of Factor X.[7]

Blood coagulation activation in cancer has a complex, multifaceted etiology. However, the function played by the production of tumor cell-associated clot-promoting characteristics is a distinctive aspect of malignancy. These characteristics cause the production of thrombin and fibrin, the activation of the clotting cascade, and the stimulation of platelets, leukocytes, and endothelial cells, causing them to display procoagulant cellular characteristics.[8]

Tumor growth, neoangiogenesis, and malfunction of the affected organs are the causes of several test indicators of coagulation activation in cancer patients. Chemotherapeutic chemicals used to treat cancer may make the patient's condition worse overall and hasten coagulation issues.[9]

The integral membrane protein known as tissue factor (TF), which is often kept isolated from the blood by the vascular endothelium, is essential for the beginning of blood coagulation. A perforating vascular injury exposes TF to blood and causes it to bind plasma factor VIIa. A chain of enzymatic events that result in clot formation and vascular sealing are started by the resultant complex.[10]

As a factor influencing the growth of tumors, tissue factor has drawn a lot of interest. Integrin function is regulated and protease-activated receptor-dependent tumor cell behavior is influenced by TF upon complex formation with its ligand, coagulation factor VIIa, which promotes tumor angiogenesis.[11]

Circulating blood cells in various pathological conditions express TF after being exposed to an inflammatory stimulus that causes intravascular clotting, vessel blockage, and thrombotic disease. Concerning the impact of TF structural characteristics, its appearance, and its function, many debates have surfaced.[12]

The enzyme known as tissue plasminogen activator (tPA), which catalyzes the transformation of plasminogen into plasmin, is in charge of dissolving clots. According to one theory, plasminogen activator contributes mostly to the catalytic production of plasmin, which then promotes extracellular matrix breakdown, cell invasion, and migration. Furthermore, the activation of dormant growth factors by plasmin may potentially contribute to the induction of cell proliferation.[13]

The polypeptide plasminogen activator inhibitor is produced by endothelial cells. By preventing the TF-VIIa complex, it functions as a natural inhibitor of the extrinsic route.[14]

A key factor in controlling fibrinolysis is plasminogen activator inhibitor-1 (PAI-1). A possible biomarker for it has been found. Plasminogen activator is inhibited by PAI-1, which in turn prevents fibrinolysis.[15]

In healthy people, fibrinolysis is an ongoing process that is essential for regulating coagulation and accelerating wound healing. Under physiological circumstances, the plasminogen activator (PA) system, in which tissue-derived and urokinase PAs (tPA and uPA, respectively), cleave circulating plasminogen to its active form, plasmin, is the main regulator of fibrinolysis. By cleaving cross-linked fibrin to create fibrin degradation products that are washed away by the flowing blood, plasmin breaks blood clots. The PA inhibitor (PAI-1) binds to and prevents plasminogen activation by both tPA and uPA, hence inhibiting this process.[16]

D-dimer is a soluble fibrin degradation product that is produced when the fibrinolytic system breaks down thrombi in an organized manner. D-dimer is a useful marker of activated coagulation and fibrinolysis, according to numerous studies.[17]

It was discovered that it was high in a variety of illnesses, including cancer, infection, and arrhythmias. Increased mortality is one of the negative effects of having high levels of D-dimers in venous thromboembolism.[18]

Plasma D-dimer concentrations rise via encouraging fibrin synthesis and fibrinolysis. Elevated plasma D-dimer levels have received a lot of attention recently in relation to various malignant cancers.[19]

Aim of study:

- To assess the levels of tissue factor, tissue plasminogen activator, plasminogen activator inhibitor, D-dimer as well as coagulation profile in patients with breast cancer.
- Correlate the results with different stages of breast cancer to review them as possible prognostic factors.

Chapter Two

Review of Literature

2.1 Breast cancer

2.1.1 Epidemiology of Breast Cancer

Breast cancer is the most frequently diagnosed cancer and the main cause of death from cancer in females. With a projected 2.3 million new cases, or 11.7% of all cancer cases worldwide in 2020, female breast cancer has surpassed lung cancer as the main cause of global cancer incidence in both sexes combined. As the sixth most common cause of cancer-related death worldwide, breast cancer affects 1 in 4 women and results in 1 in 6 cancer fatalities.[1]

In Iraq in 2020, Breast cancer is the most common form of cancer in all populations, accounting for 37.9% of all new instances of cancer among women of all ages and 22.2% of all cancer cases. Breast cancer accounts for 15.3% of all cancer-related deaths, placing it at the first place.[2]

Breast cancer was previously the second most prevalent cancer diagnosed globally in 2018 with 2.1 million women receiving a diagnosis, behind lung cancer closely at 11.6%. In the vast majority of nations (154 out of 185), the condition was the most often diagnosed cancer, and it is also the main reason for cancer deaths in more than 100 nations.[20]

Breast cancer was the highest prevalence among women in Iraq in 2018 (26.26/100,000 female population), accounting for 19.55% of all new cancer cases among females and 34.27% of all new cancer cases overall. Nearly a third of all cancers in women were breast cancer, followed by colorectal and cervical cancer.[2,21]

Breast cancer had the highest fatality rate among female cancer patients (4.79/100,000 female populations) in Iraq.[3]

2.1.2 Etiology and Risk Factors for Breast Cancer

1. **Non-modifiable Risk Factors:**

I- **Sex, Age and Ethnicity:** The postmenopausal years are when most breast cancers are discovered. Breast cancer, however, can strike at any age, from infancy to old life.[22,23]

Although this age group will obviously have competing complications that will affect survival results, the older women, postmenopausal women had the best prognosis since these patients tend to be the better differentiated, positive ER, screen-detected tumors presenting at a lower stage. Regarding tumors in young women (under the age of 35 or 40), previous studies had revealed that young age considered an independent risk factor in relation to a propensity for recurrence and metastases despite the more aggressive therapy, linked with the fact that these patients appear to have adverse histologic finding, delay diagnosis, and thus present at a later stage of disease.[24]

The incidence of triple-negative breast cancer is lowest among Asian American and Pacific Islander women, whereas it is more prevalent in African American women than in women of other racial or ethnic groups.[25]

Mortality rates are still high in more industrialized countries, but they are far higher in less developed ones due to a lack of early detection and access to medical care. Contrary to breast cancer mortality rates, breast cancer incidence has increased in Western Europe, where there are now more than 90 new cases per 100,000 women annually, compared to 30 per 100,000 in eastern Africa. At around 15 per 100 000, regions are fairly comparable, indicating later diagnosis and significantly shorter survival in eastern Africa.[26]

II- **Genetic Predisposition:** Due to hereditary mutations, about 5–10% of cases of breast cancer are connected to a genetic predisposition for the disease. This includes breast cancers linked to the susceptibility genes BRCA1 and BRCA2. The BRCA1 or BRCA2 gene mutation is only responsible for 15–25% of family breast cancer cases.[27]

Less than 1% of the population has these mutations, but they are more prevalent in some ethnic groups, such as Jews of Ashkenazi (Eastern European) heritage.[28]

The Li-Fraumeni and Cowden syndromes, as well as a number of other widespread genetic abnormalities, are other inherited disorders associated with a modestly increased risk of breast cancer.[29]

Early research suggested that women with the BRCA1 mutation had a lower overall survival rate if adjuvant therapy was not administered, but a large study of women carrying the BRCA1 and BRCA2 mutations revealed that their mortality rates from breast cancer were comparable to noncarriers. The status of mutations is not regarded as a standalone clinical outcome predictor at this time.[30,31]

III- **History of Previous Benign Breast Lesion and Other Cancers:** Breast cancer risk factors with a history of the disease include a personal history of the disease as well as a family history. A first-degree relative who has breast cancer puts women at a 2- to 3-times higher risk than the general population; the risk is further higher if the relative was diagnosed with the disease at a young age and/or has bilateral disease.[32]

The likelihood of contracting the disease increases if there are many first-degree relatives who already have it. In addition, a second-degree relative with breast cancer already increases your risk.[33]

Additionally, a history of prior benign breast conditions such atypical ductal hyperplasia and atypical lobular hyperplasia is linked with increase the chance for breast cancer developing. Breast cancer incidence is 4–5 times higher in proliferative lesions with atypia than the average risk.[34]

Additionally, compared to women with the least dense breasts, those with very high breast density had a 4–6 fold higher risk of breast cancer.[35]

IV- Menstrual History: Breast cancer risk factors include early menarche, which occurs at around 12 years of age, late menopause, which occurs at around 55 years of age and later, and prolonged exposure to reproductive hormones.[36,37]

2. Modifiable Risk Factors:

I- Socio-Demographic Information:

Geographical variations, place of residence, and degree of education are sociodemographic characteristics that affect a woman's chance of developing breast cancer. The incidence of breast cancer is more common in some areas or nations than others; its incidence is high in North America and northern Europe, intermediate in southern Europe and Latin America, and low in the majority of Asian and African nations (though it has been rising quickly in some of these nations in recent years).[22]

Because urban locations are frequently associated with westernized behaviors and lifestyles, more affluent women who typically reside there face a twice the chance for breast cancer development compared with those who reside in rural areas.[38]

Higher educated women have a higher risk of developing breast cancer. Socioeconomic position is significantly influenced by education; a number of reproductive, lifestyle, and behavioral factors, including parity, age at first birth, physical activity, and food, may have an impact on breast cancer risk.[39]

II- Lifestyle Behaviors:

Physical activity level, alcohol consumption, and smoking are lifestyle factors that affect a woman's risk of developing breast cancer. Regular physical activity reduces breast cancer risk by 10–20% compared to inactive women, with postmenopausal women being more likely to benefit than premenopausal women.[40]

High alcohol intake of approximately 10 g/day of ethanol may up the risk by 10%.[41]

According to a recent meta-analysis conducted by American Cancer Society experts, women who now smoke are 12% more likely for breast cancer development than those who have never smoked.[42]

Strong evidence supports, the risk of breast cancer slightly higher in female who smoke cigarettes, and the data supporting a slight increase in the risk of passive smoking is now more significant than it was previously.[43]

III- Reproductive Factors:

Parity, age at first pregnancy, and nursing are reproductive factors that affect a woman's risk of developing breast cancer. Old age at first birth and late menopause are associated with an increased risk. Lower lifetime risk for breast cancer is linked to younger ages for first pregnancies. But pregnancy has two distinct effects: a short-term temporary increase in the risk of breast cancer and a long-term preventive benefit.[22,44]

Postmenopausal women with a plasma hormone profile that is hyperandrogenic are more likely to develop breast cancer.[22,45]

Breastfeeding for at least 4 months has been linked to a lower risk of breast cancer in parous women. Compared to women of a similar age, single and nulliparous (without children) married women have a slightly higher risk of breast cancer, while married women who already have the disease have a 15% lower risk of dying from it.[46]

Everyone agrees that during pregnancy or lactation, women who appear with breast cancer tend to be typically aggressive. An overall worse prognosis is linked to a tumor with high HER2 expression and low hormone receptor expression .[47]

IV- Other risk factors:

Studies on women who have undergone high-dose radiation therapy to the chest and survivors of atomic bombs have shown a link between radiation exposure and breast cancer, particularly for those who were first exposed when they were younger.[48]

Studies have shown that women with night shifts work for many years had a little increase risk of breast cancer; one possible explanation for the elevated risk among these workers is their exposure to light at night. Shift work and the light at night may also considered as breast cancer risk.[49]

2.1.3 Breast Cancer histopathological types:

The two most prevalent histologic types of the disease, ductal carcinoma and lobular carcinoma, which account together about 90% of all breast cancer cases, are frequently clinically classed as cancer cases depending on the origin of the disease.[50]

Breast carcinomas are classified into several distinct subgroups in the most recent WHO classification (2019), including:

- **Epithelial tumors**

- **Invasive breast carcinoma**
    - Infiltrating duct carcinoma (NOS), 8500/3
    - Oncocytic carcinoma, 8290/3
    - Lipid rich carcinoma, 8314/3
    - Glycogen rich carcinoma, 8315/3
    - Sebaceous carcinoma, 8410/3
    - Lobular carcinoma NOS, 8520/3
    - Tubular carcinoma, 8211/3
    - Cribriform carcinoma NOS, 8201/3
    - Mucinous adenocarcinoma, 8480/3
    - Mucinous cystadenocarcinoma NOS, 8480/3
    - Invasive micropapillary carcinoma of breast, 8507/3
    - Metaplastic carcinoma NOS, 8575/3

- **Rare and salivary gland type tumors**
    - Secretory carcinoma, 8502/3
    - Acinar cell carcinoma, 8550/3
    - Mucoepidermoid carcinoma, 8430/3
    - Polymorphous adenocarcinoma, 8525/3
    - Adenoid cystic carcinoma, 8200/3
        - Classic adenoid cystic carcinoma
        - Solid basaloid adenoid cystic carcinoma
        - Adenoid cystic carcinoma with high grade transformation
    - Tall cell carcinoma with reversed polarity, 8509/3

- **Neuroendocrine neoplasms**
    - Neuroendocrine tumor, NOS, 8240/3
    - Neuroendocrine tumor, grade 1, 8240/3
    - Neuroendocrine tumor, grade 2, 8249/3
    - Neuroendocrine carcinoma NOS, 8246/3
    - Neuroendocrine carcinoma, small cell, 8041/3
    - Neuroendocrine carcinoma, large cell, 8013/3

- **Epithelial - myoepithelial tumors**
    - Pleomorphic adenoma, 8940/0
    - Adenomyoepithelioma NOS, 8983/0
    - Adenomyoepithelioma with carcinoma, 8983/3
    - Epithelial-myoepithelial carcinoma, 8562/3

- Non invasive lobular neoplasia
    - Atypical lobular hyperplasia
    - Lobular carcinoma in situ NOS, 8520/2
        - Classic lobular carcinoma in situ
        - Florid lobular carcinoma in situ
    - Lobular carcinoma in situ, pleomorphic, 8519/2

- Ductal carcinoma in situ (DCIS)
    - Ductal carcinoma, non-infiltrating, NOS, 8500/2
        - DCIS of low nuclear grade
        - DCIS of intermediate nuclear grade
        - DCIS of high nuclear grade

- **Benign epithelial proliferations and precursors**
    - Usual ductal hyperplasia
    - Columnar cell lesions including flat epithelial atypia
    - Atypical ductal hyperplasia

- Adenosis and benign sclerosing lesions
    - Sclerosing adenosis
    - Apocrine adenoma, 8401/0
    - Microglandular adenosis
    - Radial scar / complex sclerosing lesion
- **Papillary neoplasms**
    - Intraductal papilloma, 8503/0
    - Ductal carcinoma in situ, papillary, 8503/2
    - Encapsulated papillary carcinoma, 8504/2
    - Encapsulated papillary carcinoma with invasion, 8504/3
    - Solid papillary carcinoma in situ, 8509/2
    - Solid papillary carcinoma with invasion, 8509/3
    - Intraductal papillary adenocarcinoma with invasion, 8503/3

- Adenomas
    - Tubular adenoma NOS, 8211/0
    - Lactating adenoma, 8204/0
    - Duct adenoma NOS, 8503/0

- **Mesenchymal tumors**

- Vascular tumors
    - Hemangioma NOS, 9120/0
        - Perilobular hemangioma
        - Venous hemangioma
        - Cavernous hemangioma
        - Capillary hemangioma
    - Angiomatosis
    - Atypical vascular lesion, 9126/0
        - Lymphatic atypical vascular lesion resembling lymphangioma
        - Vascular atypical vascular lesion resembling hemangioma
    - Postradiation angiosarcoma, 9120/3
        - Epithelioid angiosarcoma
    - Angiosarcoma, 9120/3
        - Epithelioid angiosarcoma

- Fibroblastic and myofibroblastic tumors
    - Nodular fasciitis, 8828/0
    - Myofibroblastoma, 8825/0
    - Desmoid type fibromatosis, 8821/1
    - Inflammatory myofibroblastic tumor, 8825/1

- Peripheral nerve sheath tumors
    - Schwannoma NOS, 9560/0
    - Neurofibroma NOS, 9540/0
    - Granular cell tumor NOS, 9580/0
    - Granular cell tumor, malignant, 9580/3

- Smooth muscle tumors
    - Leiomyoma NOS, 8890/0
        - Cutaneous leiomyoma
        - Leiomyoma of the nipple and areola
    - Leiomyosarcoma NOS, 8890/3

- Adipocytic tumors
    - Lipoma NOS, 8850/0
    - Angiolipoma NOS, 8861/0
    - Liposarcoma NOS, 8850/3

- Other mesenchymal tumors and tumor-like conditions
    - Pseudoangiomatous stromal hyperplasia

- **Fibroepithelial tumors**
    - Fibroadenoma NOS, 9010/0
    - Phyllodes tumor NOS, 9020/1
        - Periductal stromal tumor
    - Phyllodes tumor, benign, 9020/0
    - Phyllodes tumor, borderline, 9020/1
    - Phyllodes tumor, malignant, 9020/3
    - Hamartoma

- **Tumors of the nipple**
    - Nipple adenoma, 8506/0
    - Syringoma NOS, 8407/0
    - Paget disease of the nipple, 8540/3
- **Malignant lymphoma**
    - Diffuse large B cell lymphoma NOS, 9680/3
    - Burkitt lymphoma NOS/Acute leukemia, Burkitt type, 9687/3
        - Endemic Burkitt lymphoma
        - Sporadic Burkitt lymphoma
        - Immunodeficiency associated Burkitt lymphoma
    - Breast implant associated anaplastic large cell lymphoma, 9715/3
    - Mucosa associated lymphoid tissue lymphoma, 9699/3
    - Follicular lymphoma NOS, 9690/3

- **Metastatic tumors**
- **Tumors of the male breast**
    - Gynecomastia
    - Carcinoma
        - Invasive carcinoma, 8500/3
        - In situ carcinoma, 8500/2.[51]

Noninvasive lesions (In-situ carcinoma)

I- Ductal Carcinoma in-Situ (DCIS): Since the basement membrane is typically unharmed, DCIS develops inside the lactiferous duct lumen and is confined to the duct in which it started. As a result, the cancer is contained and does not spread to any nearby healthy breast tissue.[52]

II- Lobular Carcinoma in-Situ (LCIS): is accidentally discovered during breast pathological examination and is not typically detected during mammography by symptoms of tumor or calcification.[53]

Invasive Carcinoma:

I- Invasive Ductal Carcinoma: (No Special Type- NST): The majority of invasive breast cancers, between 70 and 75 percent, are invasive ductal carcinomas. Due to the fact that the majority of ductal carcinomas lack distinctive histological characteristics, they are referred to as NSTs. Invasive ductal carcinoma can develop from DCIS because cells start by destroying the basement membrane.[54,55]

II- Invasive Lobular Carcinoma: estimated to be about 10-14% of invasive breast carcinomas.[55]

III- Other types: including mucinous, tubular, inflammatory, medullary features, papillary carcinomas, apocrine carcinoma, juvenile (secretory) carcinoma, metaplastic carcinoma, adenoid cystic carcinoma, micropapillary, carcinoma with neuroendocrine differentiation and Paget's disease.[56]

2.1.4 Breast cancer histological grade (by Nottingham Grading System assessment)

It is based on:

1. Nuclear grade:
    - Small, regular (1.5-2x RBC diameter) = 1
    - Moderated variability = 2
    - Marked variation (>2.5x RBC diameter) = 3
2. Tubule formation:
    - Majority of tumor - tubules >75% = 1
    - Moderate - 10% to 75% = 2
    - Minimal <10% = 3
3. Mitotic rate:
    - 0-5 mitosis/10 HPF = 1
    - 6-10 mitosis/10 HPF = 2
    - >11 mitosis/10 HPF = 3

- Grade 1 or well differentiated (score 3, 4, or 5). The cells are slower-growing, and look more like normal breast cells.
- Grade 2 or moderately differentiated (score 6, 7). The cells are growing at a speed of and look like cells somewhere between grades 1 and 3.
- Grade 3 or poorly differentiated (score 8, 9). The cancer cells look very different from normal cells and will probably grow and spread faster.[57]

2.1.5 Breast cancer molecular classification:

- **Luminal A or HR+/HER2- (HR-positive/HER2-negative):** The majority of newly diagnosed instances of breast cancer fall under this molecular category, which accounts for around half of all cases. This subtype's immunohistochemistry profile was identified as ER+ (1%), high PR expression (20%), HER2- (10%), and low Ki-67 (14%) levels. This subtype typically exhibits less lymph node involvement, a more passive clinical history, and a very favorable prognosis.[58,59]

- **Luminal B or HR+/HER2+ (HR-positive/HER2-positive):** This can be divided immunophenotypically into Luminal B (HER-): ER+ (≥1%), PR- (<20%), HER2- (≤10%) and high levels of Ki-67 (≥20%); or Luminal B (HER2+): ER+ (≥1%), HER2+ (>10%) and any level of PR and Ki-67.[58,59]
- **Triple-negative or HR-/HER2- (HR/HER2-negative):** These constitutes about 10-20% of cases breast cancer, which characterized by the lack of expression of the hormone receptors ER (<1%) and PR (<20%) and the oncoprotein HER2 (≤10%).[58,59]
- **HER2-positive:** It represents 15-20% of newly diagnosed breast cancer cases. They are characterized by a high expression of HER2 (>10%), negative ER (<1%) and PR (<20%) receptors, and high expression of Ki-67 (>20%).
- A fifth subtype, called **normal-like breast cancer**, resembles luminal A.[58,59]

2.1.6 Breast Cancer Diagnosis

Breast cancer diagnosis requires a comprehensive approach. Screening imaging techniques like mammography, magnetic resonance imaging (MRI), or ultrasound are used to find the majority of breast abnormalities. more diagnostic testing, such as physical exams, lab tests, and more imaging examinations, are monitored if an anomaly is found. The aim of the various tests is to establish if the lesion is benign or malignant, and if so, to establish the stage and grade of cancer in order to evaluate the overall clinical consequences and prognosis and forecast how a patient will respond to specific therapy.[52]

A proper diagnostic evaluation is necessary for women who are exhibiting breast symptoms or changes, such as a lump, localized pain, nipple symptoms, or skin abnormalities. A triple test, consisting of a clinical

examination, imaging (often mammography and/or ultrasonography), and (needle biopsy and/or cytology), is used to diagnose breast cancer.[60]

2.1.7 Staging of breast cancer patients

Based on the evaluation of three factors, the TNM staging method depicts the anatomical extent of the disease:

T- the extent of the primary tumor.

N- the absence or presence and extent of regional lymph node metastasis.

M- the absence or presence of distant metastasis.

Numbers are added to these components to indicate the extent of malignant disease (T0, T1, T2, T3, T4, N0, N1, N2, N3, M0, M1).[61,6]

Breast cancer TNM classification is viewed in details in table (2.1).

Table 2.1: Breast cancer TNM classification[79]

		Stage	Primary tumour (T)*	Regional lymph node status (L)	Distant metastasis (M)
T- Tumour		**0**	Tis	N0	M0
T1	Tumour ≤ 2 cm	**I**	T1	N0	M0
T2	Tumour ≥ 2 cm but ≥ 5 cm		T0	N1	M0
T3	Tumour ≥ 5 cm	**IIA**	T1	N1	M0
T4	Tumour of any size with direct extension to chest wall or skin		T2	N0	M0
N- Lymph node		**IIB**	T2	N1	M0
N0	No cancer in regional node		T3	N0	M0
N1	Regional movable metastasis	**III A**	T0	N2	M0
N2	Non-movable regional metastases		T1	N2	M0
N3	Cancer in the internal mammary lymph nodes		T2	N2	M0
M- Metastasis			T3	N1/N2	M0
M0	No distant metastases	**III B**	T4	Any N	M0
M1	Distant metastases	**III C**	Any T	N3	M0
		IV	Any T	Any N	M1

Criteria for staging breast tumours according to the UICC ICD-10 TNM classification.

*Size measurements are for the tumour's greatest dimension.

2.1.8 Prognostic factors of breast cancer

Numerous clinical and pathologic characteristics have an impact on the prognosis of breast cancer:

1. Non-modifiable Factors:

I- Age: Because their tumors are more likely to be better differentiated, ER-positive, screen discovered, and appear at a younger stage, older postmenopausal women have the best prognosis. Previous studies had indicated that young age considered as an independent risk factor related to tendency for recurrence and metastases in cancers that develop in young women (35 or 40).[63]

II- Genetic Predisposition (BRCA): Mutation status is not currently thought to be a reliable predictor of clinical outcome.[18]

Gene expression profiling: Numerous studies have described the use of tumor gene signatures chosen by microarray analysis to classify patients into useful prognostic/predictive groups that may aid in the choice of therapy.[64]

III- Pathological factors:

- Size.
- Site: There was no relationship has been found in most studies between the prognosis and primary tumor quadrant location.[65]
- Histological type: There was no significant prognostic difference between the ordinary invasive ductal and the invasive lobular carcinoma.[53]
- Microscopic grade: There was correlation between cytology and architecture with prognosis.[31]
- Metastases of lymph node: considered as one of most important prognostic factors.[34]

- Tumor-infiltrating lymphocytes (TILs): Emerging data indicate that patients with breast carcinomas demonstrating a prominent lymphocytic reaction have a better response to neoadjuvant chemotherapy than those tumors without.[65]
- Skin invasion: dermal lymphatic vessels invasion as a determinant picture of "inflammatory carcinoma" is a particularly considered as bad prognostic sign.[60]
- Local recurrence: blood vessel emboli and lymphatic tumor emboli within the breast is associated with distant recurrence risk.[60]
- Paget disease: its presence or absence in invasive ductal carcinoma had no prognostic relevance.[55]
- Hormonal receptors and cell proliferation: ER-positive tumors patients have longer disease-free survival than others, while HER2 amplification is an excellent predictor of response to trastuzumab.[55]

2. Modifiable Factors:

I. Reproductive Factors:

- There is general agreement that carcinoma of the breast manifesting during pregnancy or lactation is generally an aggressive tumor.
- There is no strong proof that prior usage of oral contraceptives affects the development or prognosis of people with breast cancer.[66]

II. Therapy Related: Type of therapy and surgical margins (a higher risk of ipsilateral tumor recurrence is associated with microscopically positive surgical margins in specimens from conservative breast excisions).[67,68]

2.2 Hemostatic defects associated with malignancy

It is now commonly acknowledged that cancer and abnormal hemostasis have a close relationship. Patients with numerous malignancies, such as pancreatic, colorectal, breast, and gastric cancer, frequently experience thrombosis, or Trousseau syndrome.

Components of the coagulation cascade are reciprocally influenced by the development of cancer. Transmembrane receptor tissue factor (TF), the main coagulation initiator, has drawn a lot of interest as a role in the development of tumors.

Both in vitro and in mice models of tumor angiogenesis, TF modulates integrin activity and influences protease-activated receptor-dependent tumor cell behavior on complex formation with its ligand, coagulation factor VIIa.[11,37]

Patients with hematologic malignancies typically experience abnormal hemostasis, which can result in both hemorrhagic and thrombotic adverse effects. A significant difficulty is the rapid identification and treatment of such complications, which have an adverse effect on patients' morbidity and mortality.[69]

2.2.1 Cancer-Related Thrombosis

Cancer patients are at an increased risk of developing venous thrombo-embolism (VTE), and the risk varies depending on the type of cancer and the stage of the disease. The pathophysiology of VTE linked to cancer is complicated and involves risk factors related to therapy, disease, and demographics.

The risk of VTE is increased by hospitalization, surgery, and other treatments (such as chemotherapy, hormonal treatments, or immunosuppressive drugs that used with chemotherapy). VTE burden is decreased and outcomes are improved in cancer patients who are at high risk of thrombosis because to effective VTE prophylaxis.[70,71]

2.2.2 Epidemiology of thrombosis in malignancy

According to estimates, 1 in 200 cancer patients will develop VTE, and having an active malignancy increases their likelihood of developing VTE. Overall, cancer is linked to 18% to 29% of all VTE incidents in the general population.[72]

In a previous study, 5,451 patients hospitalized with objectively proven DVT, 39% of them having cancer; of these, about 62% had cancer that was still present and 38% had cancer that had previously existed. Thrombosis risk in patients with cancer was further elevated by hospitalization, anticancer surgery, and vigorous treatment plans, notably chemotherapy.[72]

2.2.3 Risk of VTE associated with cancer

Previous studies depend on hospital discharge have reported incidence of VTE about 0.6% to 7.8% in cancer patients, more than twice of patients without cancer. Studies have demonstrated that VTE risk increased between 2-7-fold in patients with cancer. However, retrospective studies that only include symptomatic or objectively confirmed VTE events are likely to underestimate VTE incidence of patients with cancer. A high frequency of undiagnosed pulmonary embolism has been found in cancer patients undergoing routine-staging CT scans.[73]

Compared to persons without thrombosis, cancer patients who have a VTE episode have a poor prognosis for survival. In compared to localized tumors, advanced metastatic cancer is linked to a higher incidence of VTE.[73]

Effect of anticancer treatments on the incidence of VTE:

There are many anticancer treatments associated with increased risk of VTE, such as:

- Surgical procedures.
- Nonsurgical treatments, that include: chemotherapy, hormonal therapy, antiangiogenic agents, and supportive therapy (e.g., recombinant human erythropoietin)
- Use of a central venous catheter (CVC).[74]

2.2.4 Pathophysiology

Cancer patients can exhibit a variety of hemostatic diseases, such as thrombosis, bleeding, and DIC (disseminated intravascular coagulation), or they may merely show a hemostatic anomaly that is detected through laboratory testing and reflects an in vivo hypercoagulable condition.[75]

2.2.4.a The Hypercoagulable State

The fundamental idea is that the clotting system is systemically active in cancer patients, along with coagulation factor consumption and fibrinolysis activation. Up to 90% of cancer patients who have metastases and about 50% of all cancer patients have abnormalities in one or more common coagulation markers.[76]

The detection of minute changes in the hemostatic system has been made possible by the development of novel laboratory procedures with greater sensitivity. Patients with cancer typically have consistently high plasma levels of thrombin-antithrombin (TAT) complex and D-dimer, which show existence of in vivo thrombin production, fibrin formation, and fibrinolysis. With the beginning of anticancer medicines, these anomalies may get worse.[77]

2.2.4.b Hemostatic Markers and Risk of VTE

The usefulness of serial measures of hemostatic indicators for predicting the occurrence of VTE in cancer patients has only been partially examined in prospective trials. Presurgical TAT complex levels were revealed to be a significant predictor of postoperative DVT in one such research of patients having surgery for abdominal malignancy. A P-selectin level above the 75th percentile is predictive of thrombosis in cancer patients, and high level of D-dimer characterizes the highest VTE risk in cancer patients, as mentioned by Vienna Cancer and Thrombosis Study (CATS). Other studies have shown that platelet count >350,000/L before starting chemotherapy is predictive of subsequent thrombosis formation during chemotherapy.[78]

The measurement of plasma microparticles (MPs), tiny vesicles released by platelets, leukocytes, and endothelium in response to cell activation, is now possible thanks to advancements in laboratory techniques. The MPs have a diameter of less than 1 m, parental cell antigens are present on their surface, and they have procoagulant characteristics. Higher concentrations of tissue factor (TF) and the anionic plasma membrane phospholipid phosphatidylserine are associated to the procoagulant capacity. According to studies, patients with breast cancer had higher levels of MP produced from platelets than those with benign breast tumors. It is predicted that there will be

a significantly higher incidence of VTE because TF-bearing MPs are enhanced in about 60% of cancer patients with VTE and in about 27% of patients without VTE.[70,71]

2.2.5 The pathogenesis of the hypercoagulable state

The host cell inflammatory response and the prothrombotic characteristics unique to cancer cells both play a significant role in the pathophysiology of the cancer-associated hypercoagulability (Figure 2.1). The most significant procoagulant protein expressed by cancer cells is tissue factor (TF), which along with other procoagulant features of cancer tissue significantly contributes to the procoagulant phenotype of malignant cells. [79,80]

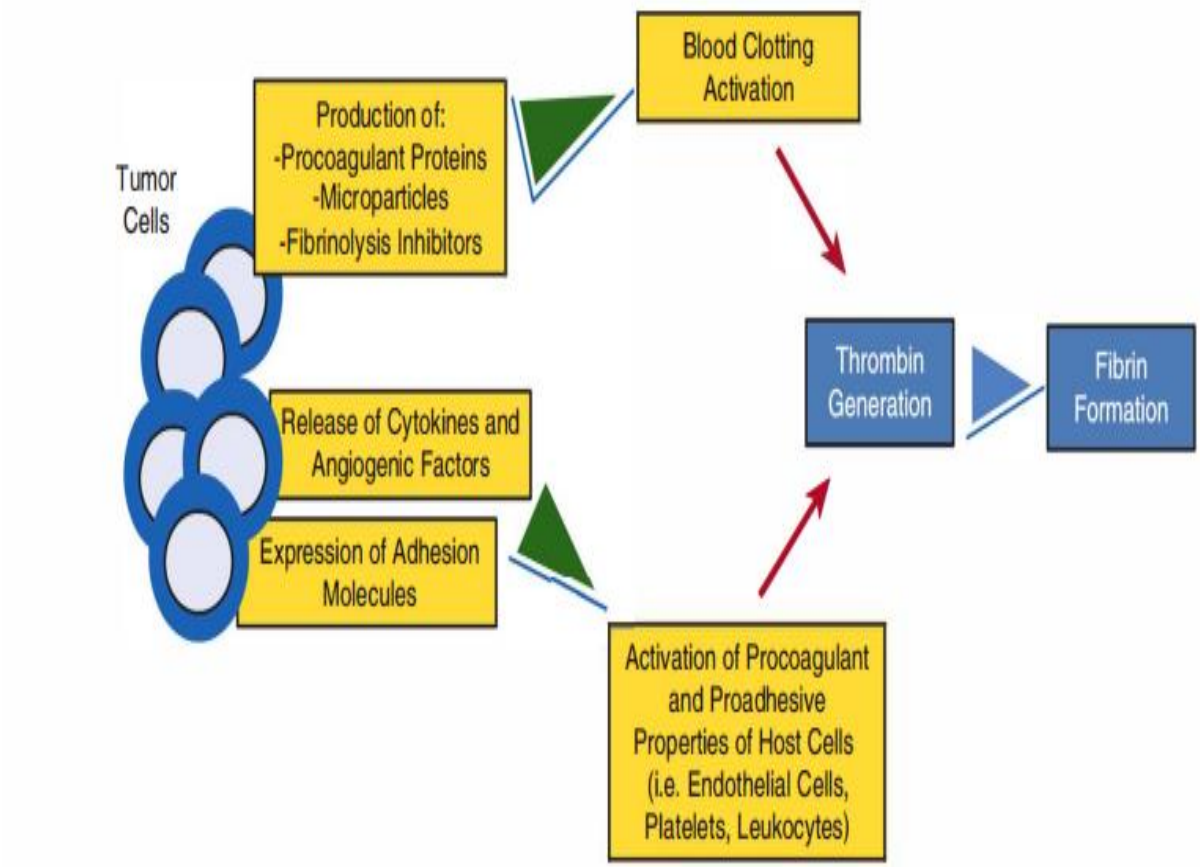


Figure 2.1: Principal prothrombotic mechanisms associated with cancer cells[81]

2.2.5.A Activation of the Hemostatic System by Tumor Cells

There are many complex interactions between malignant cells and coagulation that change the hemostatic balance toward a procoagulant state by several ways.

2.2.5.B Tumoral Production of Hemostatic Factors

1. Tissue plasminogen activator

Plasminogen is synthesized and secreted by the liver. In processes, such as wound healing and tissue remodeling that occurs in the tumor microenvironment, uPA is the predominant form of plasminogen activator, whereas tPA is the primary activator in the circulation. While tPA is found in the ECM of most tissues, uPA is localized to cell surfaces. The activities of both tPA and uPA are regulated by plasminogen activator inhibitor (PAI)-1 and PAI-2, whereas the activity of plasmin itself is inhibited by α2-antiplasmin and α2-macroglobulin. Overall, a complicated interplay between the plasminogen activators and inhibitors determines the extent of tissue remodeling, fibrinolysis, and tumor invasion and metastasis. In the tumor microenvironment, this is further complicated by the fact that the expression of the activators and inhibitors are not restricted to tumor cells alone, but the stromal cells are also involved in the expression and secretion of activators and inhibitors. [82]

The tPA is reported to show an improved chemotherapeutic activity of anticancer drugs by the degradation of fibrin in tumor blood vessels and matrix in mouse models. These results showed that treatment with tPA led to the decompression of blood vessels and improved the perfusion of anticancer drugs into tumors. However, tPA exhibits an extremely short half-life (<5 min) when used alone. The decomposition of fibrin in tumors by tPA may

be therapeutically effective if the half-life of tPA is prolonged by delivery approaches, such as tPA encapsulated nanoparticles. [83]

2. Plasminogen activator inhibitor (PAI-1)

Migration and invasion of cancer cells constitute fundamental processes in tumor progression and metastasis. Migratory cancer cells commonly upregulate expression of PAI-1, and PAI-1 correlates with poor prognosis in breast cancer. However, mechanisms by which PAI1 promotes migration of cancer cells remain incompletely defined. [84]

PAI-1 is associated with thrombosis in a variety of diseases, including obesity, diabetes, and metabolic syndrome. It reduces the generation of plasmin by inhibiting tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). PAI-1 circulates in blood in 3 different forms: active; latent inactive; or complexed with either tPA, uPA, or vitronectin. Different assays can be used to measure either total or active PAI-1 in plasma. [85]

Both tissue-type and urokinase-type plasminogen activators, as well as PAI-1 and PAI-2, are present in tumor cells. On tumor cells, certain receptors help with the assembly of the fibrinolytic building blocks, which facilitates the activation of the fibrinolytic cascade. Blast cell production of these activities may contribute to bleeding symptoms in leukemia patients. On the other hand, patients with solid tumors have been reported to have a malfunction in the synthesis of normal plasma fibrinolytic activity, which could be caused, for instance, by increased PAI-1 production. This could be another method by which these patients acquire VTE.[86]

3. Inflammatory Cytokines

Proinflammatory (TNF-a, IL-1/3) and proangiogenic (VEGF, basic fibroblast growth factor [bFGF]) cytokines are produced and released by tumor cells. Related to host's inflammatory response for tumor, inflammatory tissues also contribute to the overproduction of cytokines. The majority of these cytokines cause endothelial cells and monocytes to display procoagulant phenotypes. In response to cytokines, endothelial cells produce more PAI-1, endothelial cell surface TF expression is upregulated, and thrombomodulin expression is downregulated. Thrombomodulin is a potent anticoagulant that interacts with thrombin for activation naturally occurring anticoagulant protein.[87]

4. Adhesion molecules

The direct contact of these cells with host cells, such as endothelial cells, platelets, and leukocytes, is made possible by the production of tumor cell-surface adhesion molecules and/or their receptors. To encourage localized clotting activation to arterial wall and to initiate the thrombus formation, attachment of tumor cells to vascular endothelial cells is important. Adhesion can happen directly or with granulocyte assistance. Different routes of adhesion molecules expressed by different tumor types mediate the contact between tumor cells and endothelial cells. Related to the individual adhesive qualities of both endothelium and tumor cells, the interaction in flow conditions involves rolling and firm attachment. While some tumor cell types adhere without rolling via vascular cell-adhesion molecules, others adhere without rolling using E-selectin.[88,89]

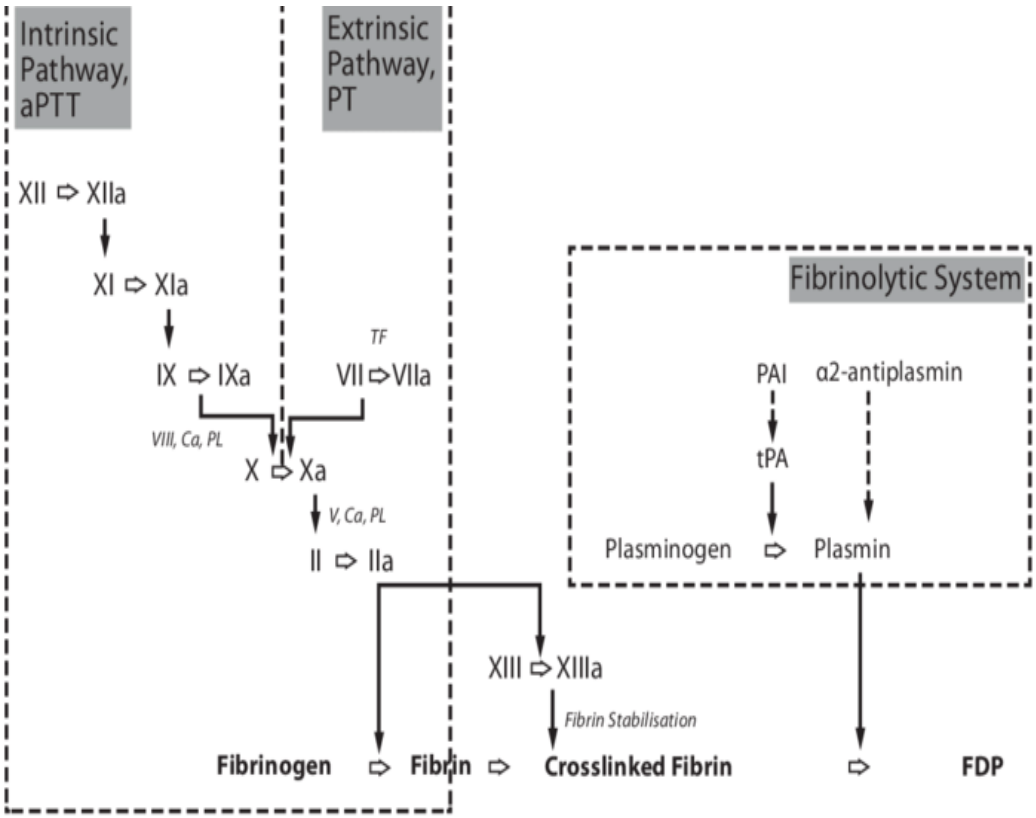
5. Procoagulant Proteins

Cancer procoagulant is a cysteine protease that, in the absence of FVIIa, directly activates FX. Malignant cells produce CP, and extracts from several tumors have been discovered to contain evidence of its action.

Although CP is almost exclusively present in cancerous cells, there is little evidence to support its use as marker for tumor or as clinical thrombosis predictor factor. Patients with various cancers, including pancreatic and breast adenocarcinomas, colorectal cancer, and acute leukemia, have been found to have circulating MPs in their plasma. By exposing phosphatidylserine and procoagulant proteins like TF, MPs aid in the formation of intravascular thrombin and may account for the elevated TF level found in the blood of cancer patients. The development of VTE in humans is highly correlated with increased MP-associated TF activity.[8,81]

2.2.6 Role of the coagulation system in progression of tumor:

The coagulation cascade is illustrated in (Figure 2.2), and the role of the coagulation system in progression of tumor is explained in (Figure 2.3).



PAI: plasminogen activator inhibitor, tPA: tissue plasminogen activator, FDP: fibrin degradation product, TF: tissue factor

Figure 2.2: The coagulation cascade[90]

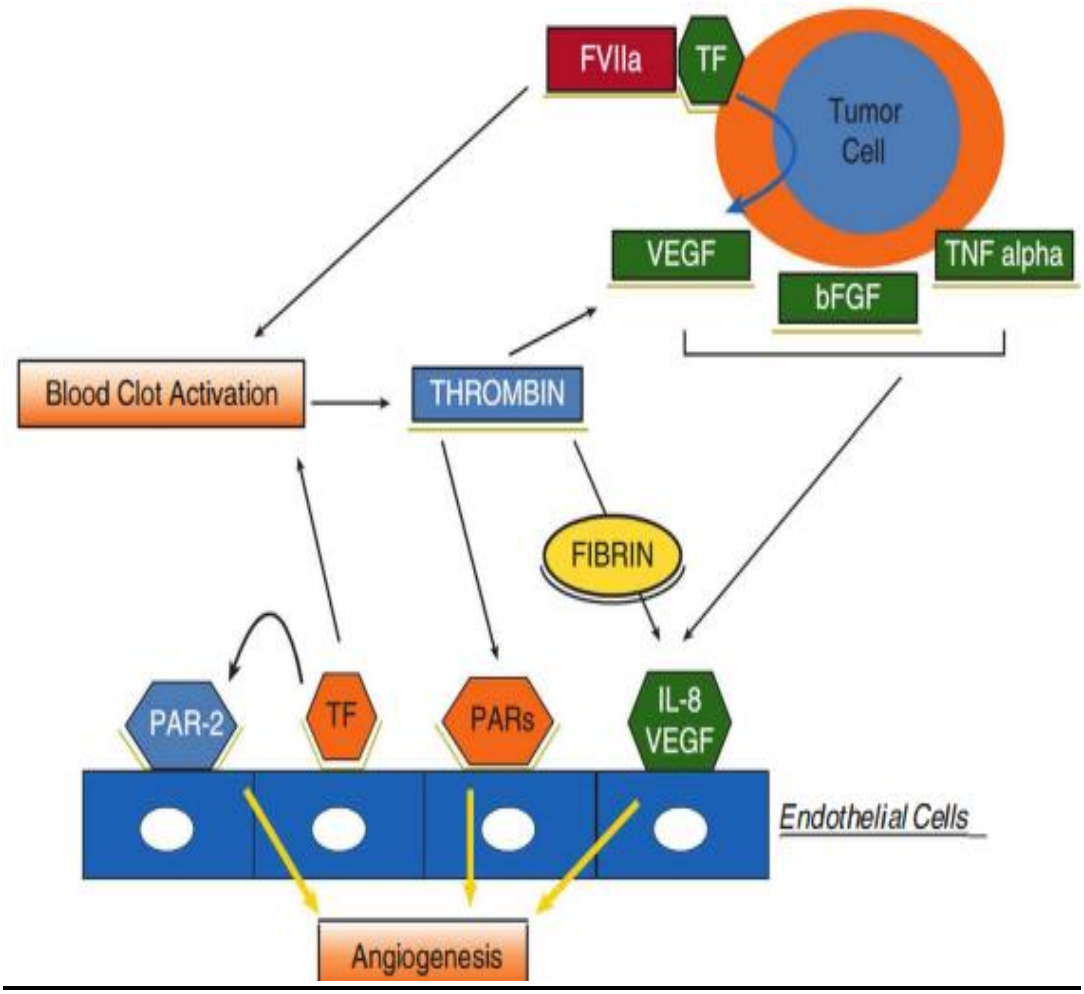


Figure 2.3: Role of coagulation system in progression of tumor, hemostatic proteins also play a role in tumor angiogenesis[90]

1. Tissue Factor

The main catalyst for hemostasis is a transmembrane glycoprotein called tissue factor (TF). With the stimulation of tissue factor signaling that supports tumor growth and angiogenesis, it is one of the most significant tumor-associated determinants for tumor progression and metastasis. Additionally, tissue factor mediates the production of (local) thrombin, which is essential for a number of pro-tumorigenic activities.[91]

By producing downstream thrombin and fibrin as well as platelet activation brought on by thrombin, it stimulates angiogenesis through clotting-dependent processes.

A vicious cycle of clot formation and tumor progression is also created when the produced fibrin causes the overexpression of TF on tumor cells as well as endothelial cells and the secretion of the proangiogenic cytokine, interleukin-8. It is also possible for TF to operate in tumor angiogenesis through pathways unrelated to thrombin production and fibrin deposition.[92]

The TF cytoplasmic tail's phosphorylation and ensuing signal transduction cascades trigger these mechanisms. TF/ FVIIa complex activates one or more protease-activated receptors (PARs) in a protease-receptor pathway, which when linked with G proteins affects several signaling pathways and promotes angiogenesis in vivo.

The vascular endothelial growth factor, an angiogenic factor, thrombospondin, an antiangiogenic peptide, can both express more frequently when TF is present.[93]

TF promotes tumor invasion and metastasis by:

A) Ability of the TF-Vlla-Xa complex to stimulate the local production of thrombin, which in turn causes the platelet and endothelial PARs to become proteolytically activated and deposit fibrin.;

B) Ability of the tumor-associated TF-Vlla-Xa to activate PAR-1 and PAR-2 signaling pathways directly;

C) Ability of TF/FVIIa complex to help the tumor vasculature that express TF pathway inhibitor to cause cellular adhesion and migration in a thrombin-independent circle.[94,95]

Actually, TF expression by several tumor-associated cells (neoplastic or stromal cells) has been specifically linked to tumor growth and processes associated with metastasis. Included among these are the production of thrombin and its subsequent interactions with a variety of targets, as well as the stimulation of a few surface receptors that are turned on by a variety of proteolytic enzymes, such as blood coagulation serine proteases.[96]

2. Thrombin

Through clotting-dependent pathways including activation of platelet and deposition of fibrin, thrombin is a powerful angiogenesis stimulator. Additionally, through clotting-independent processes mediated by PAR activation and ensuing signal transduction cascades, thrombin causes angiogenesis.

Numerous angiogenesis-related genes, such as VEGF, VEGF receptors (VEGFR), TF, bFGF, and metalloproteinase (MMP)-2, are upregulated as a result of thrombin activation of PARs. Additionally, thrombin increases platelet VEGF and angiopoietin production, which causes endothelial cells to form tubes.[97]

Thrombin contributes significantly to a more malignant phenotype by inducing:

a) Platelet-tumor aggregation,
b) Tumor adhesion to endothelial cells and extracellular matrix (ECM),
c) Tumor cell proliferation and metastasis. Ability of thrombin to cause growth factors release and extracellular proteins that encourage the proliferation and migration of tumor cells is crucial in this situation.[98]

3. Fibrin

Fibrin plays crucial functions in both thrombogenesis and the development of tumors, and the fact that it accumulates inside the vascular endothelium of newly formed tumor arteries or is connected to inflammatory or malignant cells shows that angiogenesis is aided. Particularly in conditions of severe shear stress, localized deposits of fibrin can prevent cells of tumor or tumor cell-associated emboli from endothelium adhering.

Additionally, fibrin matrix serves as a superb scaffold for the growth of new blood vessels.[94]

4. D- dimer

A popular biomarker for detecting the onset of coagulation and fibrinolysis is D-dimer. One of the most typical problems for cancer patients is coagulation issues. Patients with breast cancer and other solid tumors have higher D-dimer levels in their plasma.[99]

In common investigations of individuals with breast, colorectal, and lung cancer, it has been found that cancer frequently activates hemostatic system, and that activation degree related to a more advanced tumor stage, with negative outcomes, and the patient's prognosis.[100]

D-dimer, a stable byproduct of cross-linked fibrin breakdown, is produced as a result of increased fibrin synthesis and fibrinolysis. The level of D-dimer is used frequently to identify patient who may have myocardial infarction, thromboembolic events, or disseminated intravascular coagulation (DIC). Relation between high levels of D-dimer and cancer has been reported. The levels D-dimer have also been linked to stage, prognosis of tumor, metastasis lymph node, and the survival of patients with solid tumors like breast cancer, gastric cancer, colon cancer, and gynecological cancer, according to a number of studies. Advanced tumor stage patients have been

demonstrated to have elevated D-dimer plasma levels, which can be utilized to forecast the clinical course of cancer patients.[101]

Deep vein thrombosis (DVT) and pulmonary embolism are significant consequences with a high mortality rate in patients with malignant tumors. Numerous studies have demonstrated an association between high level of D-dimer and risk of these problems. Cancer patients frequently display abnormal coagulation and fibrinolytic activities, and their D-dimer levels typically tend to be higher than those in non-neoplastic populations. This is because fast-growing tumor cells' toxic release into the bloodstream and the fibrinolytic activator on their surface cause damage to vascular endothelial cells (Figure 2.4).[102,103]

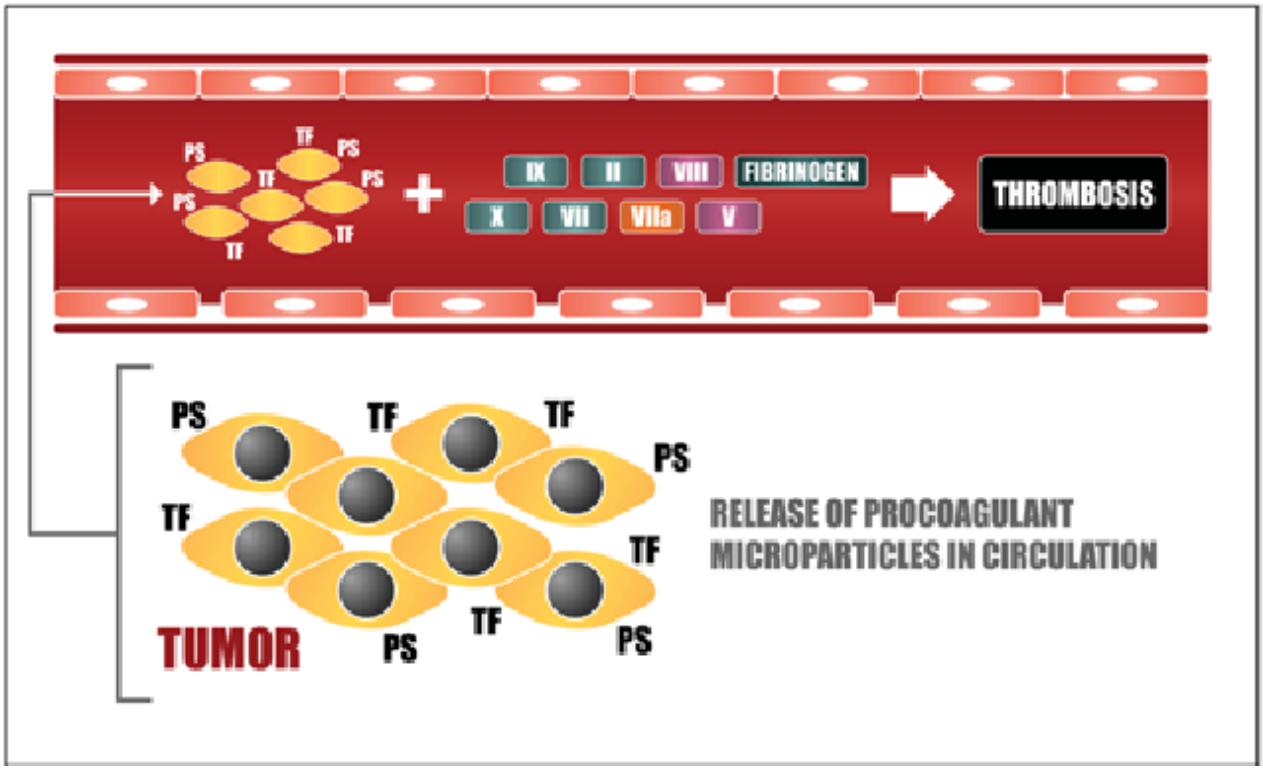


Figure 2.4: Intravascular activation of blood coagulation in cancer[103]

Chapter Three

Patients, Materials and Methods

3.1. Study design, location and timing

A cross- sectional study that was conducted in Hilla (Marjan specialized hospital, Imam Al-Sadiq teaching hospital, Al-Hilla teaching hospital) and Najaf (National hospital for oncology and blood disorders). The study extended from October 2022 to February 2023. Sample collection was completed in four months, and material purchased, laboratory work was achieved from first of September 2022 to the end of February 2023.

3.2. Patients

Sixty-two adult female patients enrolled in this study; all are cases of breast cancer who attended the centers mentioned above for treatment. All patients were newly diagnosed breast cancer cases who haven't started treatment yet. The patients are divided according to the stage of breast cancer (Stage I, II, III and IV).

All patients received verbal information explaining the aim of the study. Verbal consent was taken from each patient participating in the study.

All patients were subjected to questionnaire (Appendix 1). This questionnaire was used to collect socio- demographic data such as: Age, Sex. Symptoms …. By direct interview. Data that were collected from patient's records included:

1. Stage of breast cancer.
2. ER, PR, Her2 neu receptor status.
3. Histological subtype.
4. Molecular subtype.

3.2.1 The exclusion criteria

1. Patients having inherited bleeding disorders or any hereditary disease affecting coagulation.
2. Any patient who received chemotherapy or radiotherapy.
3. Any patient who uses drugs that affect coagulation.

3.2.2. Study design diagram

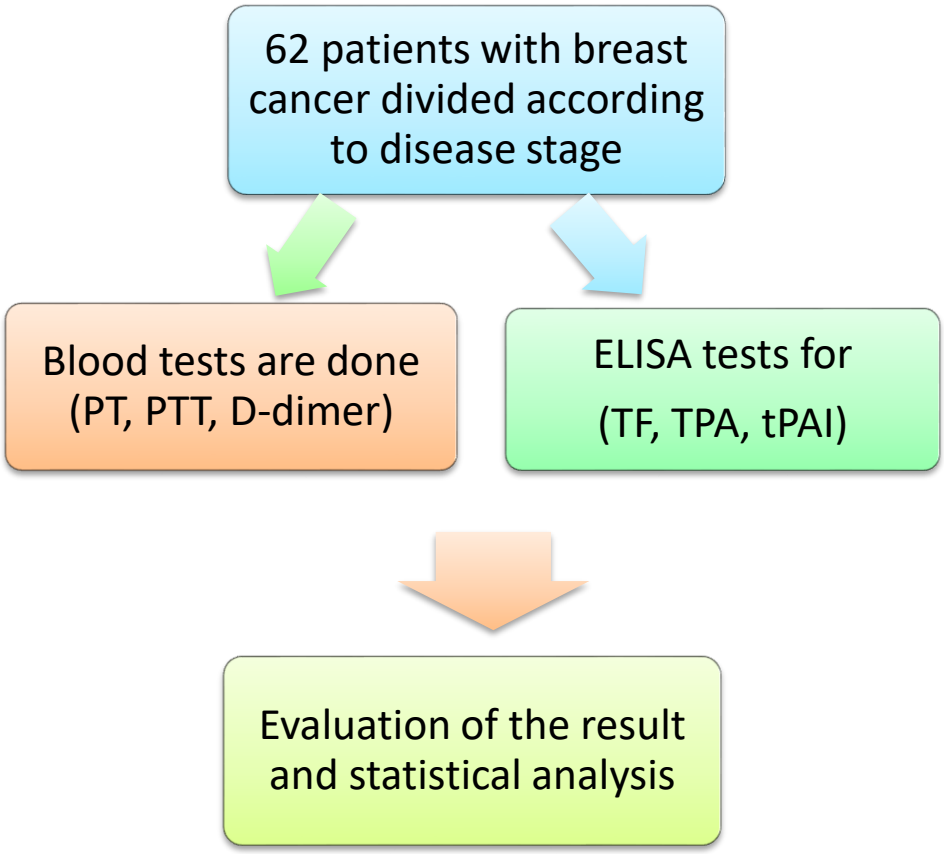


Figure 3.1: The study design

3.2.3. Ethical considerations

Ethical approval for the study was obtained from ethical committee in the department of pathology as well as from council of College of Medicine/ University of Babylon. The agreement of health authorities in the included centers was taken.

3.2.4. Sample collection

From each individual 8 ml of blood was drawn from venipuncture, by disposable 10 ml syringe under aseptic technique, the blood was drawn into test tubes. Part of the sample (4 mL) was used for tests (CBC, prothrombin time, partial thromboplastin time and D-dimer), the other part of the sample (4 mL) is maintained at room temperature for 2 hours, centrifugation of samples made for 15 minutes at 1000xg, serum was pipetted in Eppendorf tubes that were labelled and transported with ice packs and stored at -20°C until used for ELISA detection technique.

ELISA tests are done at immunity unit in the main laboratory of Imam Al-Sadiq teaching hospital while the other tests are done at private laboratory. For each patient; tissue factor, tissue plasminogen activator, plasminogen activator inhibitor, prothrombin time, partial thromboplastin time and d-dimer were measured.

3.3. Materials

3.3.1: Chemicals

The chemicals and materials used throughout this study with their origin were listed in table (3.1).

Table 3.1: Chemicals and materials used throughout the study and their origin

Chemical/material	Manufacturer
D- dimer fast test kit (Immunofluorescence assay)/ Getein 1100	Getein Biotech/ China
Deionized water	Baghdad/ Iraq
ELISA Kit 1 (tissue factor)	Elabscience Biotechnology Inc./ USA
ELISA Kit 2 (Tissue Plasminogen Activator)	Elabscience Biotechnology Inc./ USA
ELISA Kit 3 (Plasminogen Activator Inhibitor 1)	Elabscience Biotechnology Inc./ USA
Hemostat thromboplastin- SI (for determination of prothrombin time)	HUMAN/ Germany
Hemostat aPTT- EL (for determination of partial thromboplastin time)	HUMAN/ Germany

3.3.2: Instruments

All instrument used throughout this study were listed in table (3.2).

Table 3.2: Instrument used throughout the study and their origin

Instrument/equipment	Manufacturer
Absorbent paper	China
Calibrated micropipettes	Slammed (Germany)
Centrifuge REF 2300	Hettich (Germany)
Deep Freeze	Naïve (Turkey)
Disposable pipette tips	Kartell labware (Italy)
EDTA tube (2 mL)	Wafi medical laboratories (Iraq)
ELISA (chromate 4300)	Awareness technology (USA)
Eppendorff tube (1.5 mL)	Eppendorf (Germany)
Gel tube	Plasmatic laboratory (UK)
Gloves	Broche (Malaysia)
Incubator	Smi1E- 2 (USA)
Plastic syringes; 5Ml	Meheco (China)
Rack	Meheco (China)
Sodium citrate tube (3.2 %)	Plasmatic laboratory (UK)
Timer	Eppendorf, Germany
Vortex 3	IKA (USA)

3.3.2. A. ELISA Kit 1 (human tissue factor (TF)):

ELISA kit for in vitro quantitative determination of Human TF concentrations in serum, plasma and other biological fluids.

Table3.3: ELISA Kit for human tissue factor (TF) contents[104]

Item	Specifications
Micro ELISA Plate (Dismountable)	8 wells ×12 strips
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL
Concentrated HRP Conjugate (100×)	1 vial, 120 µL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	1 vial, 30 mL
Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Plate Sealer	5 pieces
Product Description	1 copy
Certificate of Analysis	1 copy

3.3.2. B. ELISA Kit 2 (Human Tissue-type Plasminogen Activator (tPA)):

ELISA kit for in vitro quantitative determination of Human tPA concentrations in serum, plasma and other biological fluids.

Table 3.4: ELISA Kit for Human Tissue-type Plasminogen Activator (tPA) contents[104]

Item	Specifications
Micro ELISA Plate (Dismountable)	8 wells ×12 strips
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL
Concentrated HRP Conjugate (100×)	1 vial, 120 µL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	1 vial, 30 mL
Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Plate Sealer	5 pieces
Product Description	1 copy
Certificate of Analysis	1 copy

3.3.3. C. ELISA Kit 3 (Human Plasminogen Activator Inhibitor 1 (PAI1)):

ELISA kit for in vitro quantitative determination of Human PAI1 concentrations in serum, plasma and other biological fluids.

Table 3.5: ELISA Kit for Human Plasminogen Activator Inhibitor 1 (PAI1) contents[104]

Item	Specifications
Micro ELISA Plate (Dismountable)	8 wells ×12 strips
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL
Concentrated HRP Conjugate (100×)	1 vial, 120 µL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	1 vial, 30 mL
Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Plate Sealer	5 pieces
Product Description	1 copy
Certificate of Analysis	1 copy

3.4. Methods

3.4.1 Enzyme Linked Immune Sorbent Assay (ELISA)

3.4.1. A. Human TF (Tissue Factor)

Principle of the assay:

The ELISA kit uses the Sandwich-ELISA principle. An antibody specific to Human TF pre-coated the micro-ELISA plate that is provided in this kit. The standards are added to the micro-ELISA plate wells and combined with specific antibody. For each micro plate well a biotinylated detection antibody specific for Human TF and Avidin-Horseradish Peroxidase (HRP) conjugate are successively added and incubated. Then free components are washed away. For each well, substrate solution is added. Only wells that contain Human TF, biotinylated detection antibody and Avidin-HRP conjugate appeared in blue color. Enzyme-substrate reaction is terminated by the addition of stop solution and turns to yellow color. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human TF. The concentration of Human TF in samples can be calculated by comparing OD of the samples to the standard curve. The color development is stopped and the intensity of the color is measured.

Reagent preparation:

1. All reagents are brought to room temperature (18-25℃) before use. Microplate reader manual is followed for set-up, before OD measurement, it is preheated for 15 minutes.
2. Wash Buffer: 30 mL of Concentrated Wash Buffer is diluted with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.
3. The standard working solution: the standard is centrifuged at 10.000×g for 1 minutes. 1.0 mL of reference standard and sample

diluent is added and is let to stand for 10 minutes and inverted gently several times. After fully dissolved, by using a pipette it is mixed thoroughly. This reconstitution produces a working solution of 500 pg/mL. Then serial dilutions are made as needed. The recommended dilution gradient is as follows: 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 0 pg/mL. Dilution method: 7 EP tubes are taken, 500 μL of reference standard & sample diluent is added to each tube. 500uL of the 500 pg/mL working solution is pipetted to the first tube and is mixed up to produce a 250 pg/mL working solution. 500 μL of the solution is pipetted from the former tube into the latter one according to this step.

4. Biotinylated Detection Ab working solution: the required amount is calculated before the experiment (100 μL/well). In preparation, slightly more than calculated is prepared. The stock tube is centrifuged before using, the 100× Concentrated Biotinylated Detection Ab is diluted to 1×working solution with Biotinylated Detection Ab Diluent.
5. Concentrated HRP Conjugate working solution: the required amount is calculated before the experiment (100 μL/well). In preparation, slightly more than calculated is prepared. The 100× Concentrated HRP Conjugate is diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure:

All reagents and samples are brought to room temperature before use.

The sample is centrifuged again after thawing before the assay.

1. Standard working solution is added to the first 2 columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 μL for each well). The samples are added to

the other wells (100 μL for each well). Sealer provided in the kit used to cover the plate, and incubated for 90 min at 37℃.

2. The liquid is removed out of each well, and is not washed. Then for each well, 100 μL of Biotinylated Detection Ab working solution is added, and covered with the Plate sealer, then gently mixed up. It is incubated for about 1 hour at 37°C.
3. From each well, the solution is aspirated or decanted. Then 350 μL of wash buffer is added to each well, and is soaked for 1-2 minutes. The solution is aspirated or decanted from each well and is patted dry against clean absorbent paper. This wash step is repeated 3 times.
4. 100 μL of HRP Conjugate working solution is added to each well. It is covered with the Plate sealer and incubated at 37°C for 30 min.
5. The solution is aspirated or decanted from each well, wash steps are repeated for 5 times.
6. 90 μL of Substrate Reagent is added to each well. It is covered with a new plate sealer and incubated for 15 minutes at 37°C. The plate is protected from light.
7. 50 μL of Stop Solution is added to each well. The stop solution is added in same order as the substrate solution.
8. The optical density (OD value) of each well is determined with a micro-plate reader set to 450 nm

3.4.1. B. Human tPA (Tissue-type Plasminogen Activator)

Principle of the assay:

ELISA kit uses the Sandwich-ELISA principle. An antibody specific to Human tPA pre-coated the micro-ELISA plate provided in this kit. Standards are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human tPA and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The free components are washed away. The substrate solution is added to each well. Only those wells that contain Human tPA, biotinylated detection antibody and Avidin-HRP conjugate appeared in blue color. By the addition of stop solution the enzyme-substrate reaction is terminated and turns to yellow color. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human tPA. The concentration of Human tPA in the samples can be calculated by comparing the OD of the samples to the standard curve.

Reagent preparation:

1. All reagents are brought to room temperature (18-25℃) before use. The Microplate reader manual is followed for set-up and before OD measurement is preheated for 15 min.
2. Wash Buffer: 30 mL of Concentrated Wash Buffer is diluted with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.
3. Standard working solution: The standard is centrifuged at 10.000×g for 1 min. 1.0 mL of Reference Standard and Sample Diluent is added, it is let stand for 10 min and inverted gently several times.

After it fully dissolved, with a pipette, it is mixed thoroughly. This reconstitution produces a working solution of 40 ng/mL. Then serial dilutions are made as needed. The recommended dilution gradient is as follows: 40, 20, 10, 5, 2.5, 1.25, 0.63, 0 ng/mL. Dilution method: 7 EP tubes is taken, 500 µL of Reference Standard & Sample Diluent is added to each tube. 500 µL is pipetted of the 40 ng/mL working solution to the first tube and is mixed up to produce a 20 ng/mL working solution. 500 µL is pipetted of the solution from the former tube into the latter one according to this step.

4. Biotinylated Detection Ab working solution: The required amount is calculated before the experiment (100 µL/well). In preparation, slightly more than calculated is prepared. Stock tube is centrifuged before using, the 100× Concentrated Biotinylated Detection Ab is diluted to 1×working solution with Biotinylated Detection Ab Diluent.
5. Concentrated HRP Conjugate working solution: The required amount is calculated before the test (100 µL/well). In preparation, slightly more than calculated is prepared. The 100× Concentrated HRP Conjugate is diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure:

1. The Standard working solution is added to the first 2 columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µL for each well). The samples are added to the other wells (100 µL for each well). With the sealer provided in the kit the plate is covered. It is incubated for about 90 min at 37℃.

2. The liquid is removed out of each well and not washed. For each well 100 µL of Biotinylated Detection Ab working solution is added and covered with the Plate sealer. It is gently mixed up and incubated for about 1 hour at 37°C.
3. From each well the solution is aspirated or decanted, then 350 µL of wash buffer is added to each well. It is soaked for 1-2 minute and the solution is aspirated or decanted from each well and is patted dry against clean absorbent paper. This wash step is repeated for 3 times.
4. 100 µL of HRP Conjugate working solution is added to each well and is covered with the Plate sealer. It is incubated for about 30 min at 37°C.
5. The solution is aspirated or decanted from each well, the wash process is repeated for 5 times.
6. 90 µL of Substrate Reagent is added to each well and is covered with a new plate sealer. It is incubated for about 15 min at 37°C. The plate is protected from light.
7. 50 µL of Stop Solution is added to each well. The stop solution is added in the same order as the substrate solution.
8. The optical density (OD value) of each well is determined at once with a micro-plate reader set to 450 nm.

3.4.1. C. Human PAI1(Plasminogen Activator Inhibitor 1)

Principle of the assay:

ELISA kit uses the Sandwich-ELISA principle. An antibody specific to Human PAI1 pre-coated the micro-ELISA plate provided in this kit. Standards are added to the micro-ELISA plate wells and combined with the specific antibody. For each micro plate well, a biotinylated detection

antibody specific for Human PAI1 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively and incubated. The free components are washed away. For each well, the substrate solution is added. Only those wells that contain Human PAI1, biotinylated detection antibody and Avidin-HRP conjugate appeared in blue color. The enzyme-substrate reaction is terminated by the addition of stop solution and turned to yellow color. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human PAI1. The concentration of Human PAI1 in the samples is calculated by comparing the OD of the samples to the standard curve.

Reagent preparation:

1. All reagents are brought to room temperature (18-25℃) before use. The Microplate reader manual is followed for set-up and before OD measurement it is preheated for about 15 min.
2. Wash Buffer: 30 mL of Concentrated Wash Buffer is diluted with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.
3. Standard working solution: The standard is centrifuged at 10.000×g for 1 min. 1.0 mL of Reference Standard & Sample Diluent is added, it is let stand for 10 min and inverted gently several times. After it is fully dissolved, with a pipette, it is mixed thoroughly. This reconstitution produces a working solution of 10 ng/mL. Then serial dilutions are made as needed. The recommended dilution gradient is as follows: 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0 ng/mL. Dilution method: 7 EP tubes is taken, 500uL of Reference Standard & Sample Diluent is added to each tube. 500uL of the 10ng/mL working solution is pipetted to the first tube and mixed up to

produce a 5ng/mL working solution. 500uL of the solution from the former tube is pipetted into the latter one according to this step.

4. Biotinylated Detection Ab working solution: The required amount is calculated before the experiment (100 μL/well). In preparation, slightly more than calculated is prepared. The stock tube is centrifuged before using, the 100× Concentrated Biotinylated Detection Ab is diluted to 1×working solution with Biotinylated Detection Ab Diluent.
5. Concentrated HRP Conjugate working solution: The required amount is calculated before the experiment (100 μL/well). In preparation, slightly more than calculated is prepared. The 100× Concentrated HRP Conjugate is diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure:

1. The Standard working solution is added to the first 2 columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 μL for each well). The samples are added to the other wells (100 μL for each well). By using the sealer provided in the kit, the plate is covered and incubated for 90 min at 37°C.
2. The liquid is removed out of each well and not washed. For each well 100 μL of Biotinylated Detection Ab working solution is added. Then it is covered with the Plate sealer. It is gently mixed up and incubated for about 1 hour at 37°C.
3. From each well the solution is aspirated, and 350 μL of wash buffer is added to each well. For 1-2 minutes it is soaked and the solution is aspirated or decanted from each well and patted dry

against clean absorbent paper. This wash step is repeated for 3 times.

4. 100 µL of HRP Conjugate working solution is added to each well and covered with the Plate sealer. It is incubated for about 30 min at 37°C.
5. From each well, the solution is aspirated or decanted, the wash process is repeated for 5 times.
6. 90 µL of Substrate Reagent is added to each well and covered with a new plate sealer. It is incubated for 15 min at 37°C. The plate is protected from light.
7. 50 µL of Stop Solution is added to each well. The stop solution is added in the same order as the substrate solution.
8. The optical density (OD value) of each well is determined at once with a micro-plate reader set to 450 nm.

3.4.2. Coagulation Tests

3.4.2. A. Prothrombin time

Principle

The one-stage PT measures the clotting time of plasma after adding a source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated factor Xa, with the consequent formation of thrombin and ultimately an insoluble fibrin clot.

Materials

1. Thromboplastin reagent (rabbit brain extract, sodium azide)
2. Buffer ($CaCl_2$, sodium azide)
3. Calibrator
4. Control plasma

Procedure

1. The reagent is pre-warmed to 37°C before use (stirred during use), also the test tubes are pre-warmed.
2. Plasma/ Control is pipetted into pre-warmed test tubes (100 µl).
3. It is incubated for 3 minutes at 37°C.
4. The pre-warmed reagent is added (200 µl).
5. The timer is started with addition of the reagent. The time required for clot formation is recorded.

3.4.2. B. Partial thromboplastin time

Principle

Activated partial thromboplastin time is performed by adding aPT reagent containing a plasma activator and phospholipid to the test specimen; the phospholipid serves as a substitute for platelets. The mixture is incubated for activation, then recalcified with calcium chloride and clot formation is timed.

aPTT-EL reagent can also be used to perform quantitative factor assays.

Materials

1. Reagent 1 (aPTT-EL reagent) (rabbit brain cephalin, ellagic acid, sodium azide)
2. Reagent 2 ($CaCl_2$, sodium azide, salts and stabilizers)
3. Control plasma

Procedure

1. Reagent2 is pre-warmed to 37°C and the test tubes are pre-warmed.
2. Patient plasma/ Control is pipetted into pre-warmed test tube (50 µl).
3. Reagent1 is added (mixed before use) (50 µl).
4. It is mixed gently then incubated for 3 minutes at 37°C.
5. Pre- warmed reagent2 is added (50 µl).

6. The timer is started with addition of reagent. The time required for clot formation is recorded.

3.4.2. C. Assay of D-dimer

Principle

The test uses an anti-human D-Dimer monoclonal antibody conjugated with fluorescence latex and another anti-human D-Dimer monoclonal antibody coated on the test line. After the sample has been applied to the test strip, the fluorescence latex-labelled anti-human D-Dimer monoclonal antibody binds with the D-Dimer in sample and forms a marked antigen-antibody complex. By capillary action, the complex moves to the test card detection zone. Then marked antigen-antibody complex is captured on the test line by another anti-human D-Dimer monoclonal antibody. The fluorescence intensity of the test line increases in proportion to the amount of D-Dimer in sample. Then insert test card into Getein 1100 Immunofluorescence Quantitative Analyzer, the concentration of D-Dimer in sample will be measured and displayed on the screen.

Materials

1. A kit for Getein1100 (D-Dimer test card in a sealed pouch with desiccant, disposable pipette, sample diluent)
2. Sample diluent composition: (Phosphate buffered saline, proteins, detergent, preservative, stabilizer).
3. A test card consists of: A plastic shell and a reagent strip which is composed of a sample pad, nitrocellulose membrane (one end of the membrane is coated with a fluorescence latex-labelled anti-human D-Dimer monoclonal antibody, the test line is coated with another anti-human D-Dimer monoclonal antibody and the control

line is coated with rabbit anti-mouse IgG antibody), absorbent paper and liner.

4. Getein1100 Immunofluorescence Quantitative Analyzer

Procedure

1. Specimens are collected according to user manual.
2. Test card, sample and reagent are brought to room temperature before testing.
3. SD card lot number is confirmed in accordance with test kit lot number.
4. The test card is removed from the sealed pouch immediately before use. The test card is labelled with patient identification.
5. The test card is put on a clean table, horizontally placed.
6. Using sample transfer pipette, 100 µl of sample is delivered into one tube of sample diluent, mixed gently and thoroughly. Then 100 µl of sample mixture is dropped into the sample port on the test card.
7. Reaction time: 10 minutes. After reaction time is elapsed. The result is shown on the screen and printed automatically.

3.5. Data Analysis

Statistical analysis was carried out using SPSS version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means and standard deviations. ANOVA test was used to compare means between four groups. Kruskal-Wallis Test was used to compare several independent groups when variable was not normally distributed. Fisher's Exact test was used to find the association between categorical variables. P-value of ≤ 0.05 was considered as significant.

Chapter Four

Results

4.1. Distribution of patients with breast cancer according to age

Distribution of patients with breast cancer according to age including (<40 years, 40-49 years, 50-59 years, 60-69 years and ≥ 70 years). Age (<40 years) represent eight patients (12.9%), age of (40-49 years) represent twelve patients (19.4%), age of (50-59 years) represent eighteen patients (29.0%) and age of (60-69 years) represent fifteen patients (24.2%) and age of ≥ 70 years represent nine patients (14.5%) of patients with breast cancer. Mean age of breast cancer patients was (55.24 ± 12.82) with older patient was 84 years and younger patient was 28 years (**Figure 4.1**).

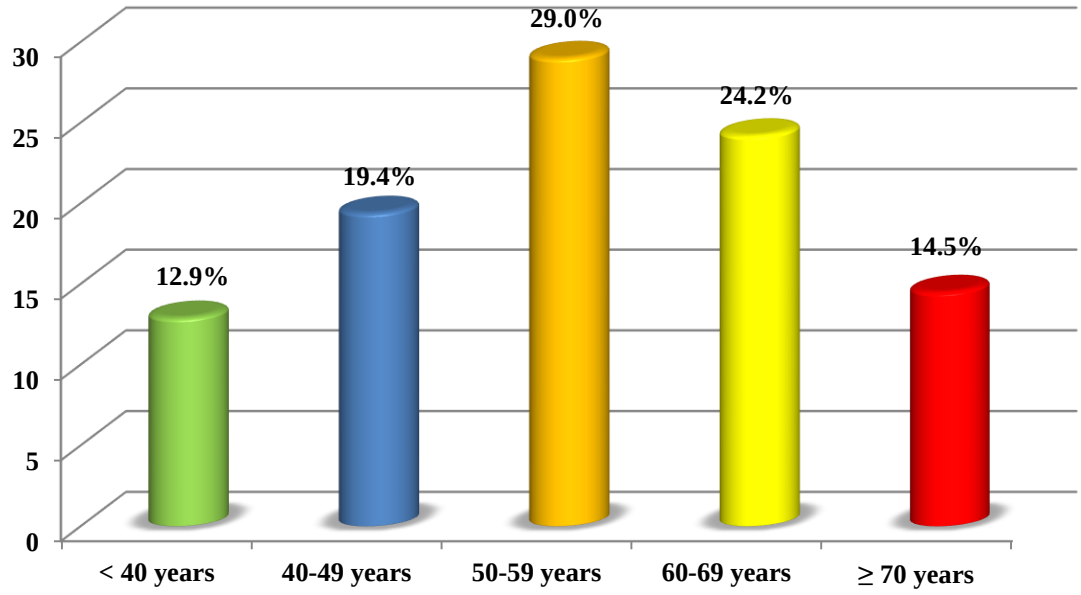


Figure 4.1: Distribution of patients with breast cancer according to age (years) (N=62)

4.2. Distribution of patients with breast cancer according to molecular subtypes

Distribution of patients with breast cancer according to molecular subtypes including (Luminal-like, Basal-like and Her2 positive). Luminal- like breast cancer represent majority of patients (N=51, 82.3%). Basal- like breast cancer represent only two patients (3.2%) and Her2 positive represent nine patients only (14.5%) of total patients (**Figure 4.2**).

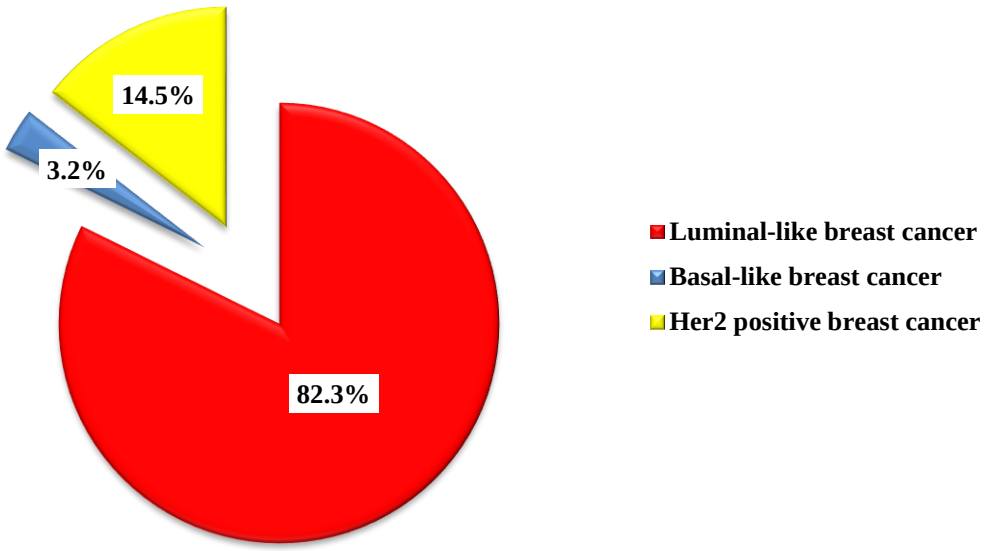


Figure 4.2: Distribution of patients with breast cancer according to molecular subtypes (N=62)

4.3. Distribution of patients with breast cancer according to histological subtypes

Distribution of patients with breast cancer according to histological subtypes including (ductal, lobular and mixed). Ductal histological subtype represents majority of patients (N=50, 80.6%). Lobular histological subtype represents only seven patients (11.3%) and mixed histological subtype represent five patients only (8.1%) of total patients with breast cancer (**Figure 4.3**).

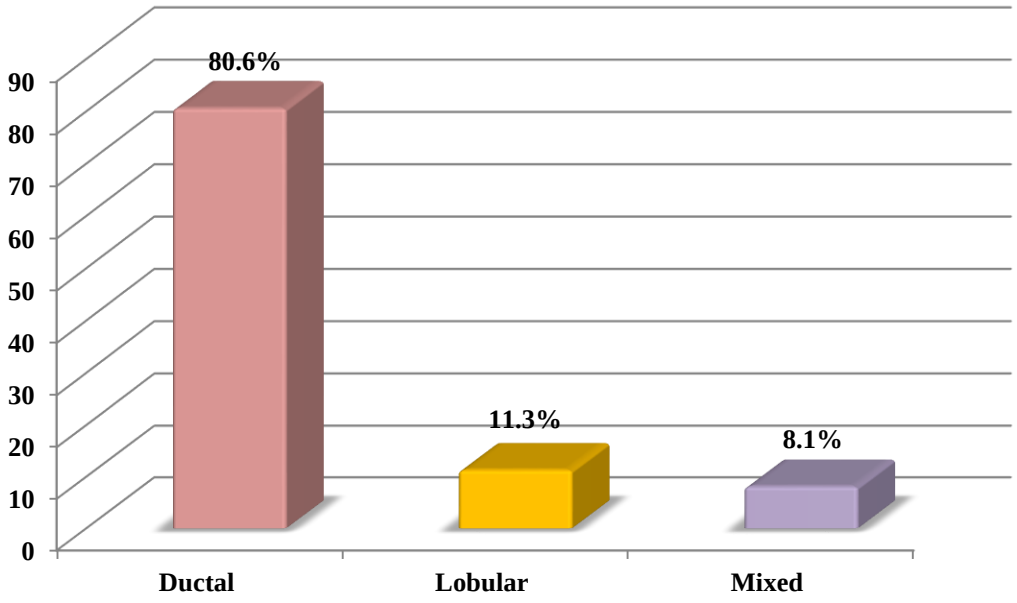


Figure 4.3: The distribution of patients with breast cancer according to histological subtypes (N=62)

4.4. Distribution of patients with breast cancer according to stages of disease

Distribution of patients with breast cancer according to stages of disease including (stage I, stage II, stage III and stage IV). Stage I represents seven patients (11.3%), stage II represents (N=26, 41.9%), stage III represents (N=15, 24.2%) and stage IV represents fourteen patients (22.6%) of total breast cancer cases (**Figure 4.4**).

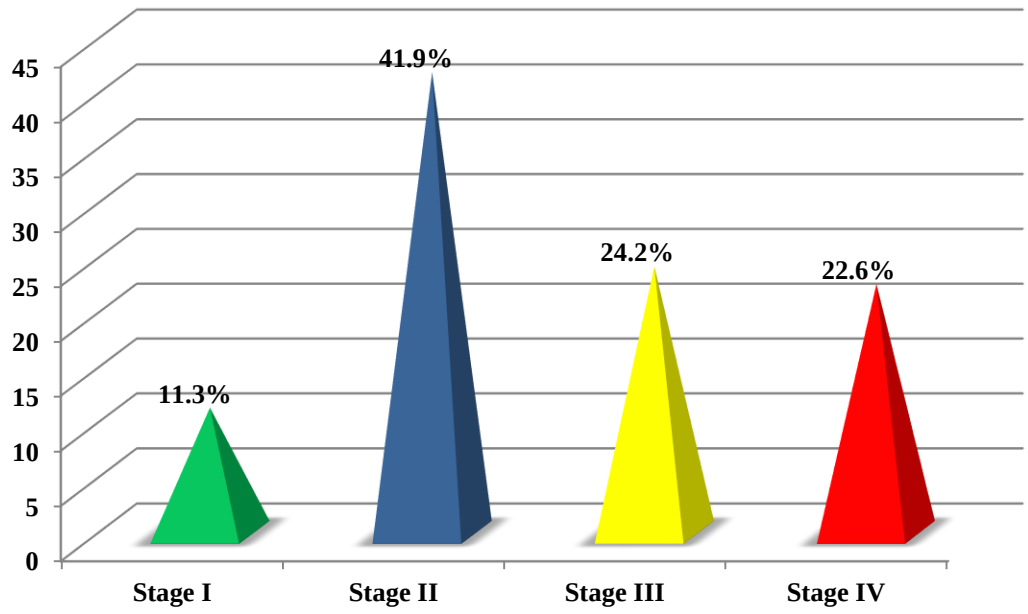


Figure 4.4: The distribution of patients with breast cancer according to stages of disease (N=62)

4.5. Distribution of mean age (in years) according to stages of breast cancer

Mean differences of age (years) related to stages of breast cancer including (stage I, stage II, stage III and stage IV) show no significant differences according to the various stages (**Table 4.1**, **Figure 4.5**).

Table 4.1: Mean differences of age (years) regarding stages of breast cancer (NO=62)

Variable	Breast cancer stages	N	Mean ± SD	F	P value
Age (years)	Stage I	7	52.29 ± 11.61	0.45	0.718
	Stage II	26	54.42 ± 12.31		
	Stage III	15	55.00 ± 12.83		
	Stage IV	14	58.50 ± 14.91		

F is the value of ANOVA test result.

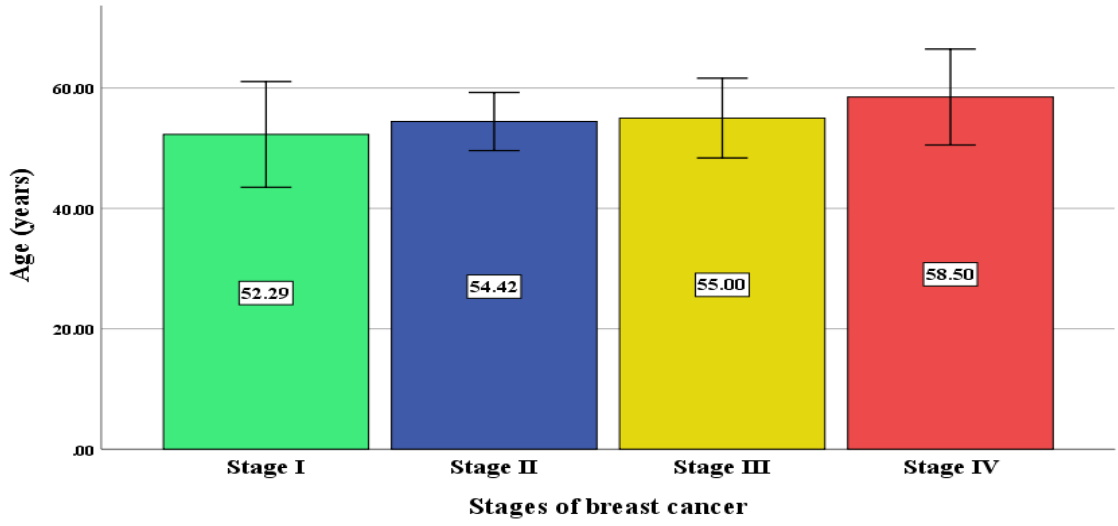


Figure 4.5: Mean differences of age (years) regarding breast cancer stages (NO=62)

4.6. Distribution of the results of study markers

These include (Tissue factor, Plasminogen activator inhibitor, Tissue plasminogen activator, Prothrombin time, Partial thromboplastin time and D- dimer). The results of study markers are shown in **(Table 4.2**). the normal values of the study markers are shown in **(Table 4.3**).

Table 4.2: The means ± standard deviations (SD) and range of study markers among breast cancer patients (N=62)

Study markers	N	Mean ± SD	Range
Tissue factor (pg/ml)	62	113.12± 190.48	(1.18-740.47)
Plasminogen activator inhibitor (ng/ml)	62	0.25 ± 0.24	(0.0 - 1.18)
Tissue plasminogen activator (ng/ml)	62	20.64 ± 15.57	(0.09-54.65)
Prothrombin time (seconds)	62	14.92 ± 1.37	(12.30-17.10)
Partial thromboplastin time (seconds)	62	32.43 ± 4.06	(25.00-39.10)
D- dimer (ng/ml)	62	627.26 ± 430.07	(200.0-2440.0)

Table 4.3: The normal values of the study markers

Study marker	Normal value
Tissue factor (pg/ml)	149 – 172
Plasminogen activator inhibitor (ng/ml)	5 – 20
Tissue plasminogen activator (ng/ml)	< 5
Prothrombin time (seconds)	13 +/- 2
Partial thromboplastin time (seconds)	30 +/- 5
D- dimer (ng/ml)	< 500

4.6.1. Tissue factor according to stages of breast cancer

There were significant differences between medians of tissue factor (pg/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of tissue factor (pg/ml) in comparison to other stages (**Table 4.4, Figure 4.6**).

Table 4.4: The median differences of Tissue factor (pg/ml) according to stages of breast cancer (N=62)

Study variable	Stages of breast cancer	N	Mean ± SD	Median	P-value
Tissue factor (pg/ml)	Stage I	7	26.13 ± 33.25	14.53	**<0.001***
	Stage II	26	42.94 ± 49.12	22.96	
	Stage III	15	37.96 ± 44.98	26.96	
	Stage IV	14	367.47 ± 269.83	308.70	

Kruskal-Wallis Test.

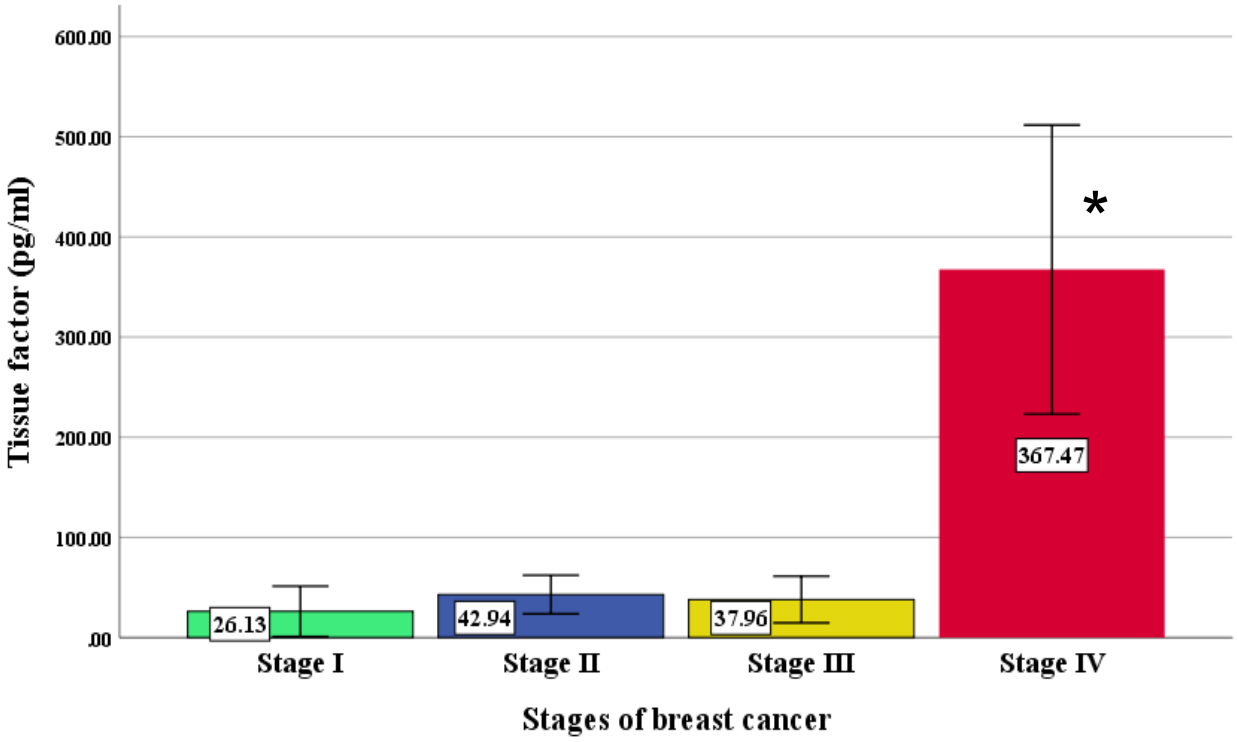


Figure 4.6: Mean differences of Tissue factor (pg/ml) regarding breast cancer stages (NO=62)

4.6.2. Plasminogen activator inhibitor according to stages of breast cancer

There were no significant differences between means of Plasminogen activator inhibitor (ng/ml) according to stages of breast cancer (**Table 4.5**, **Figure 4.7**).

Table 4.5: The mean differences of Plasminogen activator inhibitor (ng/ml) according to stages of breast cancer (N=62)

Study variable	Stages of breast cancer	N	Mean ± SD	F	P-value
Plasminogen activator inhibitor (ng/ml)	Stage I	7	0.39 ± 0.30	1.182	0.325
	Stage II	26	0.26 ± 0.27		
	Stage III	15	0.23 ± 0.19		
	Stage IV	14	0.18 ± 0.22		

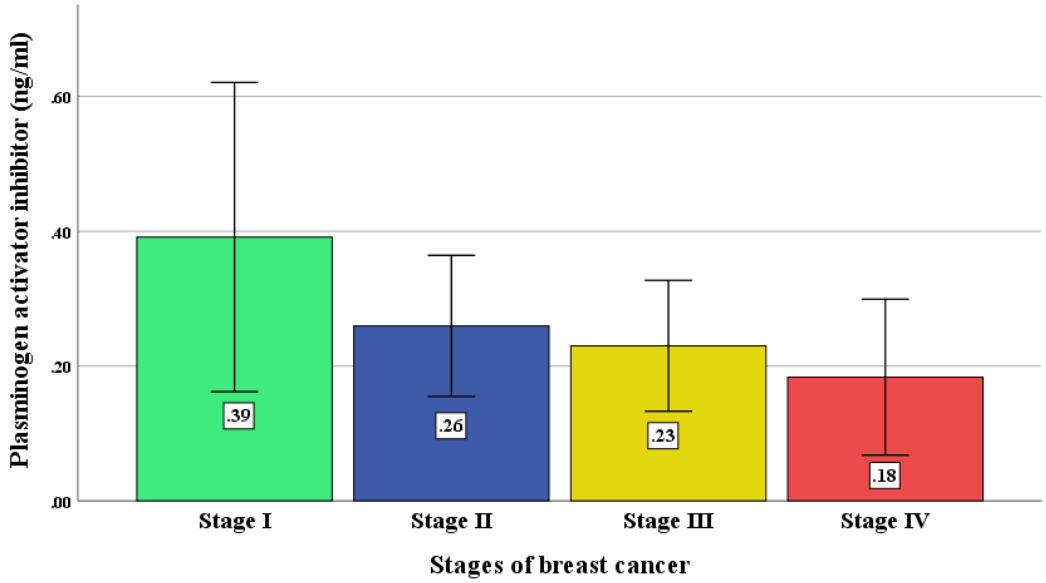


Figure 4.7: The mean differences of Plasminogen activator inhibitor (ng/ml) according to stages of breast cancer (N=62)

4.6.3. Tissue plasminogen activator according breast cancer stages

There were no significant differences between means of Tissue plasminogen activator (ng/ml) according to stages of breast cancer (**Table 4.6, Figure 4.8**).

Table 4.6: The mean differences of Tissue plasminogen activator (ng/ml) according to stages of breast cancer (N=62)

Study variable	Stages of breast cancer	N	Mean ± SD	F	P-value
Tissue plasminogen activator (ng/ml)	Stage I	7	21.12 ± 18.37	0.805	0.496
	Stage II	26	20.84 ± 15.54		
	Stage III	15	16.00 ± 13.48		
	Stage IV	14	24.99 ± 16.62		

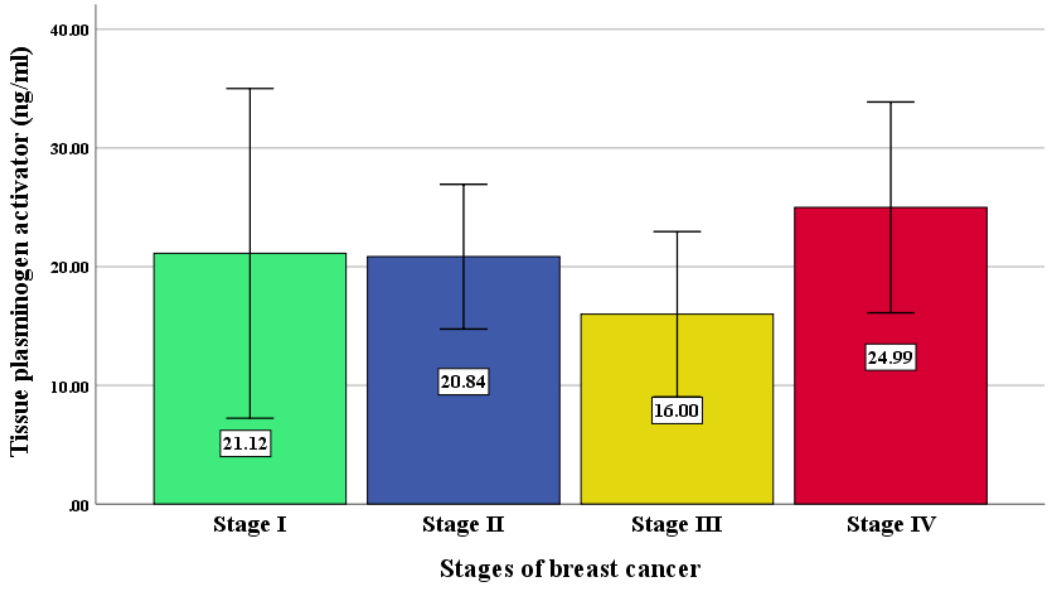


Figure 4.8: The mean differences of Tissue plasminogen activator (ng/ml) according to stages of breast cancer (N=62)

4.6.4. Prothrombin time according to stages of breast cancer

No significant differences were found between Prothrombin time means (seconds) regarding breast cancer stages (**Table 4.7**, **Figure 4.9**).

Table 4.7: Mean differences of Prothrombin time (seconds) regarding breast cancer stages (NO=62)

Variable	Breast cancer stages	N	Mean ± SD	F	P-value
Prothrombin time (seconds)	Stage I	7	15.54 ± 1.19	1.847	0.149
	Stage II	26	14.71 ± 1.42		
	Stage III	15	14.53 ± 1.39		
	Stage IV	14	15.44 ± 1.18		

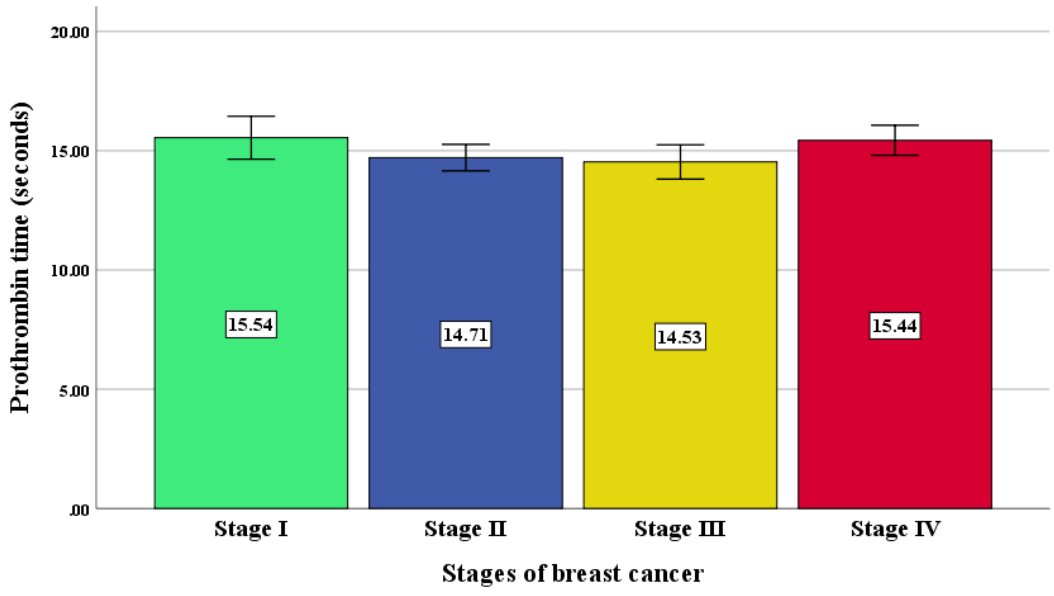


Figure 4.9: Mean differences of Prothrombin time (seconds) regarding breast cancer stages (N=62)

4.6.5. Partial thromboplastin time according to stages of breast cancer

No significant differences were found between Partial thromboplastin time means regarding breast cancer stages (**Table 4.8**, **Figure 4.10**).

Table 4.8: Mean differences of Partial thromboplastin time (seconds) regarding breast cancer stages (N=62)

Variable	Stages of breast cancer	N	Mean ± SD	F	P-value
Partial thromboplastin time (seconds)	Stage I	7	31.39 ± 4.43	1.776	0.162
	Stage II	26	31.68 ± 4.41		
	Stage III	15	32.25 ± 4.23		
	Stage IV	14	34.54 ± 2.31		

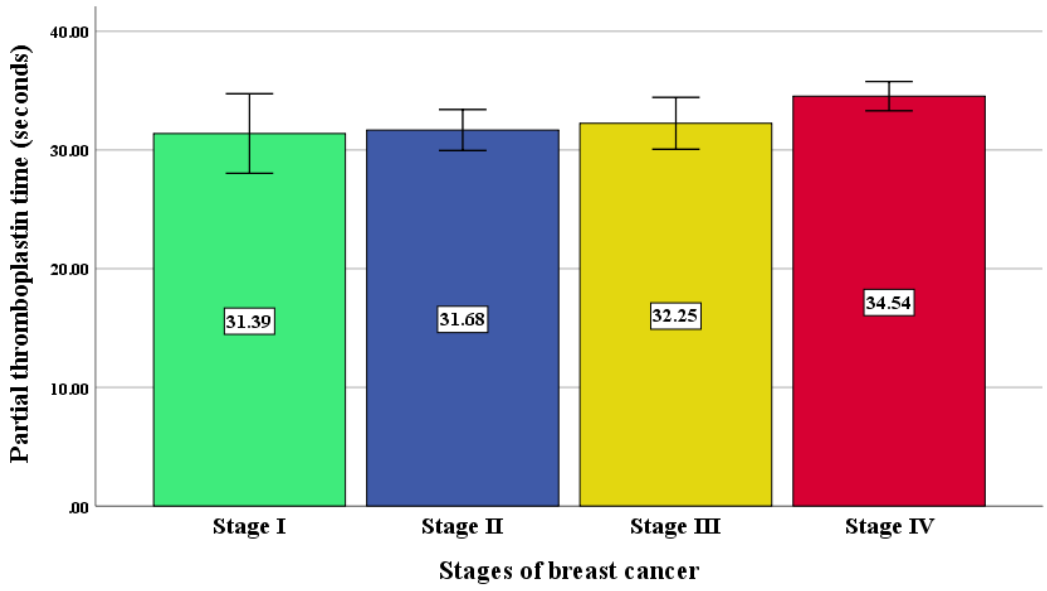


Figure 4.10: Mean differences of Partial thromboplastin time (seconds) regarding breast cancer stages (N=62)

4.6.6. D-dimer according to stages of breast cancer

There were significant differences between means of D-dimer (ng/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of D-dimer (ng/ml) in comparison to other stages of breast cancer (**Table 4.9**, **Figure 4.11**).

Table 4.9: The mean differences of D-dimer (ng/ml) according to stages of breast cancer (N=62)

Study variable	Stages of breast cancer	N	Mean ± SD	F	P-value
D-dimer (ng/ml)	Stage I	7	501.43 ± 228.65	18.819	<0.001*
	Stage II	26	455.38 ± 135.48		
	Stage III	15	466.67 ± 154.86		
	Stage IV	14	1181.43 ± 595.44		

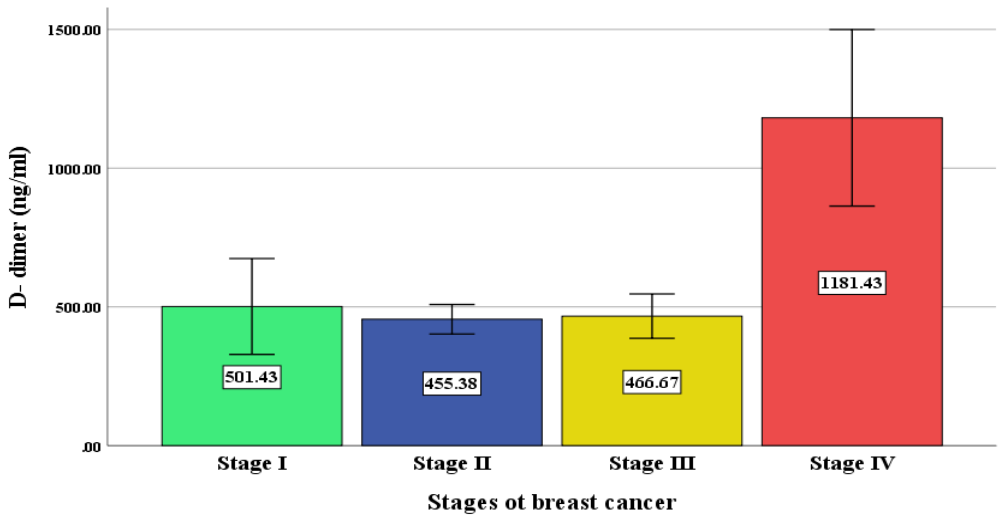


Figure 4.11: The mean differences of D-dimer (ng/ml) according to stages of breast cancer (N=62)

Chapter Five

Discussion

One of the most common female malignancies worldwide is Breast cancer. It is one of the most prevalent causes of cancer-related death.[105]

The processes of hemostasis/fibrinolysis and angiogenesis are closely linked physiological systems that remain silent under resting conditions, but in case of malignancy, these highly activated mechanisms may be involved in tumor expansion and invasive behavior.[106]

The role of coagulation and fibrinolytic systems in cancer biology and angiogenesis had been proven by many theories. Extracellular matrix remodeling is an important step in primary tumor growth as well as metastasis.

It has been indicated that an interaction between angiogenesis and hemostasis may facilitate metastasis in breast cancer and that plasma D-dimer levels are a measure of matrix remodeling in the tumor.[107]

Tissue factor (TF)-microparticles are related to cancer and exhibit increases in patients with certain cancers such as pancreatic cancer and breast cancer.[108]

This study aimed to discover the possible relationship between breast cancer in its various stages and defects in coagulation factors, and the possibility to use them as prognostic factors by comparing their levels among patients of different stages.

5.1. Breast cancer distribution related to age:

The results showed, breast cancer patients age distribution in general was (12.9% less than 40 years, 19.4% between 40- 50 years, 29.0% between 50- 60 years, 24.2% between 60- 70 years, 14.5% more than 70 years).

The study of **Abduladheem T. Jalil *et al.*** (2019) [109] in Wasit province, Iraq, found that the most prevalent age group (27%) was (40-49 years), 23.5% were in the age group (50-59 years) and 19% were in the age group (30-39 years) and 15% were in the age group (60-69 years), 0.5% were in the age group (10-19 years). The results were close to this study mostly due to geographical proximity.

The study of **Su *et al.*** (2011) [110] in China found the highest percentage (40.42%) was in the (40- 49 years) age group and the least percentage (4.51%) was patients younger than 40 years, **C. S. Vallejos *et al.*** (2010) [111] in Peru found the highest percentage (35.1%) was in the (40- 49 years) age group also and the least percentage (8.1%) was patients older than 70 years, and **Chopra *et al.*** (2014) [112] in India found the highest percentage (42%) was in the (40- 50 years) age group and the least percentage (4%) was patients older than 70 years. This indicates comparable results of age distribution of breast cancer in various countries in the world.

5. 2. Breast cancer distribution according to molecular subtypes

In this study, the distribution of patients according to molecular subtypes was: 82.3% for luminal- like subtype (including luminal A and luminal B), 3.2% for basal- like subtype and 14.5% for Her2/ neu positive subtype.

The study of **F. K. Althoubaity *et al.*** (2019)[113] in Saudi Arabia found that luminal A was (58.5%), luminal B was (14%), Her2/ neu was (11.5%) and triple negative was (16%). The study of **Cortet *et al.***

(2018)[114] in France found that luminal- like subtype was (66,8%), Her2/ neu was (11%) and triple negative was (9.2%).

S. Park *et al.* (2012)[115] in Korea found that luminal A was (53.1%), luminal B was (21.7%), Her2/ neu was (9.0%) and triple negative was (16.2%). **Caldarella *et al.*** (2012)[116] in Italy found that luminal A was (34%), luminal B was (36%), Her2/ neu was (10.2%) and triple negative was (19%). All of these studies agree with our results regarding this distribution with no significant differences.

5. 3. Distribution of breast cancer according to histological subtypes

This study found that ductal histological subtype represents majority of patients (80.6%). Lobular histological subtype represents (11.3%) and Mixed histological subtype represent only (8.1%) of total breast cancer patients.

A similar study done by **V. Ozmen *et al.*** (2014) [117] in Turkey found that ductal histological subtype represented the majority of patients (79%), lobular histological subtype represented only (7.4%) and mixed histological subtype represented (9.8%). **N. H. Alieldin *et al.*** (2014) [118] in Egypt found that ductal histological subtype represented the majority of patients (80.0%), lobular histological subtype represented only (7.8%) and other histological subtypes represented the remaining. **S. B. Abdelkrim *et al.*** (2010) [119] in Tunisia found that ductal histological subtype represented the majority of patients (91.2%), lobular histological subtype represented only (3.6%) and other histological subtypes represented (5.1%). All these studies agreed with our results with minor differences that can be attributed to the difference in sample size.

5. 4. Distribution of patients with breast cancer according to stages of disease

The distribution of patients with breast cancer according to the stages of disease including (stage I, stage II, stage III and stage IV). Stage I represents (11.3%), stage II represents (41.9%), stage III represents (24.2%) and stage IV represents (22.6%) of total cases of breast cancer. The studies of **Vondeling *et al.*** (2018) [120] in Netherlands found the highest incidence was in stages I and II and the least incidence was in stages III and IV, and **Chaari *et al.*** (2014) [121] in France found stage I patients to be more frequent than this study at time of presentation, these differences can be attributed to the insufficient primary health care programs in our country compared to developed countries, in addition to poverty, ignorance and the delay in seeking medical consultation.

5.5. Distribution of age according to stages of breast cancer

The mean age of the presentation of breast cancer patients according to the stages of the disease are: Stage I (52.29 years), stage II (54.42 years), stage III (55.00 years) and stage IV (58.50 years). No statistically significant differences were found between the means of age (years) regarding breast cancer stages.

Chaari *et al.* (2014) [121] study in France found comparable results except for the last group (stage IV) with mean age of diagnosis (49 years). **Partridge *et al*.** (2012) [122] in USA suggested that younger age was associated with more advanced stage (stage IV), this doesn't agree with our study and could be due to differences in sample size or geographical and health care conditions.

5.6. Distribution of the results of study markers

These include the following:

5.6.1. Tissue factor according to breast cancer stages:

Regarding tissue factor, there were significant differences between medians of Tissue factor (pg/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of Tissue factor (pg/ml) in comparison to other stages.

T. Ueno *et al.* (2000)[123] in Japan found tissue factor levels to be significantly elevated in most breast cancer patients with no significant association between plasma tissue factor and clinical stage suggesting that increased concentration of TF in cancer patients may result from TF production by tumor cells as well as stromal cells, while another study by **Mego *et al.*** (2015)[124] in Slovakia found no association between plasma TF levels and clinical stage, this result can be explained by the differences in sample collection and processing, technical errors or patients' variations.

Activation of clotting system often leads to more aggressive tumor biology, mechanistically, it is mainly the result of increased expression of tissue factor, which is the primary trigger in the initial activation of the clotting cascade leading to fibrin deposition. Tumor cells can express tissue factor which contributes to metastatic spread, tumor growth, and tumor angiogenesis.

5.6.2. Plasminogen activator inhibitor according to breast cancer stages:

Regarding plasminogen activator inhibitor, there were no significant differences between means of Plasminogen activator inhibitor (ng/ml) according to breast cancer stages.

Mego *et al.* (2015)[124] in Slovakia found elevated mean levels of PAI in patients with circulating tumor cells but no significant correlation with stage was found. The study of **de Jong *et al.*** (1987)[125] in Netherlands found PAI was significantly increased irrespective of the presence or absence of tumor metastasis compared to age matched healthy controls. These differences in results can be caused by smaller sample size in our study.

5.6.3. Tissue plasminogen activator according to breast cancer stages:

Regarding tissue plasminogen activator, there were no significant differences between means of Tissue plasminogen activator (ng/ml) according to stages of breast cancer.

As mentioned previously, **Mego *et al.*** (2015) [124] in Slovakia and **de Jong *et al.*** (1987) [125] in Netherlands found similar results as for PAI. In the group without metastasis a significantly decreased level of tPA activity was found but in the group with metastasis tPA activity was normal. This supports the idea that tPA plays a role in tumor progression and invasion.

5.6.4. Prothrombin time according to breast cancer stages:

Regarding prothrombin time, no significant differences were found between the means of Prothrombin time (seconds) regarding to breast cancer stages.

S. Gochhait *et al.* (2020)[126] study in India found that PT values were within normal range in different stages of breast cancer. **Chaari *et al.*** (2014)[121] study in France also found no significant correlation of prothrombin time with breast cancer which agrees with the results of this study, although another study by **F. Tas, *et al.*** (2014)[105] in Turkey showed more prolonged PT values in the more advanced stages, this result may be attributed to the differences in sample size, technique and work environment.

5.6.5. Partial thromboplastin time according to the breast cancer stages:

Regarding partial thromboplastin time, no significant differences were found between the means of Partial thromboplastin time regarding to breast cancer stages.

S. Gochhait *et al.* (2020)[126] study in India and **Chaari *et al.*** (2014)[121] study in France found no significant correlation of partial thromboplastin time with stages of breast cancer which also agrees with this study as with prothrombin time.

5.6.6. D-dimer according to stages of breast cancer

Regarding D-dimer, there were significant differences between means of D-dimer (ng/ml) according to stages of breast cancer. Patients with stage IV had significant elevation in level of D-dimer (ng/ml) in comparison to other breast cancer stages.

S. Gochhait *et al.* (2020)126 in India found that D-dimer levels were significantly raised in the group of patients with breast cancer having lymph node metastases, so we can conclude that the fibrinolytic pathway is definitely involved in carcinogenesis as well as metastases (sign of malignancy) of breast cancer, **Chaari *et al.*** (2014)[121] in France and **F. Tas, *et al.*** (2014)[105] in Turkey also agreed with our results showing significant increase in D- dimer levels in metastatic stage when compared to local stages indicating the significance of increased D-dimer level as a predictor for late-stage disease. The study of **Manjunath B. D. *et al.*** (2018)[127] in India showed elevated D- dimer levels in stages III- IV, which is a comparable result to this study. **Hermansyah *et al.*** (2022)[128] in Indonesia also found significant association with lymph nodes and metastatic status. High level of D dimer could be a marker for late stage of a patients with cancer.

Batschauer *et al.* (2010)[129] in Brazil found no significant correlation of D-dimer with lympho-nodal or metastatic status. This variation in results between the studies can be attributed to technical causes or differences in demographic variables and sample size.

Cancer is usually characterized by activation of hemostatic system and this activation extent considered correlating with a late-stage tumor. D-dimer is a biomarker that represent the activation of hemostasis and fibrinolysis.

Fibrin may increase endothelial motility, worsen angiogenesis, and contribute to an increased thrombosis risk in breast cancer patients. A previous study mentioned that various abnormalities, including thrombocytosis, an increase in fibrinogen and fibrin degradation products like D-dimer, a rise in factors V, VII, VIII, IX, and XI levels, and a decrease in antithrombin III, are seen in patients with cancer. Extracellular remodeling of fibrin is important for angiogenesis in tumors, and activation of intravascular fibrin formation and degradation had been shown to occur in patient's plasma with breast cancer.[130]

The higher D dimer levels, the higher the clinical stage will be. The plasma D-dimer levels might be used as a diagnostic marker in breast cancer patients, while elevated plasma D dimer could be a novel biomarker for late stage of a patient with solid cancer. [132]

Chapter Six

Conclusions and Recommendations

6.1 Conclusions

From the current study we can conclude the following:

1. Patients with breast cancer had higher serum tissue factor and D-dimer levels than normal ranges. Tissue factor and D- dimer levels are significantly associated with more advanced breast cancer stage (stage IV).
2. No correlation was found with tissue plasminogen activator, plasminogen activator inhibitor, prothrombin time and partial thromboplastin time between stages.
3. Tissue factor and D- dimer are considered as prognostic factors in breast cancer regarding coagulation defects when linked to clinical stage and disease progression.

6.2 Recommendations

1. A prospective cohort study with larger sample size and follow up of patients with assessment of markers' levels during the course of disease (pretreatment, during treatment and post treatment) to assess the effect of coagulation defects on patient outcome, complications and treatment responsiveness.
2. Studying the correlation of tissue factor and D- dimer with other known poor prognostic factors in breast cancer like vascular endothelial growth factor A, thrombin-antithrombin (TAT) complex, plasma microparticles and other factors.
3. Studying the association of tissue factor and D- dimer levels with other types of malignancy and comparing the results with breast cancer.
4. Educating people through health programs on the importance of routine checkup for early diagnosis of breast cancer and avoiding coagulation problems associated with advanced disease stages.
5. Educating patients with breast cancer about regular checkup of coagulation profile for early diagnosis and treatment of any defect in coagulation.
6. Suggesting treatment options involving tissue factor and D- dimer that aim to prevent or slow down metastasis.

Chapter Seven

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الطب
فرع الامراض

دراسة لبعض عوامل التخثر بين مراحل سرطان الثدي

اطروحة مقدمة إلى مجلس كلية الطب في جامعة بابل كجزء من متطلبات نيل درجة الماجستير في الطب/ الأمراض

من قبل:

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الخلاصة

الخلفية

سرطان الثدي هو أكثر أنواع السرطانات التي يتم تشخيصها والسبب الرئيسي للوفاة من السرطان بين النساء. تظهر سرطانات الثدي تباينًا كبيرًا في الإحساس بخصائصها النسيجية والتغيرات الجزيئية ومن المعروف أن هذه العوامل تؤثر على نتائج المريض وسلوكه السريري

عادة ما تصاحب تعديلات الإرقاء تطور المرض الخبيث وقد يتأثر كل مكون معروف لآلية الإرقاء بعملية المرض هذه. تنجم العلامات المختبرية المختلفة لتنشيط التخثر لدى مرضى السرطان عن نمو الورم وتكوين الأوعية الجديدة واختلال وظائف الأعضاء المصابة. قد تؤدي عوامل العلاج الكيميائي المضادة للسرطان إلى تفاقم الحالة العامة للمريض وتعجيل اضطرابات التخثر

لذلك، تركز العديد من الجهود على إيجاد مؤشرات موثوقة يمكن أن تساعد في التنبؤ بنتائج ومضاعفات سرطان الثدي وفقًا للمرحلة.

هدف الدراسة

لتقييم مستويات عامل الأنسجة، منشط البلازمينوجين النسيجي، مثبط منشط البلازمينوجين، D-dimer وكذلك ملف التخثر في مرضى سرطان الثدي.

المرضى وطرق العمل

هذه دراسة حالة ضابطة تضمنت 62 مريضة عراقية تم تشخيصها حديثًا بسرطان الثدي ولم يبدأن العلاج بعد. يتم تقسيم المرضى حسب مرحلة سرطان الثدي (المرحلة الأولى والثانية والثالثة والرابعة). تم ترتيب المرضى وفقًا لأنظمة TNM التدريجية. تم جمع عينات الدم من كل شخص، وتم إجراء الفحوصات التالية: D- ديمر، زمن البروثرومبين (PT)، وقت الثرومبوبلاستين الجزئي المنشط (aPTT) ومقايسة ELISA لـ (عامل الأنسجة، منشط البلازمينوجين النسيجي ومثبط منشط البلازمينوجين).

النتائج

تراوحت أعمار المرضى من 28 إلى 84 سنة وكانت أعلى نسبة في الفئة العمرية (50 - 59 سنة). وفقًا للأنواع الفرعية الجزيئية ، يمثل سرطان الثدي الشبيه باللمع غالبية المرضى (82.3٪) ، ويمثل سرطان الثدي الشبيه بالقاعدة فقط (3.2٪) ويمثل سرطان الثدي الموجب (14.5٪) من إجمالي المرضى. وفقًا للأنواع الفرعية النسيجية ، يمثل النوع الفرعي النسيجي القنوي غالبية المرضى (80.6٪) ، ويمثل النوع الفرعي النسيجي الفصيص (11.3٪) ويمثل النوع الفرعي النسيجي المختلط فقط (8.1٪). وبحسب مراحل المرض تمثل المرحلة الأولى (11.3٪) والمرحلة الثانية (41.9٪) والمرحلة الثالثة (24.2٪) والمرحلة الرابعة (22.6٪) من إجمالي الحالات.

كان متوسط العمر (سنوات) حسب مراحل سرطان الثدي: المرحلة الأولى (52.29 ± 11.61) والمرحلة الثانية (54.42 ± 12.31) والمرحلة الثالثة (55.00 ± 12.83) والمرحلة الرابعة (58.50 ± 14.91).

كانت القيم المتوسطة لعامل الأنسجة (pg / ml) وفقًا لمراحل سرطان الثدي: المرحلة الأولى (26.13 ± 33.25) والمرحلة الثانية (42.94 ± 49.12) والمرحلة الثالثة (37.96 ± 44.98) والمرحلة الرابعة (367.47 ± 269.83) \.

كانت القيم المتوسطة لمثبط منشط البلازمينوجين (نانوغرام / مل) وفقًا لمراحل سرطان الثدي هي: المرحلة الأولى (0.39 ± 0.30) والمرحلة الثانية (0.26 ± 0.27) والمرحلة الثالثة (0.23 ± 0.19) والمرحلة الرابعة (0.18 ± 0.22).

كانت القيم المتوسطة لمنشط البلازمينوجين النسيجي (نانوغرام / مل) وفقًا لمراحل سرطان الثدي هي: المرحلة الأولى (21.12 ± 18.37) والمرحلة الثانية (20.84 ± 15.54) والمرحلة الثالثة (16.00 ± 13.48) والمرحلة الرابعة (24.99 ± 16.62).

كانت الفروق المتوسطة في زمن البروثرومبين (بالثواني) حسب مراحل سرطان الثدي هي: المرحلة الأولى (15.54 ± 1.19)، المرحلة الثانية (14.71 ± 1.42)، المرحلة الثالثة (14.53 ± 1.39)، المرحلة الرابعة (15.44 ± 1.18).

كانت الفروق المتوسطة في زمن الثرومبوبلاستين الجزئي (بالثواني) حسب مراحل سرطان الثدي هي: المرحلة الأولى (31.39 ± 4.43)، المرحلة الثانية (31.68 ± 4.41)، المرحلة الثالثة (32.25 ± 4.23)، المرحلة الرابعة (34.54 ± 2.31).

كانت الفروق المتوسطة في D-dimer (نانوغرام / مل) وفقًا لمراحل سرطان الثدي هي: المرحلة الأولى (501.43 ± 228.65)، المرحلة الثانية (455.38 ± 135.48)، المرحلة الثالثة (466.67 ± 154.86)، المرحلة الرابعة (1181.43 ± 595.44). كانت هناك فروق ذات دلالة إحصائية بين متوسطات D-dimer (نانوغرام / مل) حسب مراحل سرطان الثدي. كان لدى المرضى الذين يعانون من المرحلة الرابعة ارتفاع كبير في مستوى D-dimer (نانوغرام / مل) مقارنة بالمراحل الأخرى من سرطان الثدي.

الأستنتاج

من الدراسة الحالية يمكننا أن نستنتج ما يلي:

المرضى الذين يعانون من سرطان الثدي لديهم مستويات أعلى من عامل أنسجة الدم ومستويات D- ديمر من الأفراد العاديين.

ترتبط مستويات عامل الأنسجة ومستويات D- ديمر بشكل كبير بمرحلة سرطان الثدي الأكثر تقدمًا (خاصة المرحلة الرابعة).

لم يتم العثور على أي ارتباط مع منشط البلازمينوجين النسيجي ، مثبط منشط البلازمينوجين ، زمن البروثرومبين وزمن الثرومبوبلاستين الجزئي بين المراحل.

يعتبر العامل النسيجي و D- ديمر عاملين تنبؤيين لسرطان الثدي فيما يتعلق بعيوب التخثر عند ارتباطهما بالمرحلة السريرية وتطور المرض. ومن ثم يمكن استخدامه للتنبؤ بنتائج عيوب التخثر في سرطان الثدي وقد تكون أهدافًا علاجية في المستقبل.