Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Medicine
Department of Chemistry and Biochemistry

Study the Effect of Receptor for Advanced Glycation End Products (RAGE) and High Mobility Group Box-1 (HMGB1)Genes Polymorphism on Breast Cancer Susceptibility in Babylon Province

A Thesis

Submitted to the Council of the College of the Medicine/ University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical Biochemistry

By

Hind Shawqi Zaki Hassan Al-Khakani

B.Sc. in Chemistry/College of Science/University of Kufa

(2009-2010)

Supervised By

Professor **Assist.Prof**

Dr. Abdulsamie H. Alta'ee **Dr.Mushtaq Q. Mohammed**

2023 A. D. **1444 A. H.**

بسم الله الرحمن الرحيم

﴿ نَرۡفَعُ دَرَجَٰتٖ مَّن نَّشَآءُۗ وَفَوۡقَ كُلِّ ذِي عِلۡمٍ عَلِيمٞ ﴾

صدق الله العظيم

(يوسف ٧٦)

Supervisor Certification

We certify that this thesis entitled " **Study the Effect of Receptor for Advanced Glycation end products (RAGE) and High Mobility Group Box-1 (HMGB1) Genes Polymorphism on Breast Cancer Susceptibility in Iraqi Female**" was carried out under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

Professor
Dr. Abdulsamie H. Alta'ee
Supervisor

Assist.Prof
Dr. Mushtaq Q. Mohammed
Supervisor

Professor
Dr. Abdulsamie Hassan Alta, ee
Head of Chemistry and Biochemistry Department College of Medicine, University of Babylon.

Committee Certification

We, the examination committee, certify that we have read thesis entitled" **Study the Effect of Receptor for Advanced Glycation End Products (RAGE) and High Mobility Group Box-1 (HMGB1) Genes Polymorphism on Breast Cancer Susceptibility in Babylon Province.** " and as examining committee examined the student " **Hind Shawqi Zaki Hassan Al-Khakani"** in its content and in our opinion it is adequate with "**Excellent"** rating as a thesis for the degree of Master in Science of Clinical Biochemistry.

Prof. Dr.
Maha Fadhil Smaism
College of Medicine
University of Babylon
(Chairman)
Date: / / 2023

Assist. Prof. Dr**.**
Manal Farhan Mohsen
College of science
University of AL-Kufa
(Member)
Date: / / 2023

Prof. Dr.
Alaa Sadeq ALaawad
College of Medicine
University of Babylon
(Member)
Date: / / 2023

Prof. Dr.
Abdulsamie Hassan Hammod Alta'ee
College of Medicine
University of Babylon
(Member and Supervisor)
Date: / / 2023

Assist. Prof. Dr.
Dr. Mushtaq Qahtan Mohammed
College of Medicine
University of Babylon
(Member and Supervisor)
Date: / / 2023

Approved by the council of the College of Medicine:

Prof. Dr.
Mohend Abbass Al-Shalah
Dean of College of Medicine/ University of Babylon
Date: / / 2023

: Dedication

This work is dedicated to:

To…… my lovely father and mother

To..... my dear husband

To.....my beloved sons

To ..... all of my family

To.....all friends and lovers

Every student has knowledge

To …… to my great country

Hind

Acknowledgment

First of all, praise is to **Allah** who enabled me to accomplish this project.

I would like to introduce my deepest thanks to my supervisor's **Prof. Dr. Abdulsamie H. Alta'ee** and **Assist.Prof. Dr. Mushtaq Q. Mohammed** for their supervision and continuous encouragement throughout the work.

I would like to express my thanks to the Head of Chemistry and Biochemistry Department **Prof. Dr. Abdulsamie Hassan Alta' ee** and all academic staff of Chemistry and Biochemistry Department, College of Medicine, University of Babylon.

I would like to thank the **Dean of the College of Medicine**, University of Babylon.

I would like to thank the working staff in **Oncology Center** in Merjan Medical City in Babylon province for their assistance in the collection of samples. Special thanks to all people participating in this study as patients and control.

Also, I'd want to express my thanks to everyone who has assisted me.

Hind

Summery

Breast cancer (BC) is the most common cancer in females worldwide. Breast cancer is an uncontrolled growth of epithelial cells originating from breast ducts or breast lobules. Breast cancers are invasive or infiltrating, it occurs when they have through the walls of the glands or ducts that originated and grown into surrounding breast tissue.

This study was designed to investigate the level of High mobility group protein box 1(HMGB1), Receptor for Advanced Glycation End Products (RAGE), Malondialdehyde (MDA) and vitamin E to find the potential association between these parameters with disease progression in women with breast cancer, and to evaluate the role of the genetic mutation (T/A RAGE and T/C HMGB1 in the gene polymorphism and associated risk of breast cancer and disease progression in Babylon Province.

To achieve the objectives of this study, a test was conducted on (100) people who were divided into two groups .The first group included 50 patients with breast cancer, while the second group included 50 control group.

The concentration of HMGB1, RAGE, MDA and vitamin E were determined by ELISA technique. DNA was extracted from blood and genotyped for RAGE T/A gene mutation rs1800624 (via RFLP-PCR technology), HMGB1 T/C gene rs1412125 (via allele-specific AS-PCR technology). The results were evaluated by various statistical analyses.

A significant increase in the level of HMGB1, RAGE and MDA was observed in breast cancer patients compared to the control group. Vitamin E level was significantly increased in patients with breast cancer. compared to control. This increase is may be due to the nutritional supplement.

On the other hand, the mutation genotypes of RAGE gene T/A rs1800624, HMGB1 T/C gene rs1412125 in breast cancer patients did not show any significant association with disease ($p > 0.05$).

Finally, the gene polymorphism is not associated with the RAGE T/A gene rs1800624 and HMGB1 T/C rs1412125 gene polymorphism associated in breast cancer.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
BMI	Body mass index
CNB	Core Needle Biopsy
ICC	cribriform carcinoma
DAMPs	Damage associated molecular patterns
DNA	Deoxyribonucleic acid
DCIS	ductal carcinoma in situ
ER	Estrogen receptors
FNA	Fine Needle Aspiration
HMGB1	High Mobility Group Box 1
Her2	human epidermal growth factor receptor 2
IDC	Infiltrating Ductal Carcinoma
ILC	Infiltrating Lobular Carcinoma
LCIS	lobular carcinoma in situ
MRI	Magnetic resonance imaging
MDA	Malondialdehyde
MRM	Modified radical mastectomy
NLSs	nuclear localizing sequences
PCR	Polymerase chain reaction
PR	progesterone receptors
ROS	reactive oxygen species
RAGE	Receptor for Advanced Glycation End Products
SNP	Single Nucleotide Polymorphisms

Chapter One

Introduction

and

Literature Review

1. Introduction

1.1. General Introduction

Breast cancer (BC) is the most common cancer in females worldwide [1]. Breast cancer is an uncontrolled growth of epithelial cells originating from breast ducts or breast lobules. This disorder includes early noninvasive breast cancer, such as ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS), breast cancer with basement membrane invasive (primary invasive breast cancer) and breast cancer that has spread to distant lymph nodes or metastasize to distant organs [2].

Breast cancer is the most common cancer among women, comprising 23% of the 1.1 million female cancers that are newly diagnosed each year [3].

In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry [4]. This shows that the breast is the leading cancer site among the Iraqi population in general, exceeding even bronchogenic cancer. The incidence of breast cancer increased in Iraq in recent years, causig a significant health problem [5].

1.2. Risk Factors for Breast Cancer include

1.2.1. Non-Modifiable Risk Factors

1.2.1.1. Age and Gender

The vast majority of cases of breast cancer, reaching 99%, occur in women. Only 1% of cases of this malignant tumor affect men. However, the incidence of breast cancer in men, like that in women, shows a steady upward trend, which is most likely associated with obesity [6].

After gender, age is the most important known risk factor for breast cancer , because the incidence of breast cancer is highly related to the increasing age. Therefore, it is necessary to have a mammography screening ahead of time in women aged 40 or older [7].

Numerous studies indicate that breast cancer in young women is characterized by high grade malignancy, frequent overexpression of the HER-2 receptor or occurs as a molecular biological subtype (“triple negative”) [8].

1.2.1.2. Genetic Predisposition

The Genetic Predisposition form about (5–10%) of breast cancer cases. The best-known genetic mutations associated with this cancer include mutations in the BRCA1 and BRCA2 genes [9],[10].

Women with BRCA1 or BRCA2 mutations have an increased risk of developing breast cancer. These women have up to 50% chance of developing breast cancer. BRCA1 mutations also increase the risk of ovarian cancer. When testing positive for these mutations, frequent monitoring of the cancer by MRI or prophylactic mastectomy should be performed [10].

1.2.1.3. Family History

The risk of developing breast cancer increases twice in women whose closest relative (mother, sister) has been treated for the malignant tumor and by three to six times if the two closest relatives have been treated [11].

1.2.1.4. Menstrual History

The hormonal history of exposure to high levels of estrogen and progesterone considered as an important risk factor. Women befor 13 years old of onset of menstruation, women having no children or having a child after age

30, and menopause women after age 50, all those considered more menstrual cycles and have a greater period of hormone exposure [12].

1.2.2. Modifiable Risk Factors

1.2.2.1. Environmental Factors and Exposure to Carcinogens

Ionizing radiation and other carcinogens that are caused mutation in the DNA of normal cells and converted it to abnormal cancerous cells were increased the risk of developing breast cancer [13].

1.2.2.2. Reproductive and Hormonal Factors

Estrogens play an important role in the pathogenesis of the development of breast cancer. Breast cancer is considered a hormone-dependent tumor in which elevated estrogen levels and longer exposure to this hormone are associated with an increased risk of its development [14],[15].

1.2.2.3. Obesity

One of the risk factors for developing breast cancer, confirmed in many studies, is obesity. many studies explained the relationship between obesity and breast cancer prevalence in premenopausal and postmenopausal women [16]. These research found that both overweight and obesity increased the risk of developing breast cancer, particularly steroid-receptor-expressed breast cancer, in postmenopausal women who did not use hormone replacement therapy [17],[18].

1.2.2.4. Smoking

Research reports on the impact of chronic nicotinism on the increased risk of breast cancer are contradictory. However, a study by Jones [19]. published in 2017 showed that smoking, especially at the beginning of early peripubertal

age or adolescence, was associated with a moderate but statistically significant increase in the risk of developing breast cancer [8].

1.2.2.5. Oxidative stress

There is increasing evidence indicating that oxidative stress is involved in the pathogenesis of breast cancer. Exposures from endogenous and exogenous oxidant sources constantly produce reactive oxygen species (ROS) including superoxide radicals, hydrogen peroxide, and hydroxyl radicals in vivo [20]. These ROS cause oxidative damage to biomolecules (e.g., DNA, protein, and lipids), and can cause genetic alterations, a process held in check only by the existence of multiple antioxidant systems that alter the balance between prooxidant cellular activity and antioxidant defenses [21],[22].

1.3. Breast Anatomy

Breasts are composed of fatty tissue that contains the glands responsible for milk production in late pregnancy and after childbirth. Within each breast, there are about 15 to 25 lobes formed by groups of lobules, the milk glands. Each lobule is composed of grape-like clusters of acini (also called alveoli), the hollow sacs that make and hold breast milk. The lobules are arranged around ducts that funnel milk to the nipples. About 15 to 20 ducts come together before they reach the nipple surface [23],[24] , as figure 1-1.

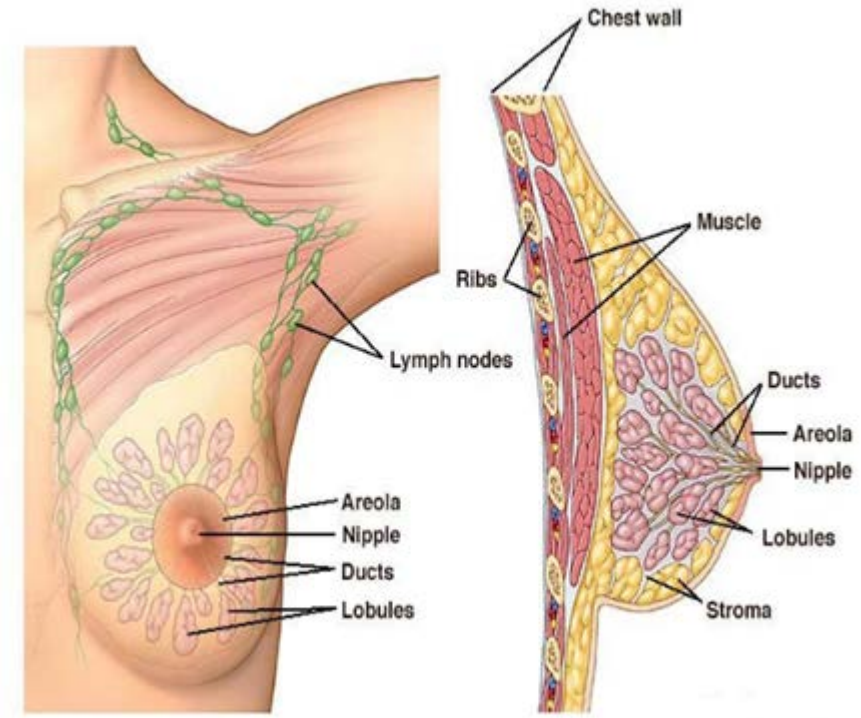

Figure 1-1 : Anatomy of the normal Breast Tissue [25].

1.4. Pathology of Breast Cancer

Breast cancer begins when cells in the breast start to grow out of control. These cells often are seen through an mammogram or its be felt as a lump when forming a tumors. The breast cancer occurs almost entirely in women, but men can get breast cancer, breast cancers can originate from different parts of the breast [26].

Sarcomas and lymphomas are also other types of breast cancer that are less common small number of growth start in other tissues in the breast. When cancer cells of the breast get into the blood or lymphaticsystem, breast cancer can metastasize and these cells are carried to other parts of the body [27].

The lymphatic system is a network of lymphatic vessels found throughout the body that connects lymph nodes. The clear fluid that is present in the lymph vessels, called lymph, contains tissue byproducts and waste material, as well as immune system cells. In breast cancer, cancer cells can enter lymph

vessels and start to grow in lymph nodes. Most of the lymph vessels of the breast drain into: [28].

1. Axillary nodes: Lymph nodes under the arm
2. Supraclavicular: Lymph nodes above the collar bone
3. Infraclavicular: Lymph nodes below the collar bone
4. Internal mammary lymph nodes: Lymph nodes inside the chest near the sternum.

If cancer cells are carried by lymph nodes, there is a higher chance of cells passing through the lymphatic system and metastasizing to other parts of the body. If there are more lymph nodes with breast cancer cells, the chance of finding cancer in other organs is higher. If cancer is present in one or more lymph nodes, the treatment plan will be affected. Often, patients need surgery to remove one or more lymph nodes to determine if the cancer has spread. However, not all women who have cancer cells in their lymph nodes will develop metastases, and some women who do not have cancer cells in their lymph nodes will later develop metastases [29].

1.5. Histopathological Types of Breast Cancer

1.5.1. Noninvasive (In situ carcinoma)

1.5.1.1. Ductal Carcinoma in -Situ (DCIS)

The most general kind of non-invasive breast cancer, is limited to the breast duct DCIS starts out as a mass that grows in a milk duct, which carries milk from the lobules, or glands, to the nipple. Over time, chances increase for the mass to break through the ductal walls into the surrounding tissue and fat of the breast [30].

DCIS may be a potential precursor to invasive cancer and is also associated with an increased risk for developing new invasive breast cancer [31].

1.5.1.2. Lobular Carcinoma in Situ (LCIS)

It called lobular neoplasia. It occurs when the abnormal cells growing within and expanding some of the lobules of the breast. LCIS may consider a risk factor for developing invasive cancer, but it's generally not considered to be a precursor of invasive cancer [32].

1.5.2. Invasive Carcinoma

About (80%) of breast cancers are invasive or infiltrative, it occurs when they invade the walls of the glands or ducts that originated and grown into surrounding breast tissue [33],[34].

1.5.2.1. Invasive Ductal Carcinoma (IDC)

Infiltrating ductal carcinoma is also recognized as invasive ductal carcinoma. IDC originates from ductules of breast and extends to the duct wall, invading the breast fatty tissues and probably other parts of the body [35].

1.5.2.2. Invasive Lobular Carcinoma (ILC)

Invasive lobular carcinoma (ILC) comprises approximately 10% of breast cancers and is less common than infiltrating ductal carcinoma (IDC). ILC is from originates (lobules) of the breast, but frequently extends to other areas of the body [35],[36].

ILC is hormonally mediated, more common in older age, tends to be multicentric, estrogen/progesterone receptor (ER/PR) positive and HER2-neu negative [37].

1.5.2.3. cribriform carcinoma (ICC)

Invasive cribriform carcinoma (ICC) is a rare type of primary breast carcinoma with an incidence of 0.3%-3.5% [38]. Its main characteristic is a

unique structure with most of its invasive components arranged in a cribriform pattern [39].

1.5.2.4. Mucinous carcinoma

Mucinous carcinoma accounts for approximately 2% of all breast cancer and is a rare subtype of infiltrating ductal carcinoma. It often presents as a lobulated, well-circumscribed mass on mammography, sonography, and magnetic resonance imaging and can therefore be mistaken for a benign [40]. The incidence of mucinous carcinoma increases with age being approximately 1% in women < 35 years of age and 7% in women> 75 years of age [40].

1.5.2.5. Comedo ductal carcinoma

Comedo-DCIS is a histologic subtype of preinvasive breast neoplasia that is characterized by prominent apoptotic cell death and has greater malignant potential than other DCIS subtypes [41]. One clinically important histologic feature is the presence of intralumenal (comedo) necrosis [42].

DCIS with comedo histology are most likely to recur following surgical excision or progress to invasive cancer. In comparison to non-comedo-DCIS, comedo DCIS lesions are populated by larger and more pleomorphic neoplastic cells. There is no information yet on the mechanisms of apoptosis in comedo-DCIS or the role of apoptosis in the transformation of comedo-DCIS into invasive carcinoma [43].

1.5.2.6. Tubular carcinoma

Tubular carcinomas are a special type of infiltrating - (invasive) breast carcinoma. Women with tubular carcinoma generally have a better prognosis than women with more common types of invasive carcinoma. Tubular carcinomas account for around 2% of breast cancer diagnoses [44].

1.5.2.6. Other types

There are several other types of breast cancer ,including medullary carcinoma also is an invasive breast cancer that designs a discrete margin normal tissue and medullary tissue. Others, inflammatory breast cancer is the form of swollen breasts (red and warm) with dimples or broad ridges due to cancer cells blocking lymphatic vessels or channels in the skin over the breast. Inflammatory breast cancer is uncommon and is tremendously fast-growing [35].

Paget's disease of the nipple is a rare form of breast cancer characterized by the presence of intra epidermal tumour cells. It is often associated with ductal carcinoma in situ (DCIS) or invasive cancer in the breast parenchyma [45].

1.5.3. Grade

The grade of breast cancer refers to how much cancer cells have similarity with healthy cells when examined under a microscope. Determining the grade of breast cancer may help the doctor to predict how quickly the cancer will metastasize. If cancer tissues look like normal tissues, it is called a low-grade tumor. If the cancerous tissues appear very different from normal tissue, it is called a high-grade tumor. In general, the lower breast cancer grade considers the better prognosis [46], as shown in Table 1-1.

Table 1-1: Grading of Breast Cancer [47]**.**

Grading	
Grade 1 (Low grade)	Cancer cells look a little different from normal cells. They are usually slow-growing.
Grade2 (Intermediate Grade)	Cancer cells do not look like normal cells. They are growing faster than grade 1 breast cancer, but not as fast as grade 3.
Grade 3 (High grade)	Cancer cells look very different from normal cells. They are fast-growing.

1.5.4. Hormone receptor

Hormonal receptors play a key role in regulating the growth and differentiation of breast epithelium and hormone receptor status is a prognostic indicator in invasive carcinoma. The expression of hormone receptors as determined by immunehistochemical stains indicates that the cells retain the ability to be manipulated by exogenous hormone therapy [48].

Estrogen receptors (ER) and progesterone receptors (PR) are uniformly expressed in normal breast tissue, ductal hyperplasia of the usual type. In DCIS, there is less uniform expression, particularly in the higher-grade lesions. The overall expression of estrogen receptors in DCIS is about 75% [48].

1.5.5. Her2

human epidermal growth factor receptor 2(HER2) is a transmembrane growth factor receptor found in normal and malignant breast epithelial cells. Phosphorylation of the intracellular tyrosine kinase results in intracellular signaling and activation of genes involved in cell growth. Overexpression of HER2 has independent prognostic significance in early breast cancer and may also predict response to hormonal and cytotoxic therapies HER2 is a relatively new prognostic marker and holds promise for predicting response to various therapies and for target specific therapy [49].

1.6. Molecular Subtypes of Breast Cancer

Breast cancer is grouped into four basic subgroups according to these:

1.6.1. Luminal A

Luminal A is typically low grade with excellent prognosis, ER/PR positive and HER2 negative, Ki-67 low, with high expression of ER-related genes and low expression of proliferation related genes [50].

1.6.2. Luminal B:

Luminal B breast cancer is having an aggressive clinical behavior, with prognosis similar to that of HER2-enriched and basal like groups. Luminal B tumours are higher grade with worse prognosis, and may be PR negative and/or HER2 positive with high expression of proliferation related genes. Luminal B cancers show increased relapse rates in the first 5 years after diagnosis, overall survival, and a metast free survival dissemination time pattern similar to basal-like and HER2-enriched cancers [51].

1.6.3. Her2\neu+ enriched

Human Epidermal Growth Factor Receptor 2-positive (HER2+) breast cancer (BC) is a highly aggressive disease commonly treated with chemotherapy and anti-HER2 drugs, including trastuzumab. HER2+ BC is caused by over-expression/amplification of the HER2/ERBB2/NEU tyrosine ki- nase receptor and constitutes 15–20% of cases [52].

1.6.4. Triple-negative

Triple-negative breast cancers are defined as tumors that lack expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. Basal-like breast cancers constitute one of four intrinsic subgroups of breast cancer, the existence of which was revealed by microarray-based expression profiling studies. This subgroup is characterized by an absence or low levels of expression of ER, an absence of HER2 overexpression, and expression of genes usually found in basal or myoepithelial cells of the normal breast [53].

1.7. Staging and Grading of Breast Cancer

1.7.1. Staging

Staging system was mainly based on: The stage is based on the size and degree of local invasion by the primary tumor and is categorized from T1 to T4. The N stage is determined by the extent of nodal involvement including axillary, internal mammary, and ipsilateral supraclavicular lymph nodes. M describes if the cancer has spread to another part of the body. The aim of cancer staging is to identify the prevalence of the disease and to help to develop the treatment plan [54], as shown in Table 1-2.

Table 1-2: Staging Breast Tumors According to TNM Classification [55].

	T category	N category	M category
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1, N2	M0
Stage IIIB	T4	N0,N1,N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

1.8. Symptoms of Breast Cancer

The most common symptom of breast cancer is a new lump or mass. A painless, hard mass that has irregular borders is more likely to be cancerous, but breast cancers can be tender, soft, or rounded. They can even be painful. For this reason, it is important to have any new breast mass or lump or breast change checked by a health care professional experienced in diagnosing breast diseases. Other possible signs of breast cancer include:

• Swelling of all or part of a breast (even if no distinct lump is felt)

• Skin irritation or dimpling.

• Breast or nipple pain.

• Nipple retraction (turning inward).

• Redness, scaliness, or thickening of the nipple or breast skin.

• Nipple discharge (other than breast milk [41].

1.9. Diagnosis of Breast Cancer

1.9.1. Mammography

Mammography is a specific type of imaging that uses a low-dose X-ray system to examine the breast, and is currently the most effective method for detection of breast cancer before it becomes clinically palpable .Mammography offers high-quality images at a low radiation dose. Current guidelines of the American Cancer Society (ACS) recommend that women aged 40–49 years have a routine mammogram every one to two years, with the first beginning at age 40 [56].

1.9.2. Ultrasound

Ultrasound imaging is one of the most effective tools as an adjunct to mammography to detect and diagnose abnormalities in the breast. Studies show that ultrasound is able to detect and discriminate benign and malignant masses with high accuracy and reduce the number of unnecessary biopsy ultrasound is more sensitive for detecting invasive cancer in dense breasts [57].

1.9.3. Magnetic resonance imaging (MRI)

Magnetic resonance imaging creates image at different cross-sections by applying strong magnetic field with RF signals, and contrast agent can be applied to increase the resolution of MRI image. Breast MRI has been recommended for subjects with high breast cancer risk, the MRI tests have been suggested for specific population groups including BRCA mutation carriers and subjects with high breast cancer risk Compared to mammography and ultrasound, MRI is less specific but more sensitive to detect small tumors in subjects with high breast cancer risk [26].

1.9.4. Fine Needle Aspiration

Fine Needle Aspiration (FNA) is a diagnostic technique of a tumor that is used in the diagnosis of breast lesions. Fine needle is inserted into the mass with very slow and the specimen is collected and placed on a slide for microscopic examinations [58].

1.9.5. Core Needle Biopsy (CNB)

This technique is used when there is no experience cytopathologist to evaluate results from an FNA. CNB provides material for histological evaluation by distinguishing between ductal carcinoma in situ and invasive cancer [59].

1.9.6. Excisional Biopsy

Excisional Biopsy is the surgical removal of a breast mass with the surrounding normal breast tissue. It's used when a Needle biopsy is not feasible or discordant with imaging results. The breast mass is removed and examined for biopsy [59].

1.10. Treatment of Breast Cancer

1.10.1. Surgery

Most patients with breast cancer have surgery to remove the cancer from the breast. Some of the lymph nodes under the arm are usually taken out and looked at under a microscope to see if they contain cancer cells . Surgery for breast cancer consists of two main options [60].

1.10.1.1. Lumpectomy

Surgery to remove a tumor (lump) and a small amount of normal tissue around it [61].

1.10.1.2. mastectomy

1.10.1.2.1. Modified radical mastectomy

Surgery is one of the mainstays of treatment. Modified radical mastectomy (MRM) typically involves removal of the entire breast, including the skin, areola, nipple, and most axillary lymph nodes, but the pectoralis major muscle is spared [62].

1.10.1.2.2. Breast-conserving surgery

Breast-conserving surgery is currently the standard treatment for most patients with early-stage breast cancer [63].

may also have some of the lymph nodes under the arm removed for biopsy [64].

1.10.2.Systemic Therapy

1.10.2.1.Cytotoxic therapy

Chemotherapy is a treatment using anti-cancer (cytotoxic) drugs. Chemotherapy can be given before surgery (this is called primary or (neo-adjuvant chemotherapy) to try and reduce the size of a tumor, or after surgery (adjuvant chemotherapy) [65].

The aim of chemotherapy given after surgery is to destroy any cancer cells that may have spread from the breast to other parts of the body [66].

Targeted therapy HER2-positive patients left untreated have a worse prognosis. trastuzumab given together with chemotherapy in early breast cancer [25].

1.10.3. Hormonal Therapy

Approximately 80% of breast cancer patients have ER-positive tumors, the growth of which is stimulated by hormones. Adjuvant endocrine therapy either blocks or lowers the circulating endogenous hormone levels, thereby reducing both local and distant recurrences. They are administered following chemotherapy since they decrease the proportion of proliferating cells, making the tumor less sensitive to chemotherapy in ER-positive patients [25].

1.10.4. Radiotherapy Therapy

Radiotherapy plays a vital role in local treatment of lymph node metastasis in breast cancer. However, radiotherapy has effect on both normal and tumor cells, which means patient have to suffer side effects that come along, and the toxicity is always dose dependent [67].

1.11. Damage Associated Molecular Patterns in Breast Cancer

Damage associated molecular patterns (DAMPs) are nuclear or cytosolic proteins with defined intracellular function that are released outside the cell following tissue injury. This transmitted from the intracellular space to the extracellular space moves the DAMPs from a reducing to an oxidizing environment causing their functional denaturation, resulting in their loss of function [68].

DAMPs are endogenous danger signals that are discharged to the extracellular space in response to damage to the cell from trauma or pathogen. They are expressed in different cell types and function in normal cellular homeostasis. They are localized in the nucleus and cytoplasm (HMGB1), cytoplasm (S100 proteins), exosomes (heat shock protein) and extracellular matrix (hyaluronic acid). On the basis of their origin and mechanism of action, the pro-inflammatory DAMP molecules can be classified as those that directly stimulate cells of the innate immune system and those that generate DAMPs from other extracellular molecules [69].

1.11.1. High Mobility Group Box 1 (HMGB1)

High mobility group protein box 1 (HMGB1) is a member of the high mobility group protein superfamily with secretory and intracellular activity superfamily with secretory and intracellular activity [70].HMGB1 is encoded by the HMGB1 gene (13q12) in human [71].

High mobility group box-1 (HMGB1) is a factor regulating malignant tumorigenesis and proliferation [72].

1.11.2. Structure of High Mobility Group Box 1 (HMGB1)

HMGB1 is a 215 amino acid long protein that consists of two DNA-binding domains (HMG A box and HMG B box) and a C-terminal acidic tail in

the nucleus, HMGB1 performs its binding and bending functions with DNA mainly through its A and B box domains respectively. The C-terminal acidic tail is composed of continuous aspartate and glutamate residues, and has a protective effect on HMG A and B box during the transport of HMGB1 from the nucleus to the cytoplasm [73] [74], figure 1-2.

Figure 1-2: Structure of High Mobility Group Box 1 (HMGB1) [74].

1.11.3. Mechanism Release of High Mobility Group Box 1 (HMGB1)

There are two nuclear localizing sequences (NLSs) present in HMGB1, one in Box-A and other in Box-B. Both NLSs have lysine AA residues. Hyperacetylation of these lysine residues enables translocation of HMGB1 from nucleus to cytoplasm [75].

The shuttling of HMGB1 protein from nucleus to cytoplasm was determined by the overall acetylation state. There are two possible mechanisms for the release of nuclear HMGB1 protein by the immune cells, active secretion or passive secretion [76].

Active release of HMGB1 from macrophage or monocytes requires a pro-inflammatory stimulus that was responsible for the acetylation of HMGB1 and leading to accumulation in cytoplasm. Active secretion of HMGB1 could not be possible either by Endoplasmic reticulum or Golgi complex secretory pathway due to absence of leader peptide sequence HMGB1 was actively secreted by inflammatory cells like macrophages or monocytes during the tissue injury and oxidative stress [77].

Passive release of HMGB1 extent of acetylation was the key factor responsible for the release and binding of HMGB1 with dsDNA . In necrotic cells, the extents of hyper-acetylation weaken the binding of HMGB1 to DNA and thus facilitated the release of HMGB1 from nucleus in passive manner. While in apoptotic cells intracellular maintenance of hypoacetylated HMGB1 was due to its strong binding with dsDNA thus limited release into nearby environment, hence responsible for causing insignificant inflammation in the neighboring tissue after apoptosis [78], figure 1-3.

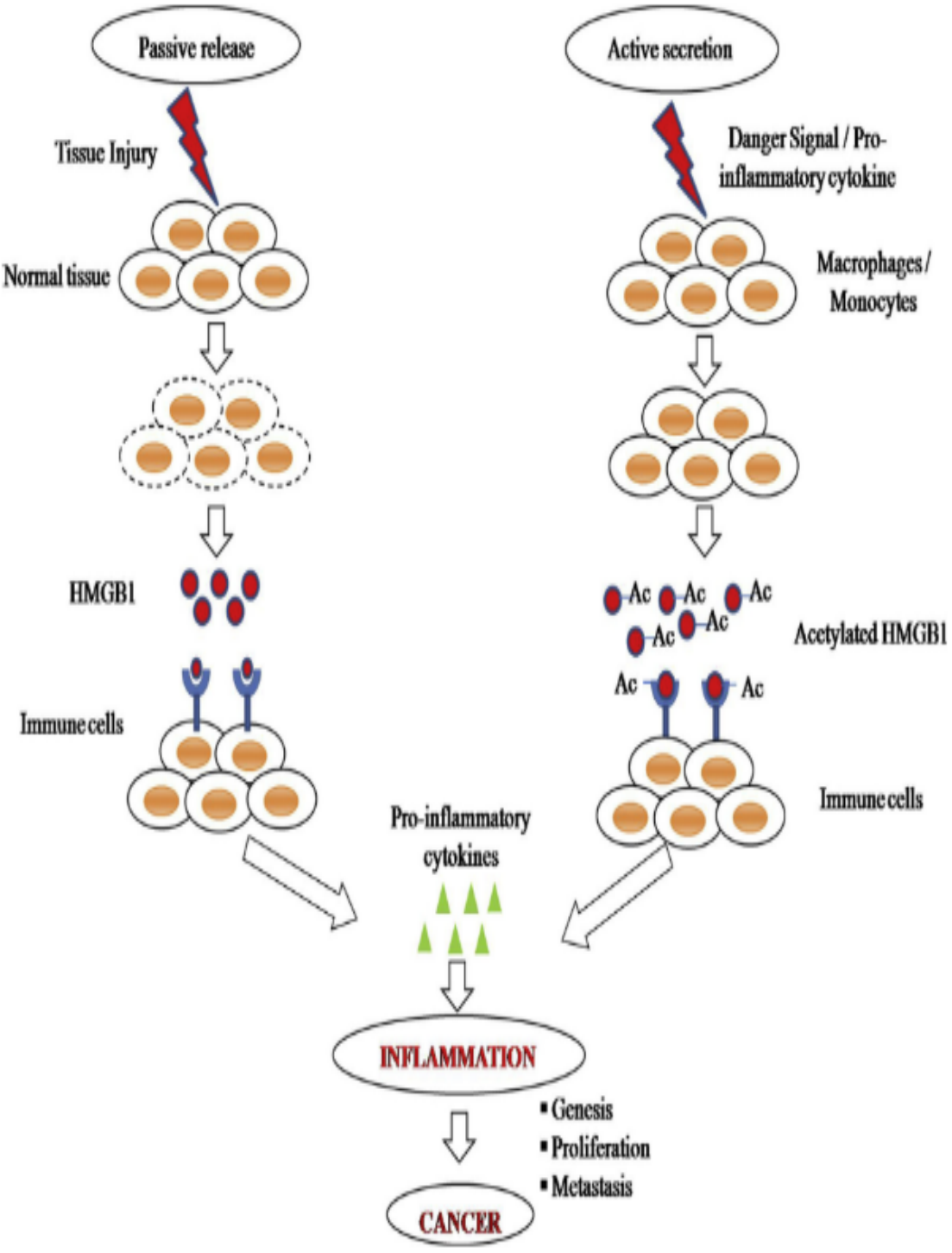

Figure 1.3: Schematic representation of active secretion and passive release mechanism of HMGB1 protein [78].

1.11.4. Role of HMGB1 Protein in Breast Cancers:

High mobility group protein box 1 (HMGB1) has a complex role in carcinogenesis because it has both pro- and anti-tumorigenic bioactivities HMGB1 up-regulation or down-regulation varies with the type of cancer [79].

Moreover HMGB1 has mixed effects on the hallmarks of cancer namely unlimited replicative potential, ability to develop blood vessels (angiogenesis) evasion of programmed cell death (apoptosis [80].

Serum HMGB1 could be a useful serological biomarker for diagnosis and screening of breast cancer [81].

Finding revealed that HMGB1 could be able to promote growth of breast cancer cells in-vitro. HMGB1 overexpression has been associated with increased apoptosis when BC cell lines have been treated with anti-HMGB1 promotes angiogenesis by binding to its receptor RAGE, activating NF-κB up-regulating leukocyte adhesion molecules and the production of proinflammatory cytokines and angiogenic factors in both cancer drugs and radiation thereby [82].

High mobility group protein box 1 (HMGB1) is a crucial factor in the development and progression of breast cancer. Binding of HMGB1 to RAGE has been associated with tumor cell survival, progression, and metastasis [83].

HMGB1 protects cells from apoptosis because it affects telomere stability and stimulates certain cellular proteins involved in the proliferation of cancer cells [84].

High mobility group protein box 1 (HMGB1) may prove to be useful biomarker to detect the progression of BC and could be targeted by gene therapy technique for cure[85].

1.12. Receptor for Advanced Glycation End Products (RAGE)

The human (RAGE) gene is located on chromosome 6p21.3, has a total length of 3.27 kb, and contains 11 exons [86].

RAGE, a Cell surface protein, is a multi-ligand receptor of the immunoglobulin super- family that is expressed in combination with certain active ligands including advanced glycosylation end-products (AGEs) and several members of the S100 family [87].

RAGE is considered a receptor for HMGB1 and several S100 proteins, which are Damage-Associated Molecular Pattern molecules (DAMPs) released during tissue damage [88].

When RAGE is combined with ligands, it promotes the downstream phosphorylation of the p38 MAPK protein, thus increasing the expression of the NF-κB signaling path. NF-κB encourages the expression of inflammatory factors, such as TNF-α and IL-1β, by regulating the target genes and triggering related inflammatory and autoimmune responses, resulting in persistent tissue damage [89], as shown in the figure1-4.

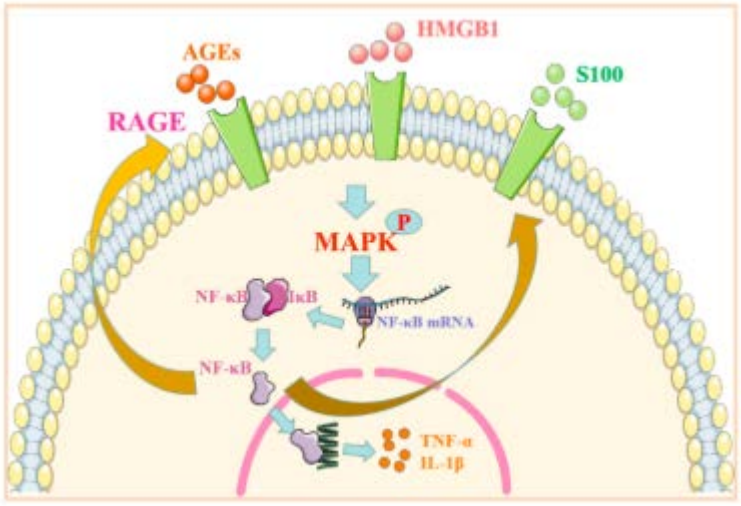

Figure 1.4: Receptor Advanced Glycation End Product (RAGE) regulates the MAPK/NF-κB signaling pathway. After binding to the ligand, RAGE

phosphorylates its downstream MAPK and activates NF-κB protein. NF-κB enters the nucleus to promote the transcriptional expression of inflammatory factors [89].

Studies have revealed that high-sugar diets, amyloidosis, oxidative stress, and other unique environments can significantly induce RAGE expression on the surface of smooth muscle cells, neurons, and other cells. RAGE participates in critical physiological processes, such as regression of inflammation, maintenance of cell homeostasis, and postinjury repair and regeneration[90] .For example, a low concentration of S100B regulates cell proliferation and differentiation through RAGE under physiological conditions. Under pathological conditions, the combination of S100B and RAGE stimulates the release of proinflammatory cytokines [91].

RAGE plays a master regulator of origin, invasion and metastasis of tumours by binding with AGEs, HMGB1, or S100 group of proteins, which are expressed largely during pathological conditions of glycation and inflammation. The receptor-ligand duo serves as the key target for prevention and successful treatment of cancers, irrespective of their site of origin, molecular subtype, and disease stage. It confers selective cytotoxicity to drugs targeting RAGE and its ligands, which when identified could be used in combination with standard conventional chemotherapy for effective control of progression and metastasis of cancers, simultaneously devoid of any adverse effects to normal cells [91].

The formation of the AGER complex primarily includes components such as non-enzymatic glycation proteins and lipoproteins, and its protein structure is comprised of an extracellular domain and a single transmembrane domain with a short cytosolic tail [92].

AGEs belong to a heterogeneous, complex group of compounds formed either exogenously or endogenously by different mechanisms and from a variety of precursors [93].

Advanced glycosylation end-products (AGEs) the products of nonenzymatic glycation condensation and oxidation between carbonyl groups of reducing sugars and free amine groups of nucleic acids, proteins or lipids, which form as post-translational modifications of proteins and lipids, primarily on lysine and arginine groups within the backbone protein [93] [94].

AGER expression is induced by excess oxidative stress and increased inflammatory responses after tissue injury, and its expression has been suggested to have devastating effects on tissue reorganization. Accordingly, scholars have suggested that the risk of development of BC might be attributed to various mutations in the AGER gene related to tumor progression [95].

1.12.1. RAGE Structure

RAGE consists of extracellular, hydrophobic, transmembrane, and intracellular segments. The extracellular V-shaped region provides RAGE–ligand binding sites. Intracellular fragments can bind to various intracellular signal molecules and mediate signal transduction to cause cascade reactions, including full-length type, truncated C-terminal type, and truncated N-terminal type [96].

The truncated C-terminal type is endogenous secretory soluble RAGE (esRAGE), which can be secreted by cells and contains only the extracellular segment. In contrast, the truncated N-terminal type consists of the transmembrane region and an intracellular component. Membrane-associated proteases can remove the transmembrane component of RAGE by hydrolysis, and the released extracellular segment can form soluble sRAGE with esRAGE. sRAGE can competitively bind to RAGE ligands, but binding to ligands

terminates intracellular signal transduction due to the loss of transmembrane and intracellular fragments [91], as Figure 1-5.

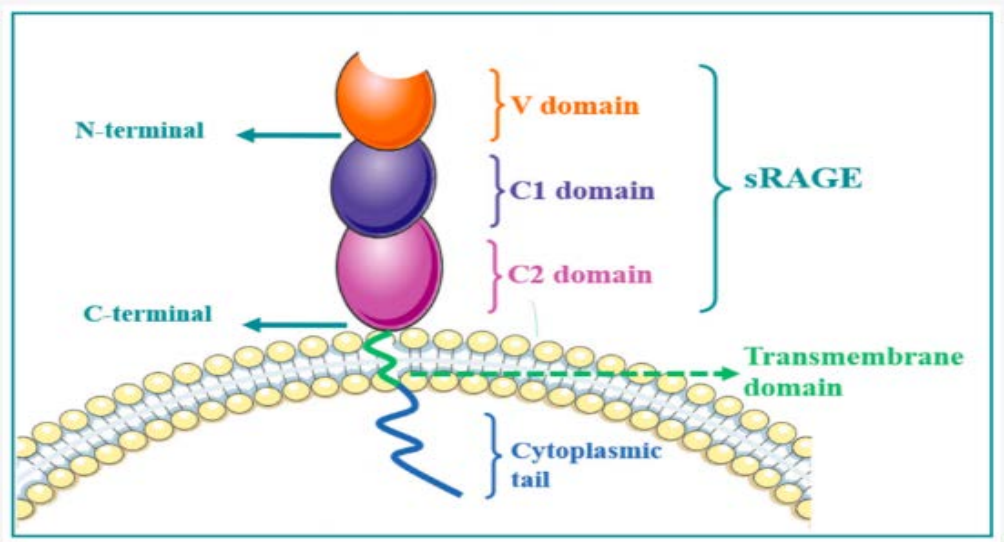

Figure 1-5: RAGE structural organization.

1.13. Single Nucleotide Polymorphisms

SNPs are variants in the genome occurring naturally in the human population." Each individual inherits one allele copy from each parent, so that the individual genotype at an SNP site is AA, BB, or AB [97]. SNP is defined as a genomic locus where two or more alternative bases occur with appreciable frequency (>1%). SNPs are the most frequent type of variation in the human genome, occurring once every several hundred base pairs throughout the genome (200). For any given SNP, the SNP can occur in a coding region but not result in a change in amino acid, it can occur in a coding region with an amino acid change, it can occur in a regulatory region where the result is a change in gene expression, or it can occur in a region between genes [98].

1.14.The Role of Oxidative Stress in Breast Cancer Risk and Prognosis

Oxidative stress can be defined as an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage. If the level of reactive species is high and overcomes the antioxidant defense mechanisms of the human body, oxidative damage can occur to lipids, proteins, or directly to DNA [99].

DNA damage is hypothesized to play an important role in the initiation of carcinogenesis Oxidative stress mechanisms are also involved in the activation of cell signaling pathways, including tumor cell proliferation, increased tumor cell migration, and increased tumor cell proangiogenic factors, and play a key role in apoptosis, mechanisms that can impact both cancer progression and metastasis. Increased reactive oxygen species (ROS) and the resulting high oxidative stress are key characteristics of malignant tumors. Many cancer treatments, such as radiotherapy and certain chemotherapy agents, act through oxidative stress pathways via the production of ROS to kill tumor cells [99].

Several biomarkers of oxidative stress have been identified for use in studies and can be measured in various biological samples including both blood and urine. Commonly used biomarkers include DNA damage biomarkers, such as 8-hydroxy-2 deoxyguanosine, protein carbonyl groups as a marker of protein oxidation, and malondialdehyde (MDA) as markers of lipid peroxidation [100],[101], as Figure 1-6.

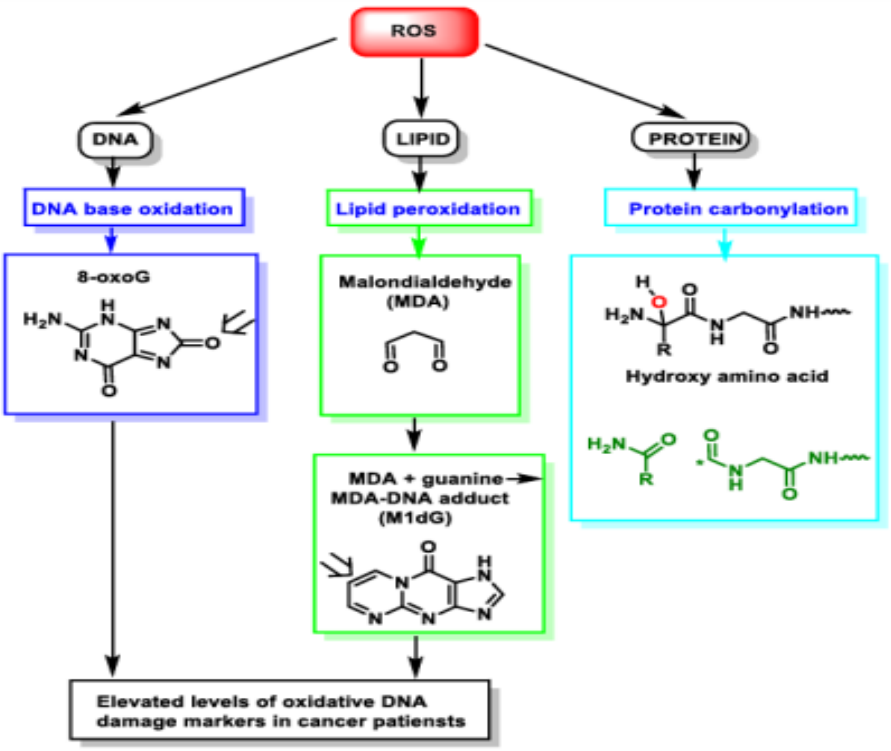

Figure 1-6: ROS action on DNA, lipids and proteins lead to DNA base oxidation, lipid peroxidation and protein carbonylation [102].

Biomarkers of oxidative stress have been investigated for their association with the development and progression of several cancer types, and in particular breast cancer, as oxidative stress mechanisms may be involved in several known breast cancer risk factors Breast cancer cells have been shown to be susceptible to oxidative damage and have high levels of oxidative stress, including protein damage, DNA damage, and lipid peroxidation several breast cancer risk factors may alter levels of endogenous oxidative stress [99],[103].

Non- enzymatic anti-oxidants include a variety of exogenous biological molecules such as glutathione, vitamin C, vitamin E, and carotenoids [104].

An anti- oxidants system may work to either prevent the formation of ROS (primary anti-oxidants) or react with the ROS to neutralize or inhibit their actions (secondary anti-oxidants) [103].

1.14.1. Malondialdehyde (MDA) as alipid peroxidation marker

MDA is a highly reactive three-carbon dialdehyde formed as a result of the depletion of antioxidant systems during the peroxidation of polyunsaturated fatty acids by reactive oxygen species. MDA comes in two forms: endogenous from lipid peroxidation and exogenous from diet [105].

Lipid peroxidation, which is the result of non- enzymatic auto-oxidation of polyunsaturated fatty acids. Malondialdehyde (MDA) is one of the final decomposition products of lipid peroxidation(polyunsaturated fatty acids peroxidation in the cells) . and it is also formed as a product of the cyclooxygenase reaction in prostaglandin metabolism. MDA may be involved in tumour promotion because it can interact with functional groups of a variety of cellular compounds, including the amino groups of proteins and nucleic acid bases, the N bases of phospholipids and the SH groups of sulphydryl compounds [106].

MDA alters the physical structure of cell membranes and is involved in the creation of protein, DNA, and RNA in an indirect manner. It also has carcinogenic and mutagenic effects. For many years, MDA has been utilized as a biomarker for lipid peroxidation and oxidative stress [107].

An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress [108].

1.14.2. Vitamin E:

The term vitamin E includes eight different chemical forms four tocopherols and four tocotrinols [109].

The most bio- logically active form of vitamin E is tocopherol. Vitamin E, the major lipid-soluble antioxidant found in cell membranes, protects against lipid peroxidation and is a free radical scavenger. are among the body's primary

defenses against cellular oxidative damage caused primarily by toxic oxygen products. Free radicals and reactive oxygen molecules are generated endogenously as a result of metabolic reactions. There are also numerous exogenous sources of these molecules, including tobacco smoke, pollutants, organic solvents, and pesticides Free [109].

vitamin E, may protect breast tissue from oxidant damage that may lead to the development of cancer [110].

Reactive oxygen species ROS is a term that includes roots that focus on oxygen (Superoxide) anion radical, Hydroxyl radical, Peroxyl radical, Lipid hydroperoxide, and Hydroperoxyl HO2 and DNA in cells and tissues, stimulate undesirable oxidation and causing membrane damage, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation . The Researches results show significant decreased in levels of vitamin E ($p<0.05$) in malignant and radiotherapic groups comparing to the control group [111] .

Aims of the study

1. Identification the genotypic and allelic frequency of HMGB1 (rs1412125) and RAGE (rs1800624) genes in breast cancer patients compared with healthy control by using AC- PCR, RFLP-PCR and their association with susceptibility to breast cancer.
2. Measure the Serum level of HMGB1 and RAGE in both breast cancer patients and healthy controls and assess their expression with progression of disease.
3. Measuring serum level of vitamin E (as antioxidant) and evaluation their expression with progression of disease.
4. Measuring serum level of Malondialdehyde (MDA) and evaluation their expression with progression of disease.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Materials

2.1.1. Study Places

This study was carried out on patients whom diagnosed by physicians in Babylon Center Treatment Cancer at Margan Hospital. The patients did not receive any chemotherapy or radiation prior to taking the blood sample to exclude the effect of these drugs on the biochemical and genetic result. The practical part of this study was conducted at the laboratory of Chemistry and Biochemistry Department /College of Medicine / University of Babylon. Sample collection was conducted during the period from the September 2022 until the of December 2022.

2.1.2. Study Design

This study was designed as case-control study.

2.1.3. Study Population

2.1.3.1. Breast Cancer Groups

The breast cancer patients whom participated in this study were (50), their age range between (29-69) years old were divided according to the molecular classification of breast cancer by immunohistochemistry technique that depends on the expression of ER, PR, and Her2-enriched proteins into four groups:

-Luminal A: 20 female patients with breast cancer.

-Luminal B: 17 female patients with breast cancer.

- Her2\neu+ enriched: 7 female patients with breast cancer.

-Triple-negative: 6 female patients with breast cancer.

2.1.3.2. Control Group

The control group whom participated in the study includes (50) apparently healthy females. Their age were ranged between (29-69) years.

2.1.4. Ethical Issues

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee.

2.1.5. Data Collection

2.1.5.1. Inclusion Criteria

2.1.5.1.1. Inclusion Criteria of Patients

- Female subjects were (29-69) years of age.
- Patients include females premenopausal and postmenopausal.
- Women primarily new diagnosed with breast cancer by physicians and confirmed through biopsy.
- The patients did not receive any chemotherapy or radiation prior to taking the blood sample.

2.1.5.1.2. Inclusion Criteria of Controls

- Female subjects were (29-69) years of age.
- include females premenopausal and postmenopausal.

- No present diagnosis of breast cancer and other type of cancers.
- No family history of breast cancer.
- No chronic diseases.

2.1.5.2. Exclusion Criteria

2.1.5.2.1. Exclusion Criteria of Patients

- Patients who received chemotherapy or radiation.
- Patients with benign breast tumor.
- Diabetic patients.

2.1.5.2.2. Exclusion Criteria of Controls

- Female with breast cancer family history
- Female with chronic diseases.
- Diabetics.

2.1.6. Demographic and Anthropometric Measurement

2.1.6.1. Questionnaire

Socio-demographic characteristics include height, weight, age, age menarche, age of menopause, histological subtype, grade , and family history of cancer, as shown in the questionnaire included in the appendix.

2.1.6.2. Anthropometric Measures

Body mass index (BMI), which is weight in kilograms divided by height in meters squared [110].

BMI (Kg/m^2) = weight (kg) / height (m^2).

- BMI < 18kg/m2 is underweight.
- BMI 19-24.9 kg/ m 2 is normal.
- BMI 25 - 29.9kg/ m 2 is overweight.
- BMI 30 - 34.9 kg/ m 2 is class I obesity
- BMI 35 - 39.9 kg/ m 2 is class II obesity.
- BMI 40 kg/m2 and above is Class III obesity.

2.1.8. Blood Collection

Venous blood samples were collected from control and patients by using disposable syringe (5 ml). Five ml of blood were obtained from each subject by vein puncture, 2 ml was put into EDTA tubes and stored at - 20˚C (deep freeze), in order to be used later in genetic part of the study, the remaining of blood 3 ml, pushed slowly into disposable tubes containing separating gel was allowed to clot at room temperature and then centrifuged at 2000 ×g for approximately 10 minutes then the serum was divided into small Eppendorf tube and kept in a deep freezer (-20º C) to be used for biochemical estimation of the levels of HMGB1, RAGE, malondialdehyde (MDA) and vitamin E by ELISA technique.

2.2. Chemicals

Chemicals and kits used in this study were listed in Table 2-1:

Table 2-1: Chemical Substances Used in the Present Study

No	Materials	Produced company	Origin
1	Agarose	Condalab	Spain
2	Absolute Ethanol (100%)	Fluka Chemika	Switzerlad
3	DNA extraction kit	Favorgen	Taiwan
4	DNA ladder 100bp	Promega	USA
5	Ethidium bromide	Promega	USA
6	Primers	Macrogen	Korea
7	Human high mobility group protein B1 ELISA Kit	Melsin	China
8	Malondialdehyde (MDA) Kit	Melsin	China
9	Nuclease free water	Promega	USA
10	PCR Master mix Kit	Promega	USA
11	Restriction enzyme	Thermo scientific	England
12	Receptor for Advanced Glycation End Products (RAGE) Kit	Melsin	China
13	TBE buffer	Promega	USA
14	Vitamin E Kit	Melsin	China

2.3. Instruments

The instruments and tools used in this study are shown in Table 2-2.

Table 2-2: Instruments and Tools Used in this Study.

No	Devices and tools	Company	Origin
1	Centrifuge	ThermoFisher	Germany
2	Deep Freeze	GFL	Germany
3	Disposable syringes(5mL)	BiozekMedical	China
4	ELISA reader and	Paramedical	Italian
5	EDTA tube (5ml)	AFCO	Jordan
6	Eppendorf tube (1.5ml)		China
7	Gel documentation	Analytik jena	Germany
8	Gel electrophoresis	Bioneer	Germany
9	Gel tube (10 mL)	AFCOVAC	Jordan
10	Hot Plate	Grant	England
11	Incubator	Memmert	Germany
12	Micro centrifuge	Wise spin	Korea
13	Nano- drop	Analytik jena	Germany
14	Oven	Memmert	Germany
15	PCR Thermo cycler	Analytik	Germany
16	Sensitive balance	Kern	Germany
17	UV-visible-Spectrophotometer	Shimadzu	Japan
18	Vortex	Kunkel	Germany
19	Water bath	GFL	Germany

2.4. Methods

2.4.1. Determination of Serum Human High Mobility Group Protein B1 (HMGB1) Levels

2.4.1.1. Principle

The Sandwich-ELISA technique is used in the determination of HMGB1level of patients and control. The plate has been pre-coated with human HMGB1 antibody. HMGB present in the sample is add and bind to antibodies coated on the microplate wells. After that biotinylated human HMGB1 antibody is added and bind to HMGB1 present in the sample. Streptavidin–HRP is added and binds to the biotinylated HMGB1 antibody. After incubation period unbound streptavidin-HRP is washed away. Chromogen A and B were added to microplate wells and incubated followed by addition of stop solution. Absorption is then measured at 450 nm [86].

2.4.1.2. Kit Contents

Table 2-3 listed the contents of kit that used to determine High Mobility Group protein B1(HMGB1) produced by Melsin company (Chaina).

Table 2-3: Contents of Kit Used to Determine HMGB1 Levels.

No	Components	Quantity
1	Microelisa Stripplate	12*8 Strips
2	Standred (1 set)	0.3mlX6
3	Sample diluent	6.0mlX1
4	HRP-Conjugate reagent	10.0mlX1
5	20X Wash solution	25mlX1

6	Chromogen Solution A	6.0mlX1
7	Chromogen Solution B	6.0mlX1
8	Stop Solution	6.0mlX1
9	Closure plate membrane	2
10	User manual	1
11	Sealed bags	1

2.4.1.3. Preparation of Reagent

Wash solution was diluted with distilled water 1:20.

2.4.1.4. Assay Procedure

1. Before use, all reagent bring to room temperature. The assay is performed at room temperature.

2. A volume of 50µl of standard was added to well standard.

3. A volume of 40µl from sample were added and then added 10µl antiHMGB1 antibody to sample well, then 100µl of HRP-conjugate reagent to both sample and standard wells, then mixed well. The over plate covered with sealer and incubated for 60 min at 37 º C.

4. Sealer was removed , and microplate was cleaned 5 times with a wash buffer. using automatic washer.

5. A volume of 50µl chromogen solution A was added and then followed by addition 50µl chromogen solution B for each wells. The coated plate incubated with new sealer for 15 min. at 37 º C in dark media.

6. To each well 50µl of stop solution was added, and the color change from the blue to yellow immediatly.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 15 min.

Detection range: 0-40000 pg/ml

Sensitivity: less than 10 pg/ml

2.4.1.5. Result Estimation

Results were determined using standard curve as shown in figure 2-2.

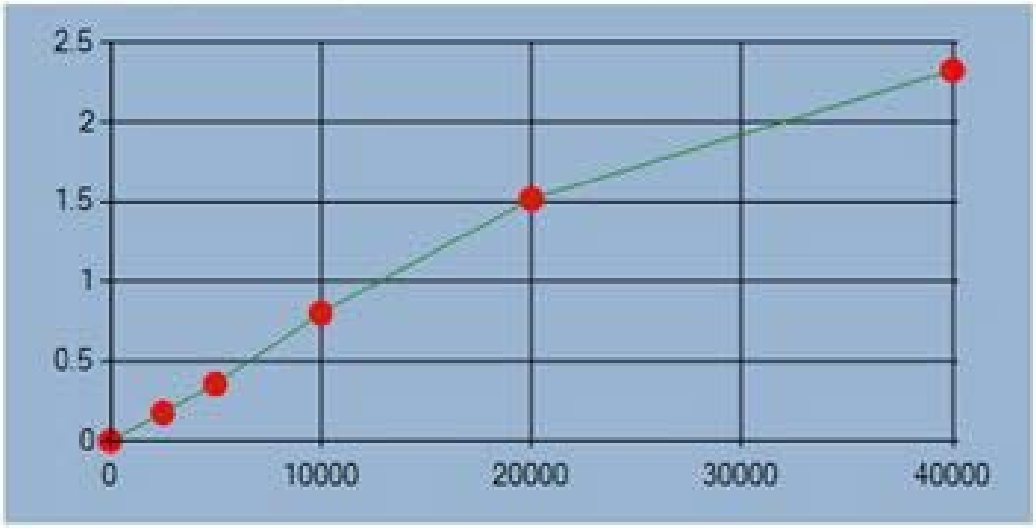

Figure 2-1: Standard curve for Human HMGB1.

2.4.2. Determination of Serum Receptor for Advanced Glycation End Products (RAGE) Levels

2.4.2.1. Principle

The Sandwich-ELISA technique is used in the determination of RAGE level of patients and control. The plate has been pre-coated with human RAGE antibody. RAGE present in the sample is add and bind to antibodies coated on the microplate wells. After that biotinylated human RAGE antibody is added and bind to RAGE present in the sample. Streptavidin–HRP is added and binds to the biotinylated RAGE antibody. After incubation period unbound streptavidin-HRP

is washed away. Chromogen A and B were added to microplate wells and incubated followed by addition of stop solution. Absorption is then measured at 450 nm [86].

2.4.2.2. Kit Contents

Table 2-4 listed the contents of kit that used to determine Receptor for Advanced Glycation End Products (RAGE) produced by Melsin company (Chaina).

Table 2-4: Kit Contents of Receptor for Advanced Glycation End Products RAGE Levels Determination.

No	Components	Quantity
1	Microelisa Stripplate	12*8 Strips
2	Standred (1 set)	0.3ml X6
3	Sample diluent	6.0ml X1
4	HRP-Conjugate reagent	10.0ml X1
5	20X Wash solution	25ml X1
6	Chromogen Solution A	6.0ml X1
7	Chromogen Solution B	6.0ml X1
8	Stop Solution	6.0ml X1
9	Closure plate membrane	2
10	User manual	1
11	Sealed bags	1

2.4.2.3. Reagent Preparation

Wash solution was diluted with distilled water 1:20.

2.4.2.4. Assay Procedure

1. Before use, all reagent bring to room temperature. The assay is performed at room temperature.

2. A volume of 50µl of standard was added to well standard.

3. A volume of 40µl from sample were added and then added 10µl anti RAGE antibody to sample well, then 100µl of HRP-conjugate reagent to both sample and standard wells, then mix well. The over plate covered with sealer and incubated for 60 min at 37 º C.

4. Sealer was removed , and microplate was cleaned 5 times with a wash buffer. using automatic washer.

5. A volume of 50µl chromogen solution A was added and then followed by addition 50µl chromogen solution B for each wells. The coated plate incubated with new sealer for 15 min. at 37 º C in dark media.

6. To each well 50µl of stop solution was added, and the color change from the blue to yellow immediatly.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 15 min.

Detection range: 0-4 ng/ml

Sensitivity: less than 0,1 ng/ml

2.4.2.5. Result Estimation

Results were determined using standard curve as shown in figure 2-3

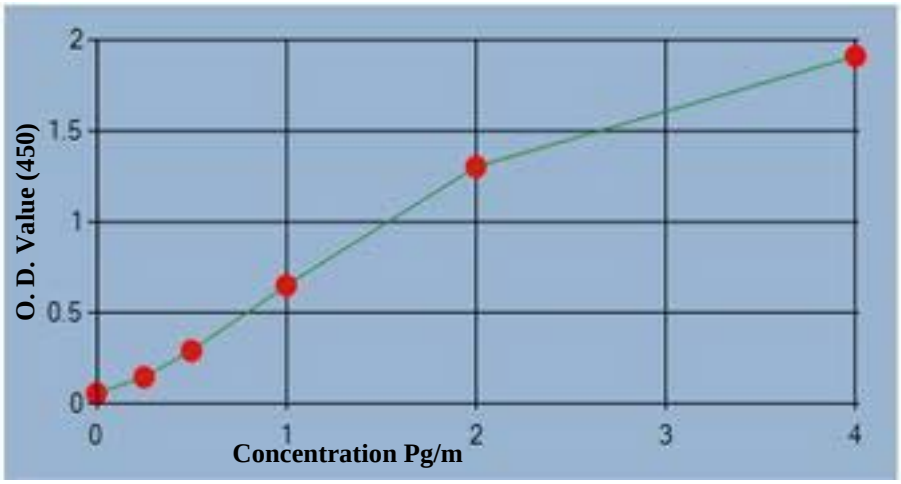

Figure 2-2: Standard Curve of RAGE.

2.4.3. Determination of Serum Malondialdehyde (MDA) Level

2.4.3.1. Principle

The Sandwich-ELISA technique is used in the determination of MDA level of patients and control. The plate has been pre-coated with human MDA antibody. MDA present in the sample is add and bind to antibodies coated on the microplate wells. After that biotinylated human MDA antibody is added and bind to MDA present in the sample. Streptavidin–HRP is added and binds to the biotinylated MDA antibody. After incubation period unbound streptavidin-HRP is washed away. Chromogen A and B were added to microplate wells and incubated followed by addition of stop solution. Absorption is then measured at 450 nm [86].

2.4.3.2. Kit Contents

Table 2-5 listed the contents of kit that used to determine Malondialdehyde (MDA) produced by Melsin company (Chaina).

Table 2-5: Kit Contents of Malondialdehyde (MDA) Levels Determination.

No	Components	Quantity
1	Microelisa Stripplate	12*8 Strips
2	Standred (1 set)	0.3ml X6
3	Sample diluent	6.0ml X1
4	HRP-Conjugate reagent	10.0ml X1
5	20X Wash solution	25ml X1
6	Chromogen Solution A	6.0ml X1
7	Chromogen Solution B	6.0ml X1
8	Stop Solution	6.0ml X1
9	Closure plate membrane	2
10	User manual	1
11	Sealed bags	1

2.4.3.3. Reagent Preparation

Wash solution was diluted with distilled water 1:20.

2.4.3.4. Assay Procedure

1. Before use, all reagent bring to room temperature. The assay is performed at room temperature.

2. A volume of 50µl of standard was added to well standard.

3. A volume of 40µl from sample were added and then added 10µl anti RAGE antibody to sample well, then 100µl of HRP-conjugate reagent to both sample

and standard wells, then mix well. The over plate covered with sealer and incubated for 60 min at 37 º C.

4. Sealer was removed , and microplate was cleaned 5 times with a wash buffer. using automatic washer.

5. A volume of 50µl chromogen solution A was added and then followed by addition 50µl chromogen solution B for each wells. The coated plate incubated with new sealer for 15 min. at 37 º C in dark media.

6. To each well 50µl of stop solution was added, and the color change from the blue to yellow immediatly.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 15 min.

Detection range: 0-8nmol/ml

Sensitivity: less than 0,1nmol/ml

2.4.3.5. Result Estimation

Results were determined using standard curve as shown in figure 2-4.

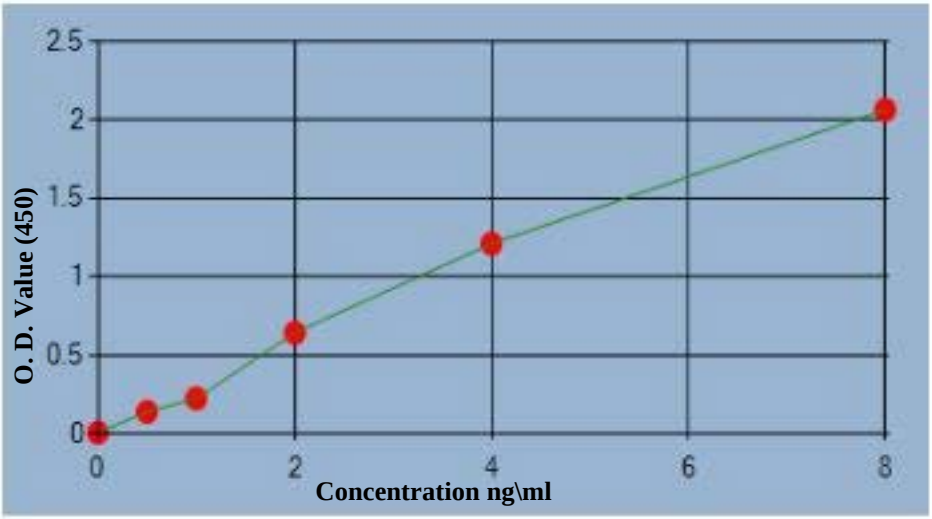

Figure 2-3: Standard Curve of Malondialdehyde (MDA).

2.4.4. Determination of Serum Vitamin E Level

2.4.4.1. Principle

The Sandwich-ELISA technique is used in the determination of vitamin E level of patients and control. The plate has been pre-coated with human vitamin E antibody. Vitamin E present in the sample is add and bind to antibodies coated on the microplate wells. After that biotinylated human vitamin E antibody is added and bind to vitamin E present in the sample. Streptavidin–HRP is added and binds to the biotinylated vitamin E antibody. After incubation period unbound streptavidin-HRP is washed away. Chromogen A and B were added to microplate wells and incubated followed by addition of stop solution. Absorption is then measured at 450 nm [86].

2.4.4.2. Kit Contents

Table 2-6 listed the contents of kit that used to determine Vitamin E produced by Melsin company (Chaina).

Table 2-6: Kit Contents of Vitamin E Levels Determination

No	Components	Quantity
1	Microelisa Stripplate	12*8 Strips
2	Standred (1 set)	0.3ml X6
3	Sample diluent	6.0ml X1
4	HRP-Conjugate reagent	10.0ml X1
5	20X Wash solution	25ml X1
6	Chromogen Solution A	6.0ml X1
7	Chromogen Solution B	6.0ml X1
8	Stop Solution	6.0ml X1
9	Closure plate membrane	2
10	User manual	1
11	Sealed bags	1

2.4.4.3. Reagent Preparation

Wash solution was diluted with distilled water 1:20.

2.4.4.4. Assay Procedure

1. Before use, all reagent bring to room temperature. The assay is performed at room temperature.

2. A volume of 50µl of standard was added to well standard.

3. A volume of 40µl from sample were added and then added 10µl anti RAGE antibody to sample well, then 100µl of HRP-conjugate reagent to both sample and standard wells, then mix well. The over plate covered with sealer and incubated for 60 min at 37 º C.

4. Sealer was removed , and microplate was cleaned 5 times with a wash buffer. using automatic washer.

5. A volume of 50µl chromogen solution A was added and then followed by addition 50µl chromogen solution B for each wells. The coated plate incubated with new sealer for 15 min. at 37 º C in dark media.

6. To each well 50µl of stop solution was added, and the color change from the blue to yellow immediatly.

7. The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate reader set at 450 nm within 15 min.

Detection range: 0-80µ mol/L

2.4.4.5. Result Estimation

Results were determined using standard curve as shown in figure 2-5

Sensitivity: less than 0,1 µmol/L.

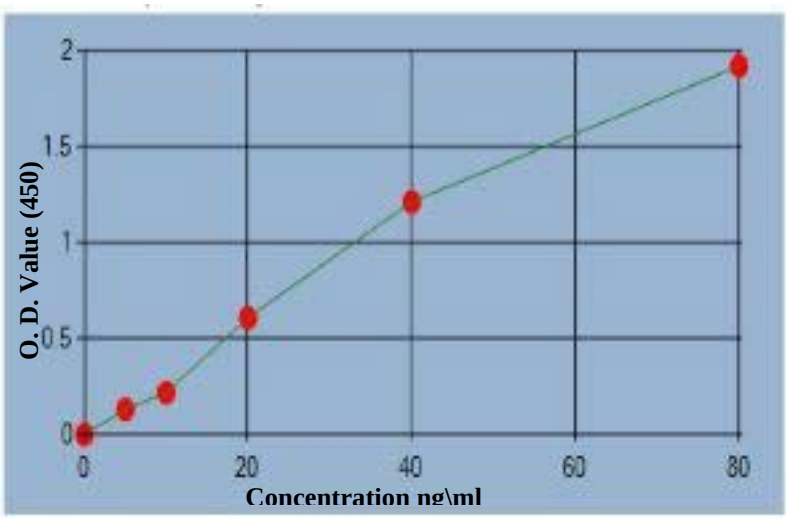

Figure 2-4: Standard Curve of Vitamin E.

2.4.5. Genomic DNA Extraction

Genomic DNA was isolated from the peripheral blood of subjects according to manufacturer manual. Table 2-7 shows the components of DNA extraction kit.

Table 2-7: Components of DNA extraction kit.

No	Item	Quantity
1	RBC Lysis Buffer	135 ml
2	FATG Buffer	30ml
3	FATG Buffer	40ml
4	W1Buffer	45ml
5	Wash Buffer	25ml
6	Elution Buffer	30ml
7	FABG Mini Column	100ml
8	Collection Tube	200ml
9	User manual	1

Preparation of Wash Buffer by adding ethanol (96-100%)			
Ethanol volume for Wash Buffer	4ml	100ml	200ml

2.4.5.1. DNA Extraction Procedure

1. A volume of 300 µl of blood was transferred to 1.5 ml micro- centrifuge tube.
2. A volume of 900 µl of RBC Lysis Buffer was added to the 300 µl of blood sample and mix it up by inversion. The sample mixture was incubated at room temperature for 10 min.
3. Sample was centerfued at 3,000 Xg for 5 min. and then the supernatant was completely removed.
4. A volume of 100 µl of RBC Lysis Buffer was added to the pellet and resuspend the cells by pipetting.
5. A volume of 100 µl of FABG Buffer was added to the sample mixture, and then mixed well by vortexing.
6. The sample mixture was incubated at room temperature for 10 min. During incubation, invert the tube was inverted every 3 min.
7. Preheat required elusion Buffer in a 70 º C water bath.
8. A volume of 200 µl of Ethanol (96-100%) was added to the sample and vortex for 10 sec. The sample was pipette to mix well if there is any precipitate formed.
9. A FABG column was placed to a collection Centrifuge tube. The sample mixture was transfered carefully to FABG column. At speed 14,000 rpm for 1 min. The collection tube was discarded and placed the FABG column into a new collection tube.

10. A volume of 400 µl of W1 Buffer added to the FABG column and centrifuged for 30 sec at speed 14,000 Xg. The flow-though was discarded and placed the FABG column back to the collection tube.

11. A volume of 600 µl of wash Buffer was added to the FABG column and centrifuged for 30 sec at speed 14,000 Xg. The flow-though was discarded and placed the FABG column and place the FABG column back to the collection tube.

Note: make sure that ethanol has been added to wash Buffer when first open.

12. Centrifugation is required for an additional 3 min at speed 14,000 Xg to dry the column.

Note: this step will avoid the subsequent enzymatic reactions from being inhibited by residual liquid.

13. The dry FABG column was Placed to a new 1.5 ml micro-centrifuge tube.

14. A volume of 100 µl of Preheated elusion Buffer added or TE to the membrane center of FABG column.

Note: for effective elusion, make sure that the elusion solution is dispensed onto the membrane center and absorbed completely.

15. The FABG column was Incubate at 37 º C for 10 min in an incubator.

16. Centrifugation is required for 1 minute at full speed 14,000 Xg to elute the DNA.

Note: standard volume for elution is 100 µl. If higher DNA yield is required, repeat the DNA elution step to increase DNA recovery and the total volume could be 200 µl.

17. The DNA fragment was stored at -20 º C.

2.4.6. Estimation the DNA Purity and Concentration by Using Scan Drop

2.4.6.1. Principle

When the DNA sample in the Scan Drop cuvette is exposed to UV light at 260 nm, the sample will absorb the light of a spectrophotometer. The more absorption light by the sample reflected a higher concentration of DNA in the sample. The absorbance of nucleic acid is maximal at 260 nm and of protein at 280nm. The absorption ratio in these wavelengths is used in protein and nucleic acid extraction as a measure of purity [112]. The ratio of 1.8-2 is generally acceptable purity to DNA. Absorbance's 230 nm is acceptable as an outcome of other contamination; Thus the ratio A260/A230 was repeat calculated [113].

2.4.7. Estimation of DNA by Agarose Gel Electrophoresis

Agarose gel electrophoresis is a commonly used technique for separating proteins and DNA. Nucleic acid molecules separated according to their size with the aid of an electric current, the negatively charged molecules traveled toward anode which is positively charged [114].

The flow is influenced solely by the molecular weight in which the smaller weight molecules traveled faster than larger ones. Also, nucleic acid fractionation by performing agarose gel electrophoresis can be considered as a first step in the purification of the interested bands. A horizontal gel electrophoresis tank was used in this method with an external power supply, a suitable running buffer, DNA sample, with suitable dye [115].

The isolated DNA sample then was loaded into agarose gel well and an electric field was exposed. DNA backbone which has a negative charge was migrated towards the anode. The small size of DNA was migrated faster, as a result, the DNA was separated by size. The Agarose percentage in the gel was

determined what size range of DNA was resolved. DNA has the same mass / charge ratio so that the size of the DNA molecules was separated and the distance traveled was inversely proportional to its molecular mass.

2.4.7.1. Gel Preparation

1- A suitable agarose mass was weighed out into an Erlenmeyer flask. By using w/v percentage solution agarose gels were prepared. The concentration of the gel was depended on the size of the DNA fragment to be separated. The concentration of the agarose gel was ranged between 1.5-2%.

2- The running buffer was added to the agarose containing flask. TBE buffer was used as a gel running buffer.

3- The agarose-buffer mixture has been heated, and this was usually achieved by hot plate heating, which can also be achieved over a heater. The flask content was swirled until the agarose dissolved fully and it was cooled down to 50°C.

4- A volume of 5 -6 µl of ethidium bromide nucleic acid staining solution was added to the agarose solution and the flask was gently swirled to mix the solution and to avoid forming bubbles.

5- The comb was placed, and the agarose solution was poured into the gel tray till the comb teeth were flood for around 1/4-1/2 into the agarose.

6- It was allowed to cool the agarose gel until solidified, the comb was removed so the gel was ready.

2.4.7.2. Loading the Samples and Electrophoresis

The DNA sample and loading dye were mixed to visualize and determine the speed of the fragments while migrating through the gel running buffer was applied up until it filled the gel 's surface. It was important that the running buffer used to prepare the gel was the same. Then DNA sample was added to the gel

well. DNA size marker was loaded with the experimental sample. After applying an electric field DNA molecules were moved from negative electrode(cathode) to positive electrode (anode) through the gel. The gel was ready for analysis. The DNA bands in the gel were visualized using UV transilluminator.

2.4.8. Detection of Genomic DNA

The presence of genomic DNA extracted by the previous procedure was detected by using agarose gel electrophoresis (1.5%). The extracted DNA is colorless, so gel loading dye was used with DNA to ease the loading step of the electrophoresis procedure, as shown in figure 2-3.

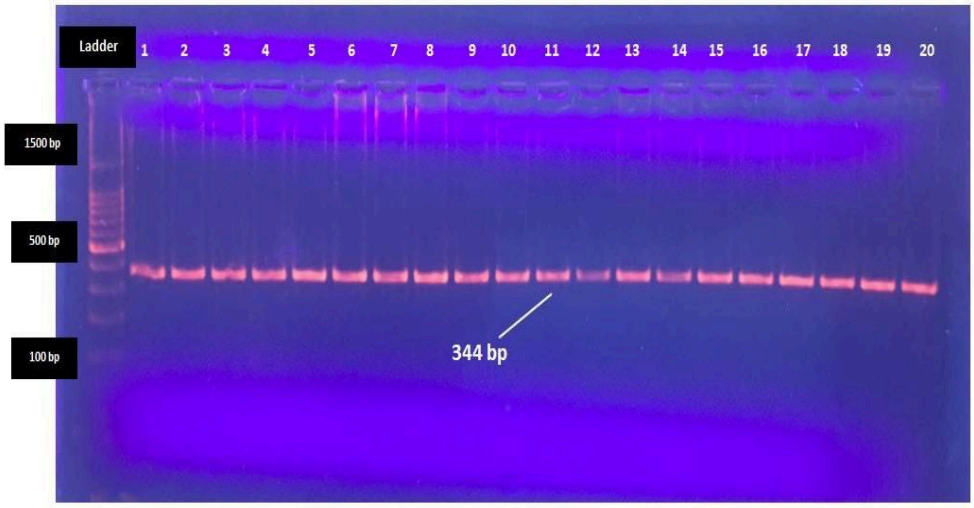

Figure 2-5: Genomic DNA.

2.4.9. Allele-specific polymerase chain reaction (AS-PCR)

An AS-PCR technique was performed to detect and genotype the HMGB1 gene polymorphism (rs1412125) from BC patients and healthy blood samples. This technique is based on allele-specific primers, which can be used for single nucleotide polymorphism (SNP) analysis [116].

2.4.9. 1. Preparations of primer

These primers produced by Macrogen Table 2-8, 2-9. which were dissolved in sterile free nucleus water. The standard solutions (100 µl) created by adding H2O to vial holding the lyophilized primers, and the working solution (10 µl) was done by combining (10 µl) of stock primers with (90 µl) of H2O. Both the stock and the working solution were kept in-20°C.

Table 2-8: The sequence of primer that used for HMGB1 (rs1412125) gene.

rs1412125 (T/C) gene polymorphism		
Primer	**Sequence (5'-3')**	**Product size**
Wild type Forward Primer	TCTTTTTAAAAGAAAATACACTATT	209bp
Mutant Forward Primer	TCTTTTTAAAAGAAAATACACTATC	
Common Reverse Primer	AGTAGAGGGAAGCAGAGGATAGT	

Table 2-9: The forward and reverse primers that used for RAGE (rs1800624) gene [95].

Primers	Sequence	Restriction Enzyme
forward	5′- TCAGAG CCCCCG ATCCTATTT 3′	TasI (Tsp509I)
reverse	5′- GGGGGCAGTTCTCTCCTC -3′	

2.4.9.2. Master Premix Components

A master premix of Promega was used as shown in Table 2-10.

Table 2-10: GoTaq® G2 Green Master Mix components.

No	Item	Concentration
1	Each: dNTP (dATP, dCTP, dGTP, dTTP)	200 mM
2	MgCl2	1.5 mM
3	Tap polymerase 1 U/µ	1 U/µ
4	Tris-HCl (pH 8.5)	75 mM
5	(NH4)2SO4	20 mM

2.4.9.3. PCR Reaction Mixture

To obtain PCR products from the (HMGB1, RAGE) genes, the polymerase chain reaction (PCR) of the HMGB1gene was performed with two reactions per sample (a wild-type allele combination reaction) and a single (mutant-type allele combination reaction), and one reaction for the RAGE gene by Using (GoTaq® G2) Green Master Mix) and this was done as in Tables 2-11, 2-12, 2-13.

Table 2-11: Wild type allele PCR Reaction mixture for HMGB1gene.

Contents of reaction mixture	Volume
Genomic DNA Template	6.5µL
Wild Type Forward Primers	2µL
Common Reverse Primer	2µL
G2 Green Master Mix	12.5µL
Nuclease free water	2µL
Total	25µL

Table 2-12: Mutant type allele PCR Reaction mixture for HMGB1 gene.

Contents of reaction mixture	Volume
Genomic DNA Template	6.5µL
Mutant Type Forward Primers	2µL
Common Reverse Primer	2µL
G2 Green Master Mix	12.5µL
Nuclease free water	2µL
Total	25µL

Table 2-13: PCR Reaction mixture for RAGE gene.

Contents of reaction mixture	Volume
Genomic DNA Template	6.5µL
Forward Primers	2µL
Reverse Primer	2µL
G2 Green Master Mix	12.5µL
Nuclease free water	2µL
Total	25µL

2.4.9.3. Polymerase chain reaction (PCR) conditions for HMGB1 gene

PCR Thermo cycler conditions were done for gene HMGB1 rs1412125 as shown in Table 2-14.

Table 2-14: PCR conditions for HMGB1 gene

AS -PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min	1
Denaturation	95°C	30 sec	35 cycle
Annealing	55°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	5min	1

Then the amplification products were separated by electrophoresis through 1.5-2% agarose gel stained with ethidium bromide.

2.4.10. Polymerase Chain Reaction Restriction Fragment Length Polymorphism PCR-RFLP

2.4.10.1. Principle

PCR-RFLP is the amplification of the fragment containing the variation. This technique involves treating the amplified fragment with an appropriate restriction enzyme.

This method began with the amplification of DNA from the gene target area using a PCR machine and then moved on to the RFLP method of cutting DNA amplicons using restriction enzyme [116].

2.4.10.2. Polymerase Chain Reaction (PCR) Amplification for (RAGE) Gene

Single nucleotide polymorphism of the RAGE Gene Amplification is (-374T/A), It was amplified by a polymerase chain reaction (PCR) with specific primers and by use restriction enzyme (Tsp509I).

2.4.10.3. PCR Conditions for RAGE gene

PCR Thermo cycler conditions were done for RAGE gene rs1800624 as shown in Table 2-15.

Table 2-15: RFLP- PCR conditions for RAGE gene.

RFLP -PCR step	Temp.	Time	repeat
Initial denaturation	94°C	5min	1
Denaturation	94°C	30 sec	35 cycle
Annealing	57°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	5min	1

Then the amplification products were separated by electrophoresis through 1.5-2% agarose gel stained with ethidium bromide.

2.4.10.4. Restriction Enzyme Assay for (RAGE) Gene Polymorphism

The PCR product digested with the restriction enzyme TasI (Tsp509I). Optimization of digestion conditions done by using:

1- Restriction enzyme prepared by mixing 1µl restriction enzyme TasI (Tsp509I) with 10µl of the PCR product.
2- Buffer B 2.5µl
3- Tango A 2.5µl
4- Sterile nuclease-free water 4µl

The time of incubation for four hours at 37°C (The digestion conditions that gave best result). The enzyme was inactivated by heat at 65°C for half an hour.

2.4.11. Photo Documentation

Agarose gel was visualized with a gel documentation package in UV transiluminators. The gel was exposed to ultraviolet light and the photos were taken using a digital computing camera for the gel documentation device.

2.5. Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0 Continuous variables are expressed as mean and standard deviation. The group of patients and control was evaluated by use analysis of variance student t test, with $P \leq 0.05$ was considered the change as significant. ANOVA test was used to compare means between three groups or more. Pearson, chi-square test were used to find the association between categorical variables. Pearson correlation coefficient was used to assess the relationship between two continuous variables. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of HMGB1, RAGE, MDA . The area under the curve(AUC) provides a useful tool to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker. A p-value of ≤ 0.05 was considered as significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Demographic Characteristics of Patients and Control

Demographic characteristics of the 100 studied subjects, 50 breast cancer and 50 control subjects, as shown in Table 3-1.

Table 3-1: Comparson of Means ± standard deviation of age and BMI between patients and control.

Parameter	Patient (50) Mean ± SD	Control (50) Mean ± SD	P-value
Age (year)	45.11 ± 6.66	42.2 ± 7.02	0.26
BMI(kg/m2)	28.4 ± 4.23	27.23 ± 2.33	0.3

P < 0.05 is consider significant, SD : standard deviation, BMI: Body Mass Index.

3.1.1. Age

Patients included in this study had age range (29-69) years, with mean±SD 45.11 ± 6.66 years Control group was with an age rang (29-69) years and mean±SD 42.2 ± 7.02 years. The mean differences of age and body mass index between the breast cancer group and the control group is statistically non-significant P > 0.05. This matching is crucial to get rid of any variations in the findings that might be caused by these parameters' discrepancies.

The age range used in this study is similar to the study conducted by Andrew *et al.*, 2015 and Dodova *et al.*, 2015, the age range used in these two studies was (30-70) years [117], [118].

3.1.2. Body Mass Index (BMI)

For weight, the results in the Table 1-3 indicate that mean differences of body mass index between the patient group and the control group is statistically not significant P > 0.05.

A higher body mass index is associated with a higher risk of breast cancer, especially in the postmenopausal period [119], [120].

3.2. Family History of Breast Cancer

The presence of family history is an important contributory factor in breast cancer. This study showed 21 (42%) of breast cancer patients have positive family history, and 29 (58%) of breast cancer patients have no family history, and these results were significantly associated with the risk of breast cancer when compared to the healthy control, (p =0.001) as shown in Table 3-2.

Table 3-2: Distribution of patients and control subjects according to family history.

Family History	Patient (50)		Control (50)		P-value
	N	%	N	%	
Positive	21	42	0	0	0.001
Negative	29	58	50	100	

N: number of cases, Chi-square test; S: significant at P > 0.05.

The results indicated the breast cancer risk was strongly associated with the family history of breast cancer in any first-degree relative, with the most convincing results observed in those whose mother and sister had breast cancer. This finding agreed with Choi *et al.*,[114], and Haber *et al.*, [115]. who reported that the risk of breast cancer was associated with the presence of a family history of breast cancer.

3.3. Distribution of Breast Cancer Patients and Healthy Controls According to Some Variables

The current study shows that the mean of menarche age were 12.52 ± 1.83 and 13.08 ± 1.06, in patients with breast cancer and healthy control group,

respectively. The mean was lower in patients group in comparison with control group and the difference was non-significant (P>0.1), as shown in Table 3-3.

Table 3-3: Distribution of breast cancer patients and healthy controls according to the menarche age.

Variable	Patient (50) Mean ± SD		Control (50) Mean ± SD		P-value
Menarche Age	12.52 ± 1.83		13.08 ± 1.06		0.1
Menopausal Age	**Patient (50)**		**Control (50)**		**P-value**
	N	**%**	**N**	**%**	
Pre-menopausal	23	46	46	92	0.05
Post-menopausal	27	54	4	8	

P < 0.05 is consider significant, SD = standard deviation.

These results consistence with results of Khalis *et al.*, (2018), which found that early menarche (≤ 13 years) was significantly associated with an increased risk of breast cancer [121].

Laamiri *et al.*, (2015), who reported a significant association between early age of menarche and an increased risk of breast cancer in Moroccan patients [122].

Although an early age of first menarche and a late age of menopause increases the risk of developing breast cancer. It is estimated that every year of delay after the age of 12 reduces the premenopausal BC risk by 7% and postmenopausal cancer by 3% [123].

The current study detects the 23(46%) premenopausal and 27(54%) postmenopausal, of BC patients. This results are similar to a study done by A. Ozsoy, *et al.*, 2017, which indicated that women with a menopause age of 55 have 2 times higher risk for developing breast cancer as compared to those with

a menopause age of 45 [124]. The results of the study agree with those of Marsden *etal.,* 2019, which indicated that the effect to be equivalent to a 2.8% increase in risk for every year of delay in menopause [123].

3.4. Distribution of breast Cancer Patients According to Some Associated Clinical Features

The results in Table 3-4 indicate that 37 (74%) have positive estrogen receptor, 32 (64 %) have positive progesterone receptor but only 24 (48%) of patients have positive HER 2. The most common breast cancer subtype which comprises 80% of all patients are estrogen receptor positive (ER) [126].

Although there is a direct correlation between the level of ER expression in invasive breast cancer and the likelihood of response to hormonal therapy, even tumors with low levels of ER expression show significant benefit compared to completely ER-negative tumors [127].

Table 3-4: Some Clinical Features of breast Cancer Patients.

Characteristic	Patients	
	N	%
Estrogen Receptor		
Positive	37	74
Negative	13	26
Progesterone Receptor		
Positive	32	64
Negative	18	36
HER2		
Positive	24	48
Negative	26	52

N: number of cases, HER2: Human epidermal growth factor receptor 2.

3.5. Distribution of Breast Cancer Patients According to Tumor Characteristics

The data presented in Table 3-5, show the frequency distribution of patients according to tumor size show that most patients with breast cancer have T2 tumor size, 28 (58.3%) of patients, as in shown figure 3-1.

Table 3-5: Tumor characteristics of breast cancer patients.

Characteristic	Patients	
	N	%
Tumor size		
T1	8	16.7
T2	28	58.3
T3	6	12.5
T4	6	12.5
Tumor Grade		
G1	3	6
G2	34	68
G3	13	26
Lymph Node		
N0	13	26.5
N1	15	30.6
N2	15	30.6
N3	6	12.3
Distance of Metastasis		
M0	43	89.6
M1	5	10.4
Stage of Cancer		
Stage I	25	50
Stage II	14	28
Stage III	6	12
Stage IV	5	10

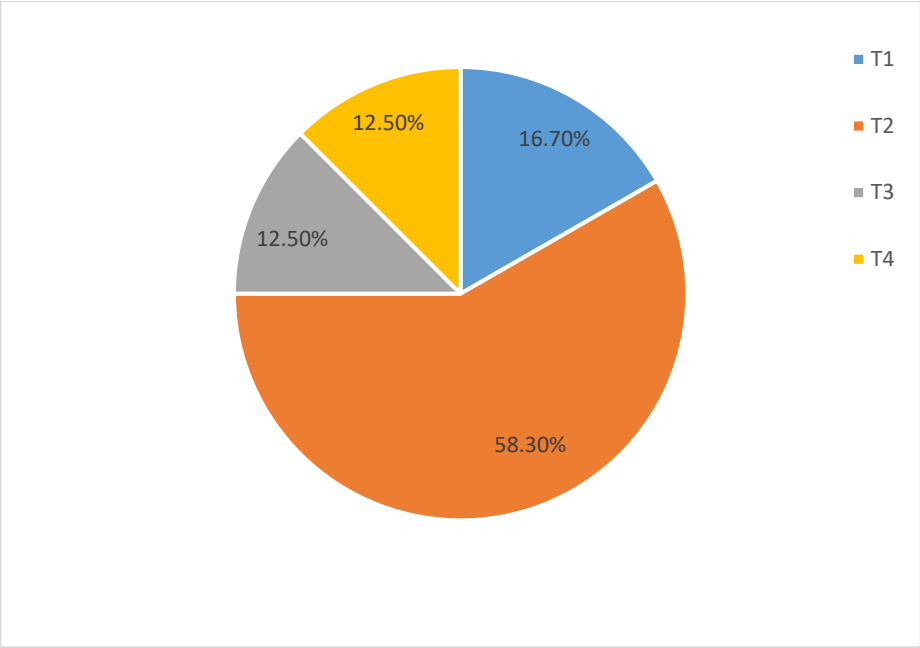

Figure 3-1: A Pie Chart showing the frequency distribution of patient with breast cancer according to tumor size.

3.6. Biochemical Parameters

3.6.1. HMGB-1

The findings demonstrated a significant difference in HMGB1 levels between patients and their control group (P< 0.025). Means, standard deviation, and statistical parameters were shown in the Table 3-6.

Table 3-6: HMGB1 level in patients and control group.

Study Group	N	HMGB1 level (ng/ml) Mean ± SD	P-value
patient	50	5.52 ± 0.74	**0.025**
control	50	4.93 ± 0.88	

P < 0.05 is consider significant, SD = standard deviation, HMGB1: High Mobility Group Box-1.

Evaluation of HMGB1 in breast cancer patients showed that the mean and SD for the patients were (5.52 ± 0.74) and for a healthy control (4.93 ± 0.99),

respectively There were a significant differences in serum HMGB1 levels (p <0.01), this is due to the fact that HMGB1 plays an important role as a cytokine involved in triggering inflammation and inflammatory-related diseases including cancer by regulating the expression of other inflammatory cytokines, it can be actively secreted by inflammatory cells like macrophages and NK-cells, released into circulation Especially apoptotic cell death [77],[128].

The results shown in Table 3-7 patients with breast cancer had greater median serum HMGB-1 levels compared to control group values, 5.474 pg/ml versus 5.055 pg/ml, the difference was a significant (P < 0.017).

Table 3-7: Median levels of Serum HMGB-1 in patients with breast cancer and control subjects.

HMGB-1 (ng\ml)	Patients N=50	Control N=50	P-value
Median	5.474	5.055	0.017

P < 0.05 is consider significant, HMGB-1: High Mobility Group Box-1.

3.6.1.1. Distribution of Serum HMGB-1 Level According to Tumor Characteristics

The results of this study are shown in the Table 3-8 that there was no significant association between mean serum HMGB-1 levels and tumor size. The mean serum HMGB-1 level increased at T1, compared to the T2, T3 and T4 volume, was 5.54 ± 0.78 5.48 ± 0.65, 5.14 ± 0.98 respectively (p = 0.48).

The levels were higher in grade two (5.66 ± 0.8) than in grade one and grade three, corresponding to (5.7 ± 0.36 and 5.13 ± 0.47, respectively (p = 0.08). There is no significant between mean levels of serum HMGB-1 and cancer stage, the value was higher in stage 1 (5.68 ± 0.7) than in stage 2, stage 3 and stage 4, 5.35 ± 0.9, 5.2 ± 0.53, 5.62 ± 0.57 respectively (p> 0.38).

There was no association between the serum HMGB1 levels and progression of the tumor size and stage. This outcome is consistent with Sun s. *et a.* 2015[129].

Table 3-8: Distribution of serum HMGB-1 level according to tumor characteristics.

HMGB-1 level (ng\ml)					
Tumor size	**T1**	**T2**	**T3**	**T4**	**P-value**
Mean ± SD	5.77±0.44	5.54±0.78	5.48±0.65	5.14±0.98	0.48 NS
N	8	28	6	6	
Tumor Grade	**G1**	**G2**	**G3**	**P-value**	
Mean ± SD	5.7±0.36	5.66±0.80	5.13±0.47	0.08 NS	
N	3	34	13		
Stage of cancer	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**P-value**
Mean ± SD	5.68±0.7	5.35±0.91	5.2±0.53	5.62±0.57	0.38 NS
N	25	14	6	5	

N: number of cases, HMGB-1: High Mobility Group Box-1, NS: Non-Significant.

3.6.2. RAGE

The findings demonstrated results RAGE showed that the difference was a highly significant (P < 0.003), as shown in Table 3-9.

Table 3-9: Comparison of RAGE level in patients and control group.

Study Group	N	RAGE level (pg/ml) Mean ± SD	P-value
patient	50	0.56 ± 0.5	0.003 HS
control	50	0.47 ± 0.14	

N: number of cases, RAGE: Receptor for Advanced Glycation End Products, HS: High Significant.

Evaluation of RAGE in breast cancer patients showed that the mean and SD for the patients were (0.56 ± 0.5) and for a healthy control (0.47 ± 0.14), respectively. There were a highly significant differences in serum RAGE levels ($p < 0.003$); Increased expression of RAGE was demonstrated in different tumors, including breast cancer and may increase with th progression of the tumor. Activation of RAGE may stimulate the proliferation, migration, and invasivity of tumor cells [130].

RAGE plays essential roles in inflammation, RAGE is the link between inflammation pathways and pathways that promote tumorigenesis and many other conditions [130].

The results shown in Table 3-10, patients with breast cancer had greater median serum RAGE levels compared to control group values, 0.557 pg/ml versus 0.533 pg/ml, the difference was highly a significant ($P < 0.003$).

Table 3-10: Median levels of Serum RAGE in patients with breast cancer and control subjects.

RAGE (pg\ml)	Patients N= 50	Patients N=50	P-value
Median	0.557	0.533	0.003 HS

N: number of cases, RAGE: Receptor for Advanced Glycation End Products, HS: High Significant.

3.6.2.1. Distribution of Serum RAGE Level According to Tumor Characteristics

The results of this study showed that there was significant association between the mean levels of RAGE in the serum and the tumor size. the mean of serum RAGE increased with increasing the size of tumor with higher levels in T4 (1.34 ±1.92) size in compared to T1, T2 and T3 size in patients group,

(0.52±0.1, 0.56±0.05, 0.51±0.12), respectively ($p < 0.023$). The levels were higher in grade three (0.92±1.31) than in grade one and grade two , (0.54±0.078 and0.55±0.082, respectively ($p = 0.24$).

RAGE and its ligand HMGB1 are considered as critical mediators of cancer development and progression through activation of oncogenic signaling cascades linked to tumor cell proliferation and metastasis [131].

RAGE may increase with tumor progression. Which leads to the activation and stimulation of the proliferation, transmission and spread of cancer cells. However, this increase, perhaps as a compensatory mechanism to counter further progress [132]. This outcome is consistent with Mueen H. *et al.* 2022 [132].

Non significant association between mean levels of serum RAGE and cancer stage, the value was higher in stage two (0.85±1.2) compared than in stage1, stage 3 and stage 4, (0.57±0.04, 0.55±0.05, 0.54±0.05) respectively, (p> 0.62). as shown in Table 3-11.

Table 3-11: Distribution of Serum RAGE Level According to Tumor Characteristics.

RAGE level (pg/ml)					
Tumor size	**T1**	**T2**	**T3**	**T4**	**P-value**
Mean ± SD	0.52±0.1	0.56±0.05	0.51±0.12	1.34*±1.92	0.023 S
N	8	28	6	6	
Tumor Grade	**G1**	**G2**	**G3**	**P-value**	
Mean ± SD	0.54±0.07	0.55±0.08	0.92±1.31	0.24 NS	
N	3	34	13		
Stage of cancer	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**P-value**
Mean ± SD	0.57±0.04	0.85±1.27	0.55±0.056	0.54±0.056	0.62 NS
N	25	14	6	5	

N: number of cases, $P < 0.05$ is consider significant, NS: Non Significant.

3.6.3. MDA

The findings demonstrated results MDA showed that the difference was a highly significant (P < 0.002), as shown in Table 3-12.

Table 3-12: Comparison of MDA level in patients and control group.

Study Group	N	MDA level (ng/ml) Mean ± SD	P-value
patient	50	1.21 ± 0.07	**0.002**
control	50	1 ± 0.29	

P < 0.05 is consider significant, MDA: Malondialdehyde.

MDA, one of the final decomposition products of lipid peroxidation, is known to be present in human plasma and to possess biological properties that may be relevant to carcinogenesis. Lipid peroxides and their products can cause damage to membrane- bound enzymes and other macromolecules, including DNA, and have been implicated in several disease processes including cancer. Many studies have examined the possibility of a connection between lipid peroxidation and cancer [106]. Our findings of raised MDA levels in patients with cancer have provided further evidence of this relationship.

This outcome is consistent with D.Tsikas *et al.* 2017 [133] , C.Yeh *et al.*, 2005 [133], who found a significantly higher MDA level in breast cancer patients than in the control group (p < 0.05).

3.6.3.1. Distribution of Serum MDA Level According to Tumor Characteristics

The results of this study showed that there was non significant association between the mean levels of MDA in the serum and the tumor size, the mean of serum MDA increased with increasing the size of tumor with higher levels in T4 (2.65 ±3.51) size in compared to T1, T2 and T3,(p = 0.44). The levels were

higher in grade three (1.89±2.38) than in grade one and grade two, (p =0.73); as shown in Table 3-13.

Increased ROS in the tumor environment plays an important role in cancer progression by altering the expression of suppressor genes involved in apoptosis, increasing the expression of cytokines involved in angiogenesis This process facilitates the migration of cancer cells to other parts of the body. Thus, increases in free radicals and oxidative stress both play important roles in the development of cancer [134].

Increased oxidative stress caused by reactive species can reduce the body's antioxidant defense against angiogenesis and metastasis in cancer cells. These processes are main factors in the development of cancer. Bimolecular reactions cause free radicals in which create such compounds as malondialdehyde (MDA) [135].

Table 3-13: Distribution of serum MDA level according to Tumor characteristics.

MDA level (ng/ml)					
Tumor size	**T1**	**T2**	**T3**	**T4**	**P-value**
Mean ± SD	1.23±0.06	1.52±1.7	1.23±0.06	2.65*±3.51	0.44 NS
N	8	28	6	6	
Tumor Grade	**G1**	**G2**	**G3**	**P-value**	
Mean ± SD	1.26±0.08	1.47±1.54	1.89±2.38	0.73 NS	
N	3	34	13		
Stage of cancer	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**P-value**
Mean ± SD	1.58±1.79	1.84±2.29	1.18±0.08	1.21±0.09	0.84 NS
N	25	14	6	5	

N: number of cases, MDA: Malondialdehyde, NS: Non-Significant.

3.6.4. Vitamin E

The findings demonstrated results vitamin E showed that the difference was a Significant (P < 0.01), as shown in Table 3-12.

Table 3-14: Comparison of vitamin E level in patients and control group.

Study Group	N	Vitamin E level (ng/ml) Mean ± SD	P-value
patient	50	11.12* ± 1.88	**0.01**
control	50	9.5 ± 2.6	

N: number of cases, P < 0.05 is consider significant.

This result was disagreed with study done by Nsaif. G *et al.* 2018, In Iraq, who found significant decreased in levels of vitamin E ($p<0.05$) in malignant groups comparing to the control group.

There are several reports, which reveal decrease vitamin E concentration in BC patients, and Ha- cisevki *et al.* have seen no changes in vitamin E concentration [136].

This difference in the results may be due to the patients taking nutritional supplements that led to this increase in them.

3.5 Diagnostic values for Serum HMGB1, RAGE and MDA

Receiver operating characteristic curve (ROC) was used to assess the diagnostic values of HMGB1 and RAGE in identifying breast cancer patients, and which of them is more specific or sensitive in the diagnosis.

3.5.1 Receiver Operating Characteristic Curve for HMGB1

Receiver operator characteristic (ROC) curve analysis was performed to assess the HMGB-1 cutoff value as well as to forecast the breast cancer as diagnostic tests or adjuvant diagnostic tests. The outcomes are displayed in figure

3-2. The HMGB-1 cutoff value was > 5.069 with sensitivity 76%, specificity 70%, positive predictive value (PPV) 89.19%, negative predictive value (NPV) 55.17, and area under curve of 0.79 (0.676-0.91).

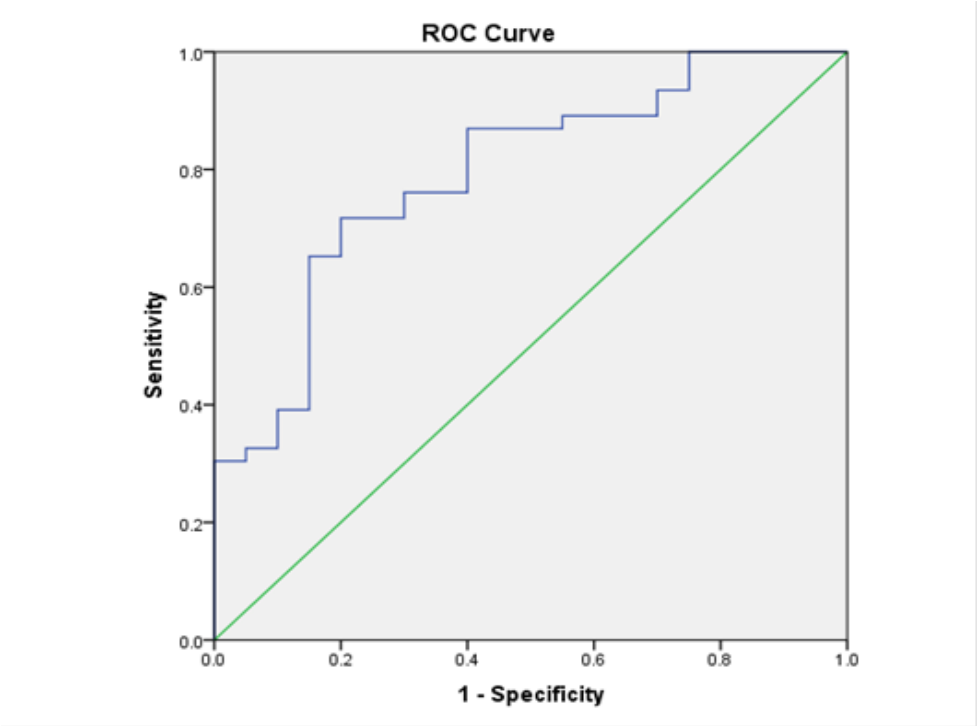

Figure 3-2: ROC curve for HMGB-1.

For HMGB1, our results stated that fair diagnostic value in the diagnosis of breast cancer.

3.5.2 Receiver Operating Characteristic Curve for RAGE

Receiver operator characteristic (ROC) curve analysis was performed to assess the RAGE cutoff value as well as to forecast the breast cancer as diagnostic tests or adjuvant diagnostic tests. The results are shown in figure 3-3. The RAGE cut off value was >0.548 with sensitivity 74 %, specificity 70 %, positive predictive value (PPV) 82.35 %, negative predictive value (NPV) 41.67 %, and area under curve of 0.81 (0.691-0.923).

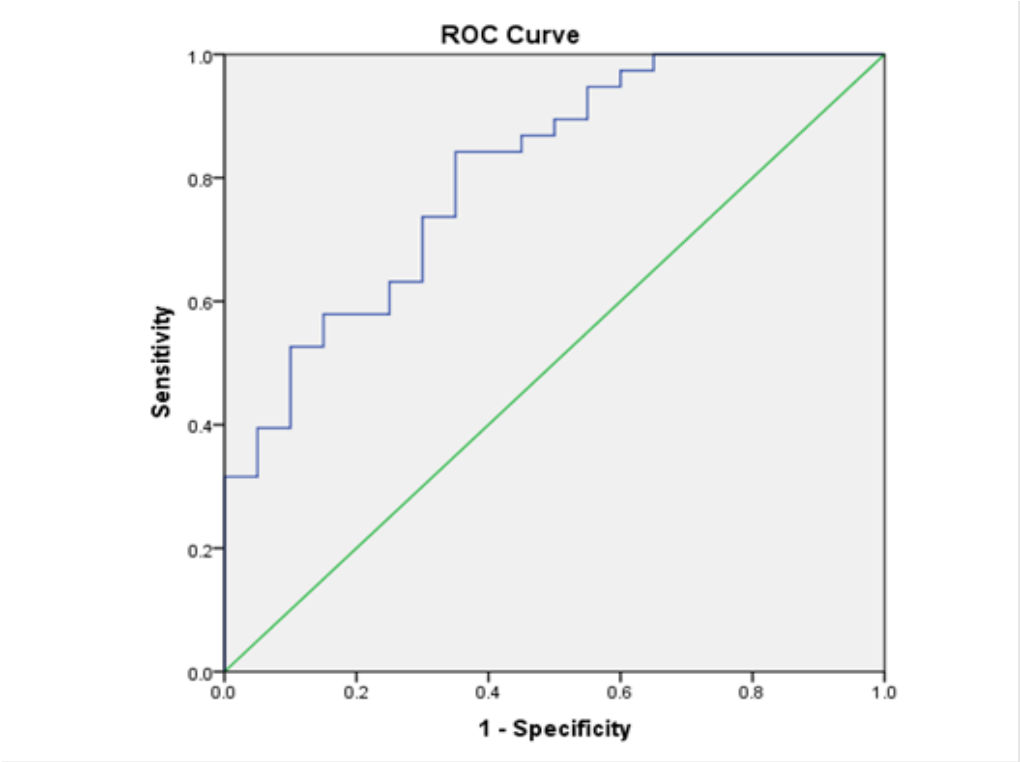

Figure 3-3: ROC curve for RAGE.

For RAGE, our results stated that good diagnostic value in the diagnosis of breast cancer.

3.5.3. Receiver Operating Characteristic Curve for MDA

Receiver operator characteristic (ROC) curve analysis was performed to assess the MDA cutoff value as well as to forecast the breast cancer as diagnostic tests or adjuvant diagnostic tests. The results are shown in figure 3-4. The MDA cut off value was >1.1397 with sensitivity 88 %, specificity 70.8 %, positive predictive value (PPV) 86.3 %, negative predictive value (NPV)73.9 %, and area under curve of 0.86(0.784-0.957).

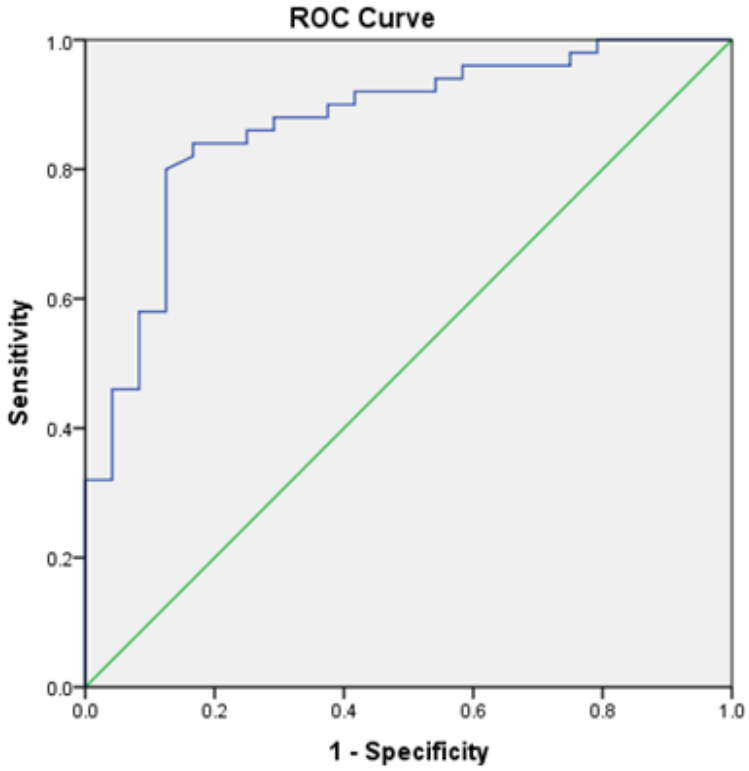

Figure 3-4: ROC curve for MDA.

For MDA, our results stated that good diagnostic value in the diagnosis of breast cancer.

3.6 Pearson Correlations of HMGB1 and RAGE, with other parameters.

There is no significant correlation of HMGB1 with RAGE r=0.13, P=0.4. So HMGB1 level no effect RAGE level, as shown in Table 3-12 and figure 3-2, While there is a statistically significant correlation with vitamin E, MDA (r=0.35, P=0.015) (r=0.41, P=0.003), respectively. So HMGB1 level indirectly effect MDA and vitamin E level as figure 3-3, 3-4.

Table 3-15: Correlations between HMGB1 and other parameters.

		HMGB1
RAGE	R	0.13
	p-value	0.4
MDA	R	0.35*
	p-value	0.015
Vitamin E	R	0.41**
	p-value	0.003

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level, r: Pearson correlation.

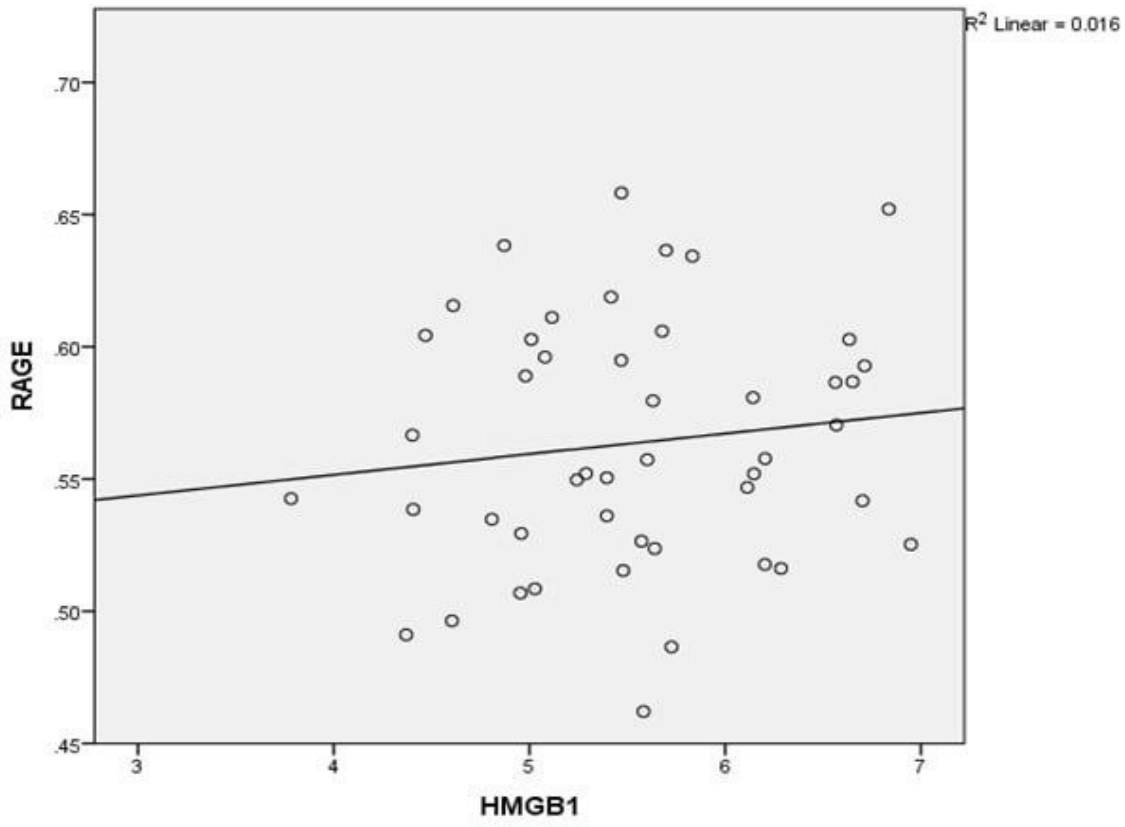

Figure 3-4: Correlations between HMGB1 and RAGE.

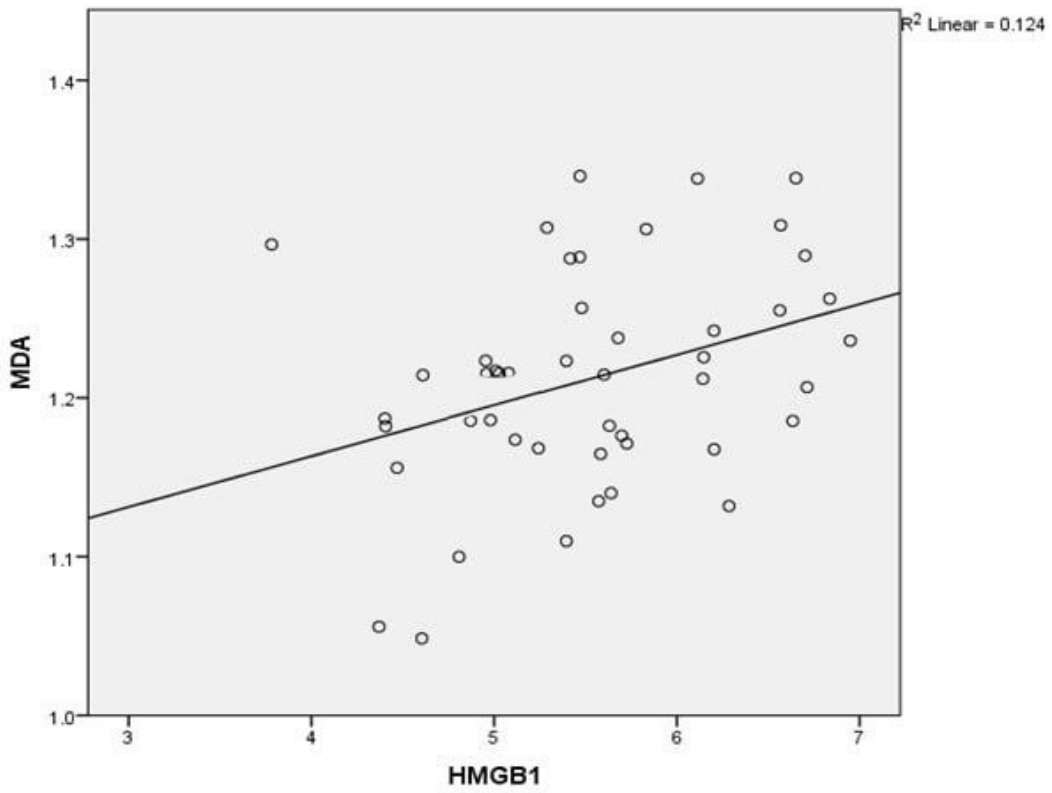

Figure 3-5: Correlations between HMGB1 and MDA.

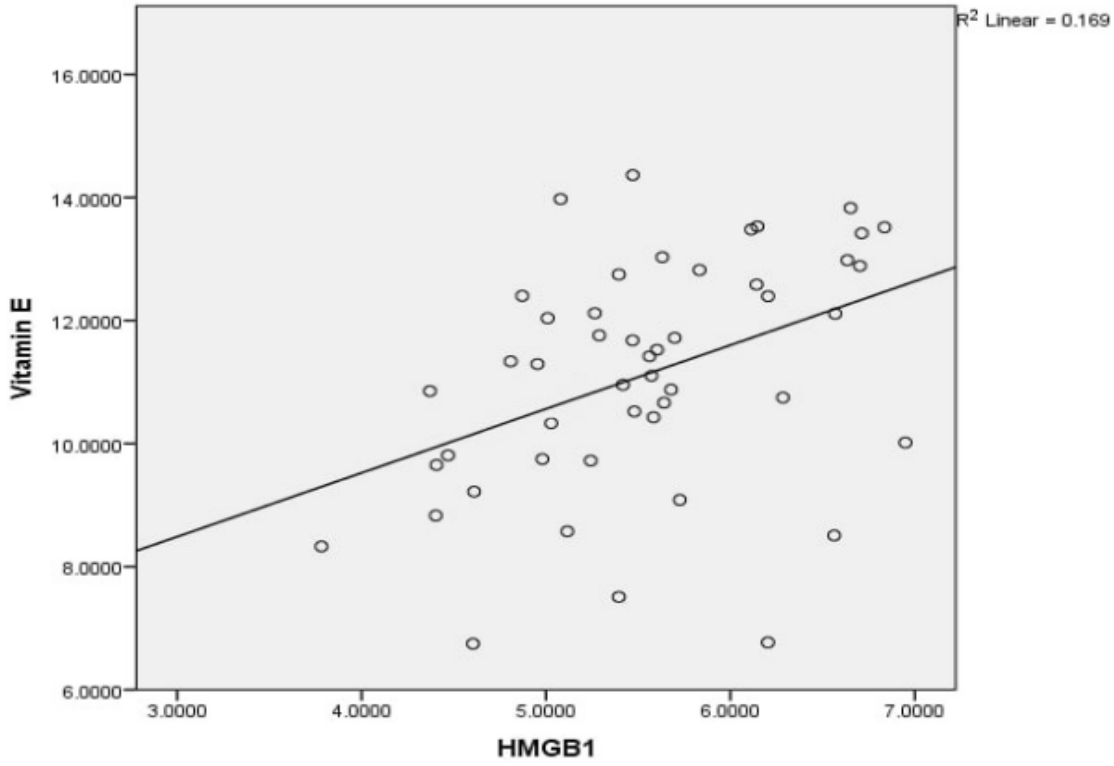

Figure 3-6: Correlations between HMGB1 and Vitamin E.

There is a statistically significant correlation of RAGE with vitamin E and MDA (r =0.43, p =0.003), (r =0.4, p =0.005) respectively, as shown in Table 3-13 and figure 3-5, 3-6.

Table 3-16: Correlations between RAGE and other parameters.

		RAGE
MDA	r	0.4**
	p-value	0.005
Vitamin E	r	0.43**
	p-value	0.003

P < 0.05 is consider significant, r:Pearson correlation, MDA: Malondialdehyde.

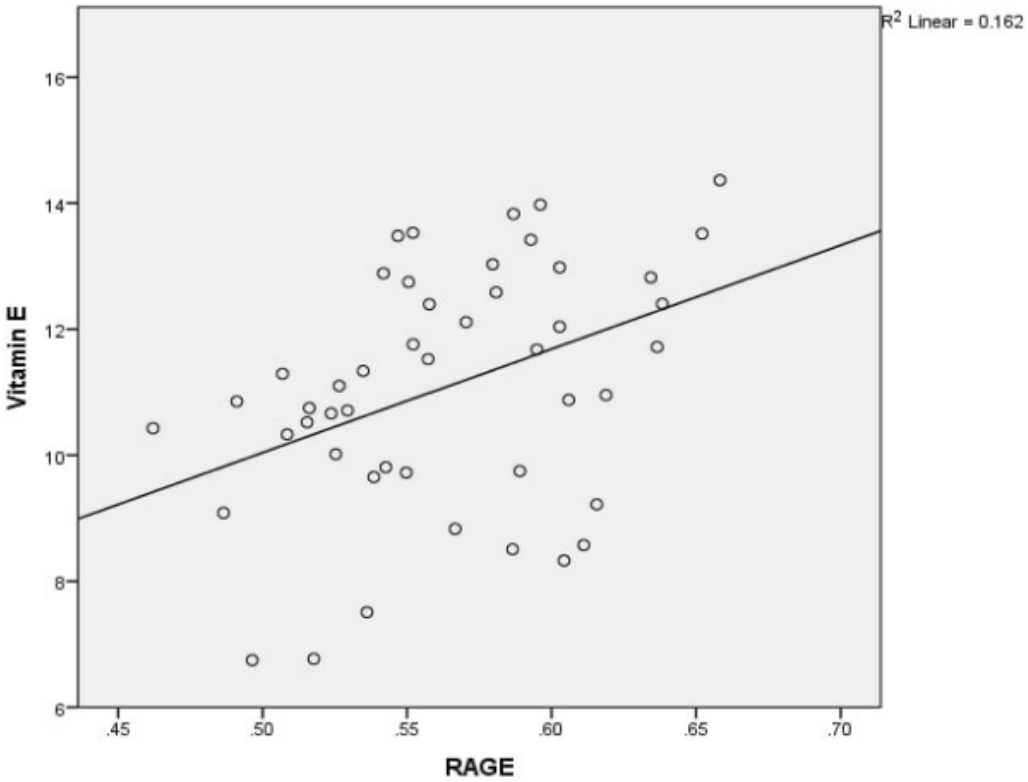

Figure 3-7: Correlations between RAGE and Vitamin E.

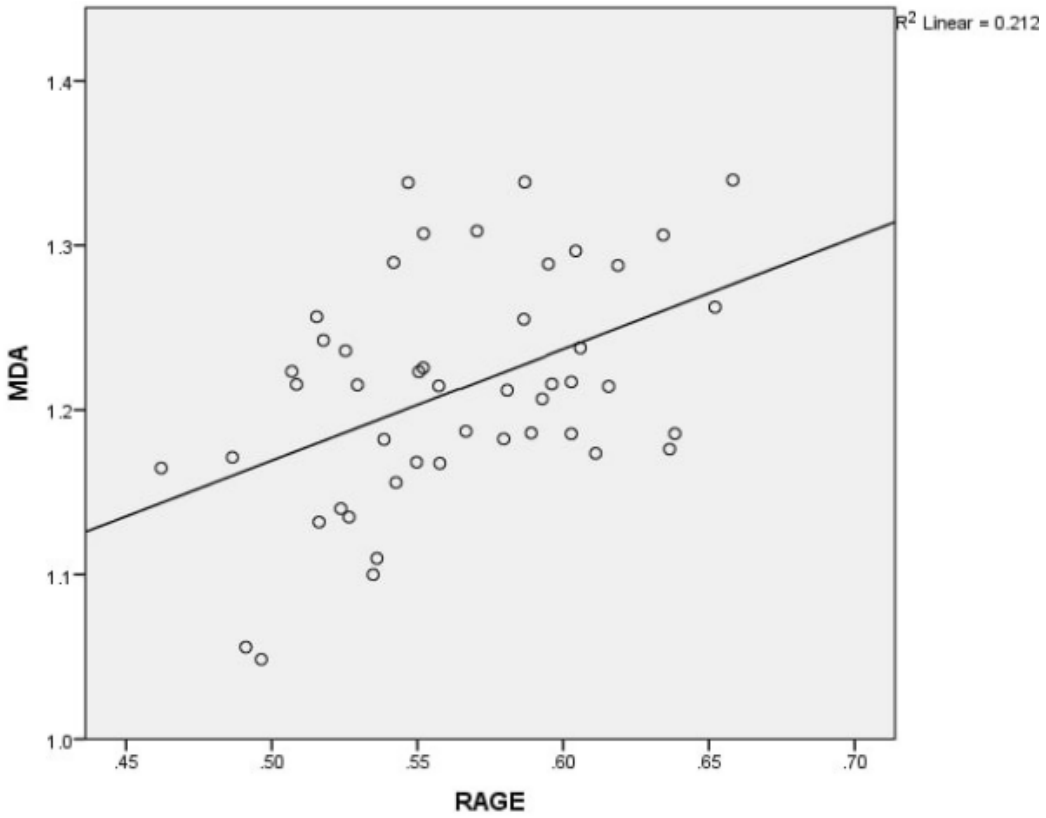

Figure 3-8: Correlations between RAGE and MDA.

3.7 Genetic Analysis

3.7.1. Detection of Genes Polymorphisms

3.7.1.1. Detection of RAGE rs18400624 Polymorphism

The two alleles of RAGE gene were separated by using the restriction enzyme method. The A allele resulted in an undigested PCR product of 344 base pairs (bp), There are three genotypes: TA,TT and AA. The homozygote genotype were showed only T allele amplification at 130, 110, 75, 29 base pairs bp. The homozygote genotype were showed only A allele amplification at 240, 75, 29 base pairs bp. Whereas, the heterozygote genotype were showed T and A alleles amplification at 240 , 130, 110, 75, 29 bp respectively.

3.7.1.2. Electrophoresis Results

The figure 3-5 shows an agarose gel of a PCR product digested with a restriction enzyme (Tsp509I) for SNP rs1800624 of the RAGE gene polymorphism. Gene polymorphisms of homozygous AA and TT homozygotes.

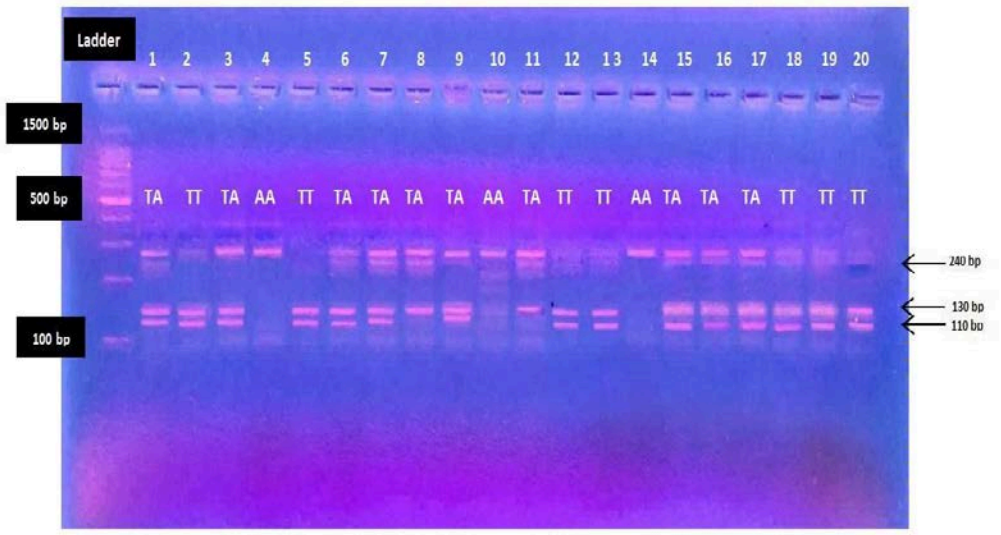

Figure 3- 9: Agarose gel (2%) electrophoresis of PCR product digested with (Tsp509I) restriction enzyme.

3.7.1.3. Distribution of Allele Frequency of RAGE (rs1800624) SNP in breast cancer Patients and Control Group

There is non-significant difference in allele frequency (P=0.06) of A allele and T allele, as shown in Table 3-5 between patient with and healthy control. This indicate that no one of the two alleles represent a risk factor for breast cancer. This result agreed with Pan H *et al.* who found no association between alleles of the RAGE polymorphism and breast cancer [137].

Table 3-17: Allele Distribution of (rs1800624) SNP of RAGE gene Polymorphism.

RAGE	Patient N=50	Control N= 50	P-value	OR	95% CI
T	74	62	**0.06**	1.194	0.985-1.447
A	26	38		0.684	0.45-1.036

OR: odds ratio, CI: confidence interval.

3.7.1.4. Genotype Distribution and Allele Frequency of rs1800624

There is non-significant difference between patients and control in TT, AA, and TA genotyping of (rs1800624) in RAGE gene (p=0.22) as in Table 3-6. This indicates that there is no association between SNP genotyping and breast cancer. This may be due to the fact that the chosen SNP (rs1800624) or the chosen gene may not be closely related to the disease or may be due to the small sample size taken for the study, it may be in larger sample size will get a significant difference.

Table 3-18: The Genotype Distribution of (rs1800624) SNP in RAGE Gene.

RAGE	Patient N=50	Control N= 50	P-value	OR	95% CI
TT	27	20			
TA	20	22	**0.22**	1.186	0.907-1.55
AA	3	8		0.489	0.146-1.64

OR: odds ratio, CI: confidence interval.

The observed genotype distributions of examined polymorphisms in RAGE gene were consistent with the Hardy-Weinberg equilibrium in both breast cancer patients and controls (P > 0.05).

This results similar with other studies such as Pan H *et al.*, 2014, In Han-China, which is case control study included 509 patients with breast cancer and 504 control group [137], Hashemi *et al*. 2012, In Iran which is case control study included 71 patients with breast cancer and 93control group there was no difference between RAGE (-374T/A) bp polymorphisms among patients with breast cancer and healthy controls, suggesting that larger studies are necessary to demonstrate any putative relation between RAGE gene polymorphisms and susceptibility to breast cancer [138].

This result was disagreed with study done by Yue L *et al.*, 2016, In Han-China, which is case control study included 524 patients with breast cancer and 518 control group, the frequency of rs1800624 polymorphism mutant A allele in RAGE gene was significantly higher in patients than in controls (24.52% versus 19.50%, P = 0.006) [138]. This study differs with the current result may be due to the low sample size and inclusion criteria chosen for the study.

3.7.2. Detection of HMGB1 rs1412125 Polymorphism

The distribution of HMGB1 rs1412125 Polymorphism was detected by specefic allele -PCR technique. At this locus there are three genotypes; TC, TT and CC. The wild type homozygote genotype were showed only T allele amplification at 209 bp product size. The mutant type homozygote genotype were showed only C allele amplification at 209bp product size. Whereas, the heterozygote genotype were showed T and C alleles amplification at 209bp product size respectively, as in the figure 3-14. The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups and agree with the reports of Yue *et al.,* (2016) [139].

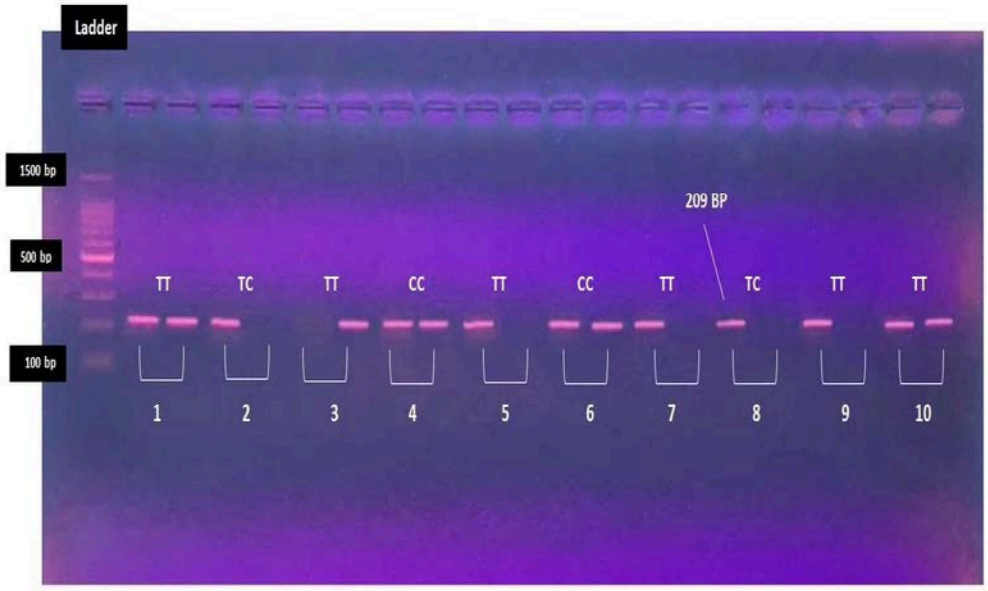

Figure 3-10: Agarose gel (2%) electrophoresis of Allele Specefic PCR product analysis of rs1412125 (T/C) gene polymorphism. (M), 100-1500 base pair ladder. The presence of (TT) wild type homozygote were showed in T wild type allele only. The presence of (CC) Mutant type homozygote were showed in C mutant type allele only. Whereas the (T/C) heterozygote were showed in both T and C allele. All genotypes observed at 209bp product size.

3.7.2.1. Distribution of Allele Frequency of (rs1412125) SNP in breast cancer Patients and Control Group

There is non-significant difference in allele frequency (P=0.2) of T allele and C allele, as shown in Table 3-5 between patient with and healthy control.

This indicate that no one of the two alleles represent a risk factor for breast cancer. This result agreed with *et al.* who found no association between alleles of the RAGE polymorphism and breast cancer.

Table 3-19: Allele Distribution of (rs1412125) SNP of HMGB1 gene Polymorphism.

HMGB1	Patient N=50	Control N= 50	P-value	OR	95% CI
T	60	72	0.237	0.828	0.6-1.136
C	40	28		1.455	0.774-2.732

OR: odds ratio, CI: confidence interval.

3.7.2.2. Genotype Distribution and Allele Frequency of (rs1412125)

There is non-significant difference between patients and control in TT, TC, and CC genotyping of (rs1412125) in HMGB1 gene (p=0.09) as in Table 3-7.

Table 3-20: The Genotype Distribution of (rs1412125) SNP in HMGB1 Gene.

HMGB1	Patient N=50	Control N= 50	P-value	OR	95% CI
TT	25	27			
TC	10	18	**0.09**	0.514	0.223-1.186
CC	15	5		2.7	0.72-10.14

OR: odds ratio, CI: confidence interval.

HMGB1 plays multiple roles inside and outside cells, such as chromatin stabilization, DNA repair, gene transcription, program cell death regulation, and immune response. The HMGB1 gene has been implicated in tumor progression in various types of cancer such as colon, liver, breast, oral, and lung cancer [140].

All genotypic frequencies were in Hardy-Weinberg equilibrium ($p > 0.05$). This results similar with other studies such as B- Huang *et al.*, 2018, In China, which is case control study included 313 patients with breast cancer and

217 control group, the frequency of rs1412125 polymorphism in HMGB1 gene was non significantly in patients and controls [141], Yue. L *et al.*, 2016, [139]. This result was disagreed with study done by Hantoosh MH *et al.*, 2022 [141].

3.7.3. Association between RAGE rs18400624 genotype and RAGE levels

The Table 3-22 shows a non significant association between RAGE levels (pg/ml) and the Genotype TT, TA, AA in patients and control, P =0.6, 0.3, respectively. This indicate non significant association between RAGE (pg/ml) and RAGE rs18400624 Polymorphism in patients with BC.

Table 3-21: Association between RAGE rs18400624 genotype and RAGE levels.

Genotype	RAGE serum level (pg/ml) Mean± SE	p-value
Patient		
TT	0.56±0.04	0.6 NS
TA	0.56±0.05	
AA	0.6±0.1	
Genotype	**RAGE serum level (pg/ml) Mean± SE**	**p-value**
Control		
TT	0.45± 0.154	0.3 NS
TA	0.44±0.139	
AA	0.58±0.02	

NS: Non-Significant, SE: Standard error.

3.7.4. Association between HMGB1 rs1412125 genotype and HMGB1 levels.

The Table 3-23 shows a non significant association between HMGB1 levels (pg/ml) and the Genotype TT, TC, CC in patients and control, P =0.8, 0.9, respectively. this indicate non significant association between RAGE (pg/ml) and HMGB1 rs1412125 Polymorphism in patients with BC.

Table 3-22: Association between RAGE rs18400624 genotype and RAGE levels.

Genotype	HMGB1 serum level (ng/ml) Mean± SE	p-value
Patient		
TT	0.59±0.1	0.8
TC	0.56±0.04	
CC	0.59±0.04	
Genotype	**RAGE serum level (pg/ml) Mean± SE**	**p-value**
Control		
TT	0.45±0.16	0.9
TC	0.46±0.14	
CC	0.44±0.23	

NS: Non-Significant, SE: Standard error.

Conclusions

1. The level of HMGB1and RAGE could be used as a prediction tool aid in the diagnosis of Breast Cancer.
2. The polymorphisim of rs1412125 of HMGB1 and rs1800624 of RAGE could not be use in the diagnosis or prediction of Breast Cancer.

Recommendations

1. Study other SNP in the candidate genes in this region.
2. To obtain a complete picture of genotype distribution in the Iraqi population, the sample size must be increased and more Iraqi provinces added. Using DNA sequencing technique to detect the genetic polymorphism of SNPs.

References

References

1. Zakariya BF, Almohaidi AMS, Akilli S, Kamal AM, Al-dabbagh WH, Al-waysi SA. Associations between single-nucleotide polymorphisms of the interleukin-18 gene and breast cancer in Iraqi women. 2022;1–7.

2. Valenzuela M, Julian TB. Ductal carcinoma in situ: Biology, diagnosis, and new therapies. Clin Breast Cancer. 2007;7(9):16–21.

3. Jemal A, Bray F, Ferlay J. Global Cancer Statistics: 2018. CA Cancer J Clin [Internet]. 1999;49(2):1,33-64.

4. Jassim MMA, Hamad BJ, Hussein MH. Review on Breast Cancer in Iraq Women. Univ Thi-Qar J Sci. 2022;9(1):92–4.

5. Aljuboury N. Breast Cancer in Iraq: A Review. Int J Med Sci. 2018;1(1):6–12.

6. Lima SM, Kehm RD, Beth M. EClinicalMedicine Global breast cancer incidence and mortality trends by region , age- groups , and fertility patterns. EClinicalMedicine. 2021;38:100985.

7. Sun Y, Zhao Z, Yang Z, Xu F, Lu H, Zhu Z, et al. Risk Factors and Preventions of Breast Cancer. 2017;13.

8. Smolarz B, Zadrożna Nowak A, Romanowicz H. Breast Cancer—Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). Cancers (Basel). 2022;14(10):1–27.

9. Nardin S, Mora E, Varughese FM, Avanzo FD, Vachanaram AR, Rossi V, et al. Breast Cancer Survivorship , Quality of Life , and Late Toxicities. 2020;10(June):1–6.

10. Lips EH, Kumar T, Megalios A, Visser LL, Sheinman M, Fortunato A, et al. Genomic analysis defines clonal relationships of ductal carcinoma in situ

and recurrent invasive breast cancer. Nat Genet. 2022;54(6):850–60.

11. Mehrgou A, Akouchekian M. The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. 2016;1–12.

12. Carey LA. Through a glass darkly: Advances in understanding breast cancer biology, 2000-2010. Clin Breast Cancer. 2010;10(3):188–95.

13. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Non-ionizing radiation, Part 1: static and extremely low-frequency (ELF) electric and magnetic fields. IARC Monogr Eval Carcinog risks to humans. 2002;80:1—395.

14. Yue W, Wang J, Li Y, Fan P, Liu G, Zhang N, et al. Effects of estrogen on breast cancer development : role of estrogen receptor independent mechanisms. 2019;1757:1748–57.

15. Shatnawi A, Ayoub NM, Alkhalifa AE, Ibrahim DR. Estrogen-Related Receptors Gene Expression and Copy Number Alteration Association With the Clinicopathologic Characteristics of Breast Cancer. Breast Cancer Basic Clin Res. 2022;16:11782234221086712.

16. Mehmood S, Yasin A, Ahmad I, Abiha Rabab S, Muhammad Soomar A. Relationship Between Obesity, Menopausal Status and Breast Cancer Risks - A Review. Pakistan J Heal Sci. 2022;02–7.

17. Jiralerspong S, Goodwin PJ. Obesity and Breast Cancer Prognosis : Evidence , Challenges , and Opportunities. 2016;34(35).

18. Fortner RT, Katzke V, Kühn T, Kaaks R. Obesity and Breast Cancer. In: Pischon T, Nimptsch K, editors. Obesity and Cancer. Cham: Springer International Publishing; 2019. p. 43–65.

19. Pandere PS, More SS, Kale V V, Nagrale DS, Pondkule A V. A systemic

review on triple negative breast cancer. Int J Chem Res Dev. 2022;4(2):07–9.

20. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. Oxygen. 2022;2(2):48–78.

21. Calaf GM. Curcumin, oxidative stress, and breast cancer. Cancer Oxidative Stress Diet Antioxidants. 2021;359–70.

22. Kuo C-L, Ponneri Babuharisankar A, Lin Y-C, Lien H-W, Lo YK, Chou H-Y, et al. Mitochondrial oxidative stress in the tumor microenvironment and cancer immunoescape: foe or friend? J Biomed Sci. 2022;29(1):74.

23. Nelson HD, Fu R, Cantor A, Pappas M, Daeges M, Humphrey L. Effectiveness of breast cancer screening: Systematic review and meta-analysis to update the 2009 U.S. Preventive services task force recommendation. Ann Intern Med. 2016;164(4):244–55.

24. Rusby JE, Brachtel EF, Michaelson JS, Koerner FC, Smith BL. Breast duct anatomy in the human nipple: Three-dimensional patterns and clinical implications. Breast Cancer Res Treat. 2009;106(2):171–9.

25. Zetterlund LH. Sentinel Lymph Node Biopsy in Breast Cancer-Aspects on Indications and Limitations. 2019.

26. Wang L. Early diagnosis of breast cancer. Sensors (Switzerland). 2017;17(7).

27. Ray-Coquard I, Cropet C, Van Glabbeke M, Sebban C, Le Cesne A, Judson I, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. Cancer Res. 2019;69(13):5383–91.

28. Polite BN, Adams-campbell LL, Brawley OW, Bickell N, Carethers JM,

Flowers CR, et al. Charting the Future of Cancer Health Disparities Research : A Position Statement from the American Association for Cancer Research , the American Cancer Society , the American Society of Clinical Oncology , and the National Cancer Institute. 2017;77(17):4548–55.

29. Ashokkumar N, Meera S, Anandan P, Murthy MYB, Kalaivani KS, Alahmadi TA, et al. Deep Learning Mechanism for Predicting the Axillary Lymph Node Metastasis in Patients with Primary Breast Cancer. Biomed Res Int. 2022;2022.

30. Effiong EB, Nweke BC, Okereke AH, Pius ANO. BREAST CANCER–REVIEW. J Glob Biosci Vol. 2022;11(3):9248–57.

31. Salvatorelli L, Puzzo L, Vecchio GM, Caltabiano R, Virzì V, Magro G. Ductal carcinoma in situ of the breast: An update with emphasis on radiological and morphological features as predictive prognostic factors. Cancers (Basel). 2020;12(3):1–11.

32. Wen HY, Brogi E. Lobular Carcinoma In Situ. Surg Pathol Clin. 2018;11(1):123–45.

33. Eusebi V, Feudale E, Foschini MP, Micheli A, Conti A, Riva C, et al. Long-term follow-up of in situ carcinoma of the breast. Semin Diagn Pathol. 1999;11(3):223—235.

34. Watkins EJ. Overview of breast cancer. J Am Acad Physician Assist. 2019;32(10):13–7.

35. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer. Biol Res. 2019;1–23.

36. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: Tumor characteristics and clinical outcome. Breast Cancer Res. 2008;6(3):7–11.

37. Thomas M, Kelly ED, Abraham J, Kruse M. Invasive lobular breast cancer: A review of pathogenesis, diagnosis, management, and future directions of early stage disease. Semin Oncol. 2019;46(2):121–32.

38. Demir S, Sezgin G, Sari AA, Kucukzeybek BB, Yigit S, Etit D, et al. Clinicopathological analysis of invasive cribriform carcinoma of the breast, with review of the literature. Ann Diagn Pathol. 2021;54:151794.

39. Adachi Y, Katsuki T, Sasaguri T, Hirata K, Nagata N. Invasive cribriform carcinoma of the breast detected incidentally on computed tomography: A case report. Clin Case Reports. 2021;9(12):5–9.

40. Ferguson MJ. Multifocal invasive mucinous carcinoma of the breast. J Med Radiat Sci. 2020;67(2):155–8.

41. Erarslan D, Schmitt F. A Review of the Recent Findings on Ductal Carcinoma In Situ of the Breast (DCIS). J Women's Heal Dev. 2019;03(01):1–24.

42. Almekinders MM, Bismeijer T, Kumar T, Yang F, Thijssen B, van der Linden R, et al. Comprehensive multiplexed immune profiling of the ductal carcinoma in situ immune microenvironment regarding subsequent ipsilateral invasive breast cancer risk. Br J Cancer. 2022;127(7):1201–13.

43. Yang M, Moriya T, Oguma M, De La Cruz C, Endoh M, Ishida T, et al. Microinvasive ductal carcinoma (T1mic) of the breast. The clinicopathological profile and immunohistochemical features of 28 cases. Pathol Int. 2009;53(7):422–8.

44. Samineni R, Chimakurthy J, Udayaratna K, Devatulasi K, Varshitha Reddy B, Keerthi Sri G, et al. a Quick Overview of Nanomedicine Applicationsin Breast Cancer Detection, Imaging, and Therapy. Asian J Adv Med Sci. 2022;4(2):44–56.

45. Dalberg K, Hellborg H, Wärnberg F. Paget's disease of the nipple in a population based cohort. Breast Cancer Res Treat. 2008;111(2):313–9.

46. Giuliano AE, Edge SB, Hortobagyi GN. Eighth Edition of the AJCC Cancer Staging Manual: Breast Cancer. Ann Surg Oncol. 2018;25(7):1783–5.

47. Schonberg MA. Breast Cancer Screening in the Older Woman. Breast Cancer Screening: An Examination of Scientific Evidence. Elsevier Inc.; 2018. 189–217 p.

48. Singh M, Rittenbach J V. Ductal and lobular proliferations: Preinvasive breast disease. Early Diagnosis Treat Cancer Ser Breast Cancer Expert Consult - Online Print. 2010;11–20.

49. LOHRISCH C. An overview of HER2. Semin Oncol. 2001;28:3–11.

50. Provenzano E, Ulaner GA, Chin S. Molecular Classification Of Breast Cancer.

51. Ades F, Zardavas D, Bozovic-spasojevic I, Pugliano L, Fumagalli D. Luminal B Breast Cancer : Molecular Characterization , Clinical Management , and Future Perspectives. 2014;1–12.

52. Liu JC, Voisin V, Bader GD, Deng T, Pusztai L, Fraser W. Seventeen-gene signature from enriched Her2 / Neu mammary tumor-initiating cells predicts clinical outcome for human HER2 + : ER α − breast cancer. 2012;

53. Young RB, Chew HK. Triple-negative breast cancer. Tumor Board Rev Evidence-Based Case Rev Quest. 2022;87–100.

54. Koh J, Kim MJ. Introduction of a New Staging System of Breast Cancer for Radiologists : An Emphasis on the Prognostic Stage. 2019;20(1):69–82.

55. Güth U, Jane Huang D, Holzgreve W, Wight E, Singer G. T4 breast cancer under closer inspection: A case for revision of the TNM classification.

Breast. 2007;16(6):625–36.

56. Tang J, Rangayyan RM, Xu J, El Naqa IE, Yang Y. Computer-aided detection and diagnosis of breast cancer with mammography: Recent advances. IEEE Trans Inf Technol Biomed. 2009;13(2):236–51.

57. Jalalian A, Mashohor SBT, Mahmud HR, Saripan MIB, Ramli ARB, Karasfi B. Computer-aided detection/diagnosis of breast cancer in mammography and ultrasound: A review. Clin Imaging . 2018;37(3):420–6.

58. Autier P, Boniol M, Middleton R, Doré JF, Héry C, Zheng T, et al. Advanced breast cancer incidence following population-based mammographic screening. Ann Oncol. 2019;22(8):1726–35.

59. Walker, S O'Carroll JK-2012-tara. tcd. i. Secondary Breast Cancer.

60. WiÅniewska M, WiÅniewski M, Lewandowska MA. Personalized and targeted therapies. Phys Sci Rev. 2021;2019.

61. Kunkler IH, Williams LJ, Jack WJL, Cameron DA, Dixon JM. Breast-conserving surgery with or without irradiation in early breast cancer. N Engl J Med. 2023;388(7):585–94.

62. Srivastava A, Jamil S, Khandelwal A, Raj M, Singh S. Thoracic Epidural Anesthesia for Modified Radical Mastectomy in a High-Risk Patient: A Case Report With Literature Review. Cureus. 2021;2021.

63. Bosma SCJ, Van Der Leij F, Van Werkhoven E, Bartelink H, Wesseling J, Linn S, et al. Very low local recurrence rates after breast-conserving therapy: Analysis of 8485 patients treated over a 28-year period. Breast Cancer Res Treat. 2016;156(2):391–400.

64. Kubota S, Takigawa M. The CCN family acting throughout the body:

Recent research developments. Biomol Concepts. 2013;4(5):477–94.

65. Ganesh N. Sharma*1, Rahul Dave1, Jyotsana Sanadya2, Piush Sharma2 KKS. March 2010 Revised: 19. 2010;1(2):109–26.

66. Cheung-Ong K, Giaever G, Nislow C. DNA-damaging agents in cancer chemotherapy: Serendipity and chemical biology. Chem Biol [Internet]. 2013;20(5):648–59.

67. Wu X, Wu MY, Jiang M, Zhi Q, Bian X, Xu MD, et al. TNF-α sensitizes chemotherapy and radiotherapy against breast cancer cells. Cancer Cell Int. 2017;17(1):1–12.

68. Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol. 2007;28(10):429–36.

69. Rock, Kenneth L; Kono H, Adriano Aguzzi, Christina Sigurdson and MH. The inflammatory response to cell death. Annu Rev Pathol Dis. 2018;3:67–97.

70. Dong H, Zhang L, Liu S. Targeting HMGB1 : An available Therapeutic Strategy for Breast Cancer Therapy. 2022;18.

71. Sohun M, Shen H. The implication and potential applications of high-mobility group box 1 protein in breast cancer. 2016;4(9):1–13.

72. Andersson U, Yang H, Harris H. High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells. Semin Immunol. 2018;38(February):40–8.

73. Blair RH, Horn AE, Pazhani Y, Grado L, Goodrich JA, Kugel JF. The HMGB1 C-Terminal Tail Regulates DNA Bending. J Mol Biol. 2016;428(20):4060–72.

74. Wang Q, Zeng M, Wang W, Tang J. The HMGB1 acidic tail regulates HMGB1 DNA binding specificity by a unique mechanism. Biochem Biophys Res Commun. 2017;360(1):14–9.

75. Yang H, Antoine DJ, Andersson U, Tracey KJ. The many faces of HMGB1 : molecular structure-functional activity in inflammation , apoptosis , and chemotaxis. 2013;93(June):865–73.

76. Valdes-ferrer SI, Hotamisligil S, Tracey KJ. Novel role of PKR in inflammasome activation and HMGB1 release. 2015;1–6.

77. Tripathi A, Shrinet K, Kumar A. HMGB1 protein as a novel target for cancer. Toxicol Reports. 2019;6(February):253–61.

78. Kazama H, Ricci J, Herndon JM, Hoppe G, Green DR, Ferguson TA. Article Induction of Immunological Tolerance by Apoptotic Cells Requires Caspase-Dependent Oxidation of High-Mobility Group Box-1 Protein. 2008;21–32.

79. Flohr AM, Rogalla P, Meiboom M, Borrmann L, Krohn M, Thode-Halle B, et al. Variation of HMGB1 expression in breast cancer. Anticancer Res. 2001;21(6 A):3881–5.

80. Mantovani A, Garlanda C, Allavena P. Molecular pathways and targets in cancer-related inflammation. Ann Med. 2018;42(3):161–70.

81. Sun S, Zhang W, Cui Z, Chen Q, Xie P, Zhou C, et al. High mobility group box-1 and its clinical value in breast cancer. Onco Targets Ther. 2015;8:413–9.

82. Jiao Y, Wang HC, Fan SJ. Growth suppression and radiosensitivity increase by HMGB1 in breast cancer. Acta Pharmacol Sin. 2017;28(12):1957–67.

83. El-far AH, Sroga G, Jaouni SK Al, Mousa SA. Role and Mechanisms of

RAGE-Ligand Complexes and RAGE-Inhibitors in Cancer Progression. :1–21.

84. Smolarczyk R, Cichoń T, Jarosz M, Szala S. [HMGB1--its role in tumor progression and anticancer therapy]. Postepy Hig Med Dosw . 2012;66:913–20.

85. Pan S. m i c n h i b i t s M e t a s t a s i s o f B re a s t C a n c e r C e l l s b y T a r g e t i n g.

86. Yin NC, Lang XP, Wang XD, Liu W. AGER genetic polymorphisms increase risks of breast and lung cancers. Genet Mol Res. 2015;14(4):17776–87.

87. Gao J, Shao Y, Lai W, Ren H, Xu D. Association of polymorphisms in the RAGE gene with serum CRP levels and coronary artery disease in the Chinese Han population. J Hum Genet. 2019;55(10):668–75.

88. Sessa L, Gatti E, Zeni F, Antonelli A, Catucci A, Koch M, et al. The Receptor for Advanced Glycation End-products (RAGE) is only present in mammals, and belongs to a family of Cell Adhesion Molecules (CAMs). PLoS One. 2018;9(1).

89. Laczko R, Chang A, Watanabe L, Petelo M, Kahaleua K, Bingham J-P, et al. Anti-inflammatory activities of Waltheria indica extracts by modulating expression of IL-1B, TNF-α, TNFRII and NF-κB in human macrophages. Inflammopharmacology . 2020;28(2):525–40.

90. Jangde N, Ray R, Rai V. RAGE and its ligands: from pathogenesis to therapeutics. Crit Rev Biochem Mol Biol. 2020;55(6):555–75.

91. Yue Q, Song Y, Liu Z, Zhang L, Yang L, Li J. Receptor for Advanced Glycation End Products (RAGE): A Pivotal Hub in Immune Diseases. Molecules. 2022;27(15):4922.

92. Fang F, Lue L-F, Yan S, Xu H, Luddy JS, Chen D, et al. RAGE-dependent signaling in microglia contributes to neuroinflammation, Aβ accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease. FASEB J. 2010;24(4):1043–55.

93. Twarda-clapa A, Olczak A, Białkowska AM, Koziołkiewicz M. Cells-11-01312-V2.Pdf. 2022;

94. Rehman S, Aatif M, Rafi Z, Khan MY, Shahab U, Ahmad S, et al. Effect of non-enzymatic glycosylation in the epigenetics of cancer. Semin Cancer Biol. 2022;83:543–55.

95. Tiszlavicz Z, Gyulai Z, Bencsik K, Szolnoki Z, Kocsis ÁK, Somogyvári F, et al. RAGE gene polymorphisms in patients with multiple sclerosis. J Mol Neurosci. 2009;39(3):360–5.

96. Oh S, Son M, Choi J, Lee S, Byun K. sRAGE prolonged stem cell survival and suppressed RAGE-related inflammatory cell and T lymphocyte accumulations in an Alzheimer's disease model. Biochem Biophys Res Commun. 2018;495(1):807–13.

97. de Aguiar BRL, Ferreira EB, Normando AGC, Mazzeu JF, Assad DX, Guerra ENS, et al. Single nucleotide polymorphisms to predict acute radiation dermatitis in breast cancer patients: A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2022;173:103651.

98. Brody T. Chapter 19 - Biomarkers. In: Brody TBT-CT (Second E, editor. Boston: Academic Press; 2016. p. 377–419.

99. Lee JD, Cai Q, Shu XO, Nechuta SJ. The Role of Biomarkers of Oxidative Stress in Breast Cancer Risk and Prognosis: A Systematic Review of the Epidemiologic Literature. J Women's Heal. 2017;26(5):467–82.

100. Omofuma OO, Peterson LL, Turner DP, Merchant AT, Zhang J, Thomson

CA, et al. Dietary Advanced Glycation End-Products (AGEs) and Mortality After Breast Cancer in the Women's Health Initiative (WHI). 2022;1–20.

101. Niki E. Lipid peroxidation products as oxidative stress biomarkers. BioFactors. 2008;34(2):171–80.

102. Andrés Juan C, Manuel Pérez de la Lastra J, Plou FJ, Pérez-Lebeña E, Reinbothe S. Molecular Sciences The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. Int J Mol Sci. 2021;22:4642.

103. Annor F. ScholarWorks @ Georgia State University Psychosocial and Oxidative Stress and Health of Adults. 2015;

104. Gasparetto C, Malinverno A, Culacciati D, Gritti D, Prosperini PG, Specchia G, et al. Antioxidant vitamins reduce oxidative stress and ventricular remodeling in patients with acute myocardial infarction. Int J Immunopathol Pharmacol. 2005;18(3):487–96.

105. Wang R, Liang L, Matsumoto M, Iwata K, Umemura A, He F. Reactive Oxygen Species and NRF2 Signaling, Friends or Foes in Cancer? Biomolecules. 2023;13(2).

106. Gönenç A, Özkan Y, Torun M, Şmşek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J Clin Pharm Ther. 2001;26(2):141–4.

107. Klaunig JE. Oxidative Stress and Cancer. Curr Pharm Des. 2019;24(40):4771–8.

108. Gaweł S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek. 2004;57(9–10):453–5.

109. Zaaboul F, Liu Y. Vitamin E in foodstuff: Nutritional, analytical, and food technology aspects. Compr Rev Food Sci Food Saf. 2022;21(2):964–98.

110. Baum M, Ziv Y, Colletta A. Prospects for the chemoprevention of breast cancer. Br Med Bull. 2014;47(2):493–503.

111. Nsaif GS, Abdallah AH, Ahmed NS, Alfatlawi WR. Evaluation of Estridiol and Some Antioxidant in Breast Cancer Iraqi Women. 2018;21(1):35–40.

112. Tataurov A V., You Y, Owczarzy R. Predicting ultraviolet spectrum of single stranded and double stranded deoxyribonucleic acids. Biophys Chem. 2018;133(1–3):66–70.

113. Nzilibili SMM, Ekodiyanto MKH, Hardjanto P, Yudianto A. Concentration and Purity DNA Spectrophotometer: Sodium Monofluorophosphate forensic impended effect. Egypt J Forensic Sci. 2018;8(1).

114. Mohanty AK, Yadav ML, Choudhary S. Gel Electrophoresis of Proteins and Nucleic Acids BT - Protocols in Semen Biology (Comparing Assays). In: Srivastava N, Pande M, editors. Singapore: Springer Singapore; 2017. p. 233–46.

115. Anguilm TN. CSU ePress Recombinant Expression of Preptin Analogs for Alanine Scanning Mutagenesis of Residues 27-29. 2020;

116. Nie YC, Wang B, Zhao ZQ, Zhou HG. Allele-specific PCR and its application in forensic science. Fa Yi Xue Za Zhi. 2014;30(4):282–7.

117. Of E of A on the D and M, Breast Cancer. Effects of age on the detection and management of breast cancer. Cancers (Basel). 2015;7(2):908–29.

118. Dodova RI, Mitkova AV, Dacheva DR, Hadjo LB, Vlahova AI, Hadjieva MST, et al. Spectrum and frequencies of BRCA1/2 mutations in Bulgarian high risk breast cancer patients. BMC Cancer. 2015;15(1).

119. Picon-Ruiz M, Morata-Tarifa C, Valle-Goffin JJ, Friedman ER, Slingerland JM. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. CA Cancer J Clin. 2017;67(5):378–97.

120. Balalis D, Tsakogiannis D, Kalogera E, Kokkali S, Tripodaki E, Ardavanis A, et al. Serum Concentration of Selected Angiogenesis-Related Molecules Differs among Molecular Subtypes , Body Mass Index and Menopausal Status in Breast Cancer Patients. 2022;

121. Aziz S, Zangana A. Association between Breast Feeding And Breast Cancer: A Case Control Study in Erbil. Indian J Forensic Med Toxicol. 2020;14(2):2087–93.

122. Laamiri FZ, Bouayad A, Hasswane N, Ahid S, Mrabet M, Amina B. Risk factors for breast cancer of different age groups: Moroccan data? Open J Obstet Gynecol. 2015;5(02):79.

123. Hashim HT, Ramadhan MA, Theban KM, Bchara J, El-Abed-El-Rassoul A, Shah J. Assessment of breast cancer risk among Iraqi women in 2019. BMC Womens Health. 2021;21:1–9.

124. Özsoy A, Barça N, Dölek BA, Aktaş H, Elverici E, Araz L, et al. The Relationship Between Breast Cancer and Risk Factors : A Single-Center Study. 2017;(November 2013):145–9.

125. Marsden J, Society BM. British Menopause Society consensus statement: the risks and benefits of HRT before and after a breast cancer diagnosis. Post Reprod Heal. 2020;25(1):33–7.

126. Sarhadi M, Aryan L, Zarei M. The estrogen receptor and breast cancer: a complete review. CRPASE Trans Appl Sci [Internet]. 2020;06(04):309–14.

127. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen Receptor Status

by Immunohistochemistry Is Superior to the Ligand-Binding Assay for Predicting Response to Adjuvant Endocrine Therapy in Breast Cancer. J Clin Oncol. 2023;41(7):1331–8.

128. Hamann U, Braun M, Feller A, Heinemann V, Siegele B, Nagel D, et al. Circulating immunogenic cell death biomarkers HMGB1 and RAGE in breast cancer patients during neoadjuvant chemotherapy. 2013;81–90.

129. Sun S, Zhang W, Cui Z, Chen Q, Xie P, Zhou C, et al. High mobility group box-1 and its clinical value in breast cancer. Onco Targets Ther. 2015;8:413–9.

130. Tesařová P, Kalousová M, Jáchymová M, Mestek O, Petruzelka L, Zima T. Receptor for advanced glycation end products (RAGE) - Soluble form (sRAGE) and gene polymorphisms in patients with breast cancer. Cancer Invest. 2007;25(8):720–5.

131. Kwak T, Drews-Elger K, Ergonul A, Miller PC, Braley A, Hwang GH, et al. Targeting of RAGE-ligand signaling impairs breast cancer cell invasion and metastasis. Oncogene. 2017;36(11):1559–72.

132. Santolla MF, Talia M, Cirillo F, Scordamaglia D, De Rosis S, Spinelli A, et al. The AGEs/RAGE Transduction Signaling Prompts IL-8/CXCR1/2-Mediated Interaction between Cancer-Associated Fibroblasts (CAFs) and Breast Cancer Cells. Cells. 2022;11(15):1–21.

133. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017;524:13–30.

134. Yeh CC, Hou MF, Tsai SM, Lin SK, Hsiao JK, Huang JC, et al. Superoxide anion radical, lipid peroxides and antioxidant status in the blood of patients with breast cancer. Clin Chim Acta. 2005;361(1–2):104–11.

135. Paskeh MDA, Entezari M, Mirzaei S, Zabolian A, Saleki H, Naghdi MJ, et al. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. J Hematol Oncol. 2022;15(1):1–39.

136. Nourazarian AR, Kangari P, Salmaninejad A. Roles of oxidative stress in the development and progression of breast cancer. Asian Pacific J Cancer Prev. 2014;15(12):4745–51.

137. Aghvami T, Djalali M, Keshavarz A, Sadeghi MR, Zeraati H, Sadrzadeh Yeganeh H, et al. Plasma level of antioxidant vitamins and lipid peroxidation in breast cancer patients. Iran J Public Health. 2006;35(1):42–7.

138. Mohammad Hashemi a b , Abdolkarim Moazeni-roodi c , Farshid Arbabi d , Aliakbar Fazaeli b , Ebrahim Eskandari Nasab b M, Taheri a CK e & SG f. Genotyping of–374A/T,–429A/G, and 63 bp Ins/del polymorphisms of RAGE by rapid one-step hexaprimer amplification refractory mutation system polymerase chain reaction in breast cancer patients. Nucleosides, Nucleotides and Nucleic Acids. 2012;31(5):401–10.

139. Yue L. Genetic predisposition of six well-defined polymorphisms in HMGB1 / RAGE pathway to breast cancer in a large Han Chinese population. 2016;20(10):1966–73.

140. Yue L, Zhang Q, He L, Zhang M, Dong J, Zhao D, et al. Genetic predisposition of six well-defined polymorphisms in HMGB 1/RAGE pathway to breast cancer in a large Han Chinese population. J Cell Mol Med. 2016;20(10):1966–73.

141. Huang BF, Tzeng HE, Chen PC, Wang CQ, Su CM, Wang Y, et al. HMGB1 genetic polymorphisms are biomarkers for the development and progression of breast cancer. Int J Med Sci. 2018;15(6):580–6.

الخلاصة

سرطان الثدي : هو أكثر أنواع السرطانات شيوعًا بين الإناث في جميع أنحاء العالم. سرطان الثدي هو نمو غير متحكم فيه للخلايا الظهارية التي تنشأ من قنوات الثدي أو فصيصات الثدي. سرطانات الثدي هي سرطانات غازية أو متسللة ، وتحدث عندما تمر عبر جدران الغدد أو القنوات التي نشأت ونمت في أنسجة الثدي المحيطة.

تم تصميم هذه الدراسة للتحقيق في مستوى HMGB1، RAGE ، MDAوفيتامين E للعثور على الارتباط المحتمل بين هذه المعلمات مع تطور المرض لدى النساء المصابات بسرطان الثدي ، ولتقييم دور الطفرة الجينية RAGE T / A) و HMGB1 T / C في تعدد الأشكال الجيني والمخاطر المرتبطة بالثدي السرطان وتطور المرض في محافظة بابل.

ولتحقيق أهداف هذه الدراسة تم إجراء اختبار على (100) شخص تم تقسيمهم إلى مجموعتين ، المجموعة الأولى ضمت 50 مريضة بسرطان الثدي ، في حين ضمت المجموعة الثانية 50 مجموعة ضابطة.

تم تحديد تركيز MDA, RAGE, HMGB1 وفيتامين E بواسطة تقنية الاليزا ELISA. تم استخراج الحمض النووي من الدم والتنميط الجيني لطفرة جين RAGE T / A rs1800624 (عبر تقنية RFLP- PCR) ، جين HMGB1 T / C rs1412125 (عبر تقنية AS -PCR الخاصة بالأليل). تم تقييم النتائج من خلال التحليلات الإحصائية المختلفة.

لوحظ زيادة كبيرة في مستوى HMGB1 و RAGE MDA في مرضى سرطان الثدي مقارنة بمجموعة التحكم. كان مستوى فيتامين E زيادة معنوية في مرضى سرطان الثدي . مقارنة بالاصحاء. من المحتمل أن تكون هذه الزيادة بسبب المكملات الغذائية.

من ناحية أخرى ، لم تظهر الأنماط الجينية للطفرة الجينية RAGE gene T / A rs1800624 ، جين HMGB1 T / C rs1412125 في مرضى سرطان الثدي (التي تمت دراستها في البحث) أي ارتباط معنوي بالمرض ($p> 0.05$) .

أخيرًا ، لا يرتبط تعدد الأشكال الجيني لجين RAGE T / A rs1800624 ، HMGB1 T / C rs1412125 في سرطان الثدي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعه بابل / كلية الطب
فرع الكيمياء والكيمياء الحياتية

دراسة تاثير تعدد الاشكال الجينية لمستقبلات منتجات الكلايكيشن المتقدمة ومجموعة التنقل عالية الحركة على قابلية الاصابة بسرطان الثدي في محافظة بابل

رسالة

مقدمه الى مجلس كلية الطب / جامعة بابل كجزء من متطلبات نيل درجة ماجستير العلوم في الكيمياء الحياتية السريرية

من قبل

هند شوقي زكي حسن الخاقاني

بكالوريوس علوم كيمياء / كلية العلوم / جامعة الكوفة / 2009-2010

اشراف

الأستاذ **الاستاذ المساعد**

الدكتور عبد السميع حسن الطائي **الدكتور مشتاق قحطان محمد**

2023م **1444هـ**