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Evaluation of *BRCA-1,2* & *P53* gene polymorphisms and CEA & CA 15-3 Tumor Markers among Females with Breast Cancer in Al-Diwaniya Province

A Dissertation

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
يَرْفَعِ اللّٰهُ الَّذينَ آمَنوُاْ مِنْكُمْ
وَ الَّذينَ أوتوا العِلْمَ دَرَجاتٍ
وَاللّٰهُ بما تَعْمَلُونَ خَبيرٌ
صَدَقَ اللّٰهُ العَظيمْ
سورة المجادلة (الآية 11)

Dedications

To ... My Dears: father, mother, husband ,brothers and sisters For their support and encouragement, I hope this dissertation serves to repay some of their contributions.

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Supervisor's Certificate

We certify this dissertation (**Evaluation of *BRCA-1,2* & *P53* gene polymorphisms and CEA & CA 15-3 Tumor Markers among Females with Breast Cancer in Al-Diwaniya Province**) was prepared under our supervision at the Department of Microbiology, College of Medicine/ University of Al-Qadisiya, as partial fulfillment of the requirements for the degree of Philosophy doctoral of Medical Sciences in Microbiology.

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Summary

The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality.

This case-control study was arranged to investigate the possible role of selected genetic and immunological parameters in a random samples of patients with breast cancer in the Al-Diwanyia province. Ten ml blood samples obtained from fifty females with breast cancer in post-operative stage attending the outpatient department of oncology in Al-Diwaniyia teaching hospital ,in period from March-July 2016, have been recruited in the study and compared to 50 healthy control females, ages of patients and control were ranged between 19-80 years. Six ml blood samples were assessed for serum measurement Cancer Antigen 15-3(CA 15-3) and Carcinoemberionic Antigen (CEA) tumor markers by using Fully-auto chemiluminescence immunoassay – CMIA. four ml blood sample was utilized for genomic DNA extraction for detection of single nucleotide polymorphism of *BRCA1* , *BRCA2* and *P53* genes using polymerase chain reaction–restriction fragment length polymorphism technique (RFLP- PCR). Results showed no statistically significant differences in mean age in the two study groups, but more frequent in 40-50 years age group in both patients and control group (40% and 46% respectively). In the association between breast cancer and selected tumor markers the results showed a significant association between serum concentration of tumor markers (CA15-3 and CEA) and breast cancer disease ($p<0.05$) in comparison with control group. The mean serum levels CA15-3 and CEA were significantly higher in patients as compared to control group ($P <0.0001$). The statistical analysis of the present study showed no significant association between CA 15-3 and CEA in patients group ($p=0.185$) ,but moderate positive correlations is appeared ($r = 0.2432$). In the other hand the association between patients age and selected tumor markers

in study (CA15-3 and CEA) and the results show the serum concentrations of CA-15.3 and CEA not effected strongly by Age (r = 0.20 and r = 0.114 respectively). In the context of the family history our results showed a significant association between breast cancer and family history (P< 0.0001) in patients group, 28 (56%) of patients group with positive history family of breast cancer and 22 (44%) in negative history family patients group (P > 0.05).

This work included three candidate susceptible genes, *BRCA*-genotypes had significant predictive fraction. In *BRCA-1* GG genotype has obviously suggested as a risk factor for tumor, as had an (OR 5.3191) and Etiologic Fraction (EF 0. 065) , In contrast, the AG & AA genotypes had rather preventive role as it had Protective Fraction (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively), and patient have 16% and 84% of patients have G and A alleles respectively . In *BRCA-2* AG genotype has obviously suggested an etiology for tumor, as had an (OR 13.4146) and Etiologic Fraction (EF 0.1851), In contrast, the AA genotype had rather preventive role as it had Protective Fraction (PF) of 0.9103 and low OR (0.0731). Patients have 10% of G and 90% of A alleles compared with control they have 100% of A only .In *P53* CC genotype has obviously suggested an etiology for tumor, as had an (OR 1.2941) and Etiologic Fraction (EF 0.091) , In contrast, the GC genotype had rather preventive role as it had Protective Fraction (PF) of 0.087 and low OR (0.4565) and patients have 56% of G allele and 44% of C allele compared with control they have 52% of G and 48% of C. In present results declared significant correlation between *BRCA-1* genotype and history family (P<0.0085) and that appear more clearly in GG genotype that have 4(100%) in patients compared (0%) in negative history family , *BRCA-2* genotype also have significant correlation with positive history family (p< 0.0027) and AG genotype in patient have highest frequency 90% among positive family history patients in compared in negative patient 10%. In contrast , *P53* genotype show significant association with family history (P< 0.0001) and GC genotype show highest 100% in patients with positive history family in compared with negative history family patients

between genetic 0%, the attempts in this study to determine the association markers and immunological markers, it were found that there are no significant correlation between the genes (*BRCA-1, BRCA* & *P53*) and tumor markers (CEA &CA15-3) , no significant association between *BRCA -1* genotype and tumor markers "CEA and CA15-3" (P< 0.838& p<0.896 respectively) , also do not significant correlation between *BRCA-2* gene and tumor markers" CEA&CA-15.3" (P < 0.595 & p< 0.157 respectively) ,as well as in *P53* gene too no significant association between this gene and tumor markers "CEA&CA15-3" (P < 0.750& p< 0.619 respectively).

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List of Abbreviation

Abbreviation	Meaning
AFP	Alpha-Pheto Protein
ASCO	American Society of Clinical Oncology
ATM	Ataxia Telangiectasia Mutated
ATR	Ataxia Telangiectasia and *Rad3* Related
BASC	BRCA1-Associated Genome Surveillance Complex
BBD	Benign Breast Disease
BMI	Body-Mass Index
BRCA-1	Breast Cancer-1
BRCA-2	Breast Cancer-2
BTs	Breast Tumors
CA 125	Cancer Antigen 125
CA 19-9	Cancer Antigen 19.9
CA 27.29	Cancer Antigen 27.29
CA-15.3	Cancer Antigen 15.3
CAs	Chromosomal Aberrations
CEA	Carcinoembryonic antigen
CHEK2	*CHEK2* Checkpoint Homolog
CIS	Carcinoma In Situ
CMIA	Chemiluminescence Immunoassay
DCIS	Ductal Carcinoma in Situ
DSS1	Deleted in Split Hand/Split-foot 1

ER	Estrogen Receptor
FANCD1	Fanconi Anemia Complementation Group D1
FCCs	Fibrocystic Changes
HCG	Human Chorionic Gonadotropin
HER-2	Human Epidermal Growth Factor Receptor-2
HIV	Human Immunodeficiency Virus
HR	Homologous Recombination
HRT	Hormone Replacement Therapy
IBC	Inflammatory Breast Cancer
IDC	Invasive (or infiltrating) Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
kDa	Kilo Dalton
Kg	Kilo gram
LCIS	Lobular Carcinoma in Situ
MDC	Mammary Duct Ectasia
MLH1	MutL Homolog 1
MRE11A	Meiotic Recombination 11 Homolog A
MRN	Meiotic Recombination Nucleotide
MSH2	MutS Homolog 2
MSH6	MutS Homolog 6
NBS1	Nijmegen Breakage Syndrome 1
NCCN	National Comprehensive Cancer Network
NER	Nucleotide Excision Repair

NHEJ	Non-Homologous End Joining
NLS	Nuclear Localization Signals
NS	Not Significant
OCs	Oral Contraceptives
OR	Odd Ratio
p	P-value
P53	Protein 53
PBLs	Peripheral Blood Lymphocytes
PCNA	Proliferating Cell Nuclear Antigen
PgR	Progesterone Receptor
PSA	Prostatic Specific Antigen
RAP80	Receptor Associated Protein 80
RFC	Replication Factor C
RFLP-PCR	Restriction Fragment Length Polymorphism-Polymerase Chain Reaction
SCD	SQ-Cluster Domain
SD	Standard Deviation
SE	Standard Error
SNPs	Single Nucleotide Polymorphisms
TNM	Tumor , lymph Node status and Metastasis
UTR	Untranslated Region
WHO	World Health Organization

1. Introduction and Literature Review

1.1 Introduction

The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality (Bray *et al*., 2012). In 2008, approximately 1.4 million women were diagnosed with breast cancer worldwide with a corresponding of 460,000 deaths (Ferlay *et al*., 2010). 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 (Iraqi Cancer Registry, 2011). and is the second cause of cancer related deaths (Saaed *et al*., 2011). No specific etiological factor has been documented, but different breast cancer-associated risk factors have been suggested by epidemiological studies; for instance, age, menarche, menopause, breastfeeding, use of exogenous hormones or oral contraceptive, obesity, lack of exercise, diet, smoking, alcohol consumption and family history of breast cancer or other cancers (Davies,2012). However, these risk factors have been shown to have different relations to breast cancer in different ethnic populations of the world (Abdulrahman and Rahman, 2012).Accordingly, breast cancer is clinically regarded as a heterogeneous and complex disease, encompassing a wide variety of pathological entities and a range of clinical behavior. This heterogeneity is strictly linked to individuals and tumors genetic variability; therefore it is now widely accepted that accumulation of genetic anomalies contributes to the acquisition of an increasingly invasive or chemo-resistant tumorphenotype (Cavallaro *et al*., 2012). *BRCA1* and *BRCA2* proteins appear to share a number of functional similarities that may suggest why mutations in these genes lead to specific hereditary predisposition to breast cancer, *BRCA* genes contribute to DNA repair and transcriptional regulation in response to DNA damage and cell cycle control. Studies suggest that *BRCA*

proteins are required for protecting the genome from damage (Yoshida *et al*.,2004). The frequency and spectrum of mutations within *BRCA1/2* genes vary widely among populations. In some ethnic or geographically isolated groups, founder mutations can explain the majority of inherited breast and ovarian cancer cases, genetic influence on mammary carcinogenesis has long been implicated and it is estimated that approximately 10 % of breast cancer patients are carriers of gene mutations susceptible for the development of breast cancer , of these genes, perhaps the most extensively studied are breast cancer 1, early onset (*BRCA1*), breast cancer 2, early onset (*BRCA2*) and Tumor protein *p53* (*TP53*) genes. These are associated with a high risk of developing breast cancer in carriers and hence they are referred to as high penetrance genes (Turnbull *et al*.,2010).

In parallel, the measurement of tumor markers in breast cancer has been studied for nearly 20 years, their usefulness remains unclear. In patients with metastatic breast carcinoma, tumor markers appear to be useful during follow-up, but a wide range in rates of marker positivity has been reported: 50%–80%. The CA 15-3 and CEA concentrations increase was observed in various malignant tumors, but this is a useful marker for breast cancer metastasis and is determined in monitoring disease progression and success of therapy, It is not used as screening test or as a test for primary diagnosis because it has low diagnostic sensitivity (Ebeling *et al*., 2002). CA 15-3 and CEA , however, are not recommended as a marker for either diagnosis or detection of early recurrence of breast cancer according to the American Society of Clinical Oncology (ASCO) guidelines, because of insufficient data, the ASCO also does not recommend the use of CA 15-3 and CEA as a markers for monitoring response (Kumpulainen *el al*.,2002 ; Jiang and Shapirob, 2013).

Aim of Study

Study of some predisposing genes and tumor markers to reach to more frequent and dangerous factor among breast cancer patients through the following objectives :

1-Study of genetic variation in *BRCA-1 &-2* , and *P53* as a predisposing genes and response to tumor by using RFLP-PCR.

2-Study and measure some standers of tumor markers(CA-15.3 & CEA) which may have prognostic value in breast cancer patients by using CMIA.

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1.2 Literature Review

1.2.1 Breast Cancer

Breast cancer is a type of malignancy caused by the abnormal growth and uncontrolled cell division within the terminal duct and lobular units of the breast that can invade and destroy surrounding normal tissue, and spread throughout the body via blood or lymph fluid to new sites (Liang, 2011). It is the most frequent malignant disease and the leading cause of cancer death among women

in both economically developed and developing regions of the world. Globally, 1.4 million new breast cancer cases are estimated each year and approximately one-third of the diagnosed patients are reported to die of the disease (Jemal *et al*., 2011). The incidence rates are highest in the Western world, where the life-time risk of developing breast cancer is found to be one in nine, due to increased awareness, early detection, and availability of better treatment options, breast cancer mortality rates have declined in recent years (Coleman *et al*., 2011). Despite the common occurrence, the exact aetiology of the disease is still under investigations. Breast cancer is believed to be a multifactorial disease which is a result of the interaction of different genetic and environmental factors (Ponder, 2001). Over the past decade, significant progress has been done in defining risk factors, determining susceptibility of individuals to developing breast cancer as well as the genetic factors that contribute to this risk, despite this improvement of the knowledge, the unraveling of the complex genetic and environmental influences on the disease is still at an initial stage, an even better understanding of the genetic mechanisms underlying the development and progression of breast cancer would be a major advance for improved prevention, detection and treatment strategies (Loizidou, 2009).

1.2.2Epidemiological Profile

Based on a recent estimation, a total of 1,384,000 females were diagnosed with breast cancer globally in 2008, and this corresponded to an age standardized rate of 42.3 new cases per 100,000 of populations. It represented almost a quarter (23%) of all invasive cancers diagnosed among females in 2008, and accordingly, breast cancer had the highest incidence of any cancer among females in most regions of the world, with the exception of several countries in Eastern and Western Africa, as well as parts of Central and South America and Southern Asia (most notably India), where cervical cancer was the more common (Ferlay *et al*., 2010). The overall number of new diagnosed cases was almost similar in more developed countries, but as compared to less developeing countries, incidence

rates were almost 2.5 times higher in developed countries (71.7/100,000 and 29.3/100,000, respectively) after adjusting for population size and age structure. The highest incidence rates were recorded in Western Europe, Australia/New Zealand and Northern Europe, whilst the rateswere lowest in Eastern Africa, Middle Africa and Melanesia (Youlden *et al*., 2012). In Arab countries, it has been estimated that breast cancer constitutes 13- 35% of all female cancers; however the age-standardized incidence rate was the lowest (9.5 per 100,000) in Algeria and the highest (46.7 per 100,000) in Lebanon, and the rate mean was 21.3 per 100,000 (El-Saghir *et al*., 2007; in this study, data about Iraqi women were not presented). With respect to Iraqi women, Ad'hiah *et al*. (2002) examined the Iraqi Cancer Registry records from 1975 to 1997, and recorded 12,665 cases of breast cancer for a period of 23 years. The incidence rate per 100,000 per year showed a gradual increase from 1975 to 1997, with a mean incidence rate of 6.9 per 100,000 per year. However, such rate was increased to 9.4 per 100,000 in 2009 (Iraqi Cancer Registry, 2011). In terms of mortality rates, 459,000 females were estimated to have died from breast cancer worldwide during 2008, and an age-standardized rate of 13.9 deaths per 100,000 was recorded. Accordingly, breast cancer remains the leading cause of cancer-related mortality among females internationally, responsible for almost 14% of all cancer deaths (Ferlay *et al*., 2010). However, the average life expectancy of women in developing countries (including Arabs) is shorter than in women in developed countries resulting in a lower age of breast cancer in developing countries. Most Arab and non-Arab developing countries have a cone shape population pyramid compared to cylinder shape in the developed countries such as Australia (El-Zaemey *et al*., 2012).

1.2.3 Breast Development and Risk of Tumor

The breast consists of milk lobules, milk ducts, fat tissue, blood vessels and lymph ducts. Development of the breast is initiated during embryonic life when the ducts are formed, Such development is subjected to a regulation by the

ovaries that produce ovarian steroid hormones; for instance estrogens and progesterone , Moreover, the human growth factor is essential to breast development (Laban *et al*., 2003).There are four types of lobules, lobules 1 to 4; the most differentiated and proliferated state being lobule 4, in the normal breast of an adult woman, three types of lobules are present; lobule types 1 to 3. In nulliparous women lobule type 1 is the most predominant lobule, whereas in parous women, type 3 lobule is the most common structure during pregnancy and lactation, lobule 3 develops into lobule 4. The proportion of lobule 3 in porous women peaks during early reproductive years, and then it decreases with age, Porous postmenopausal women therefore have almost the same breast composition, i.e. lobule type 1, as the nulliparous woman (Russo *et al*., 2005). However, lobule 1 in nulliparous and postmenopausal parous women may be biologically different. Since most cancers are initiated in lobules 1 and 2; the time window between menarche and birth of first child, when most lobules mature to lobule 3, has been considered to be a period when the breast cells are sensitive to hormonal stimuli. Additional full-term pregnancies further reduce the number of remaining type 1 lobules into more mature lobules. After menopause, the glandular tissue of breast atrophies, the connective tissue becomes less cellular, and the amount of collagen decreases. In some women, marked fatty infiltration of the breast occurs at this stage; in others, the breasts shrink considerably (Ellis and Mahadevan, 2013). Normal growth of the mammary gland involves endocrine signaling from the hypothalamic-pituitary-gonadal axis. However, autocrine and paracrine hormones and growth factors also play critical roles in development and regulation of the mammary gland. Some of the many endocrine hormones and growth factors known to modulate mammary gland development include: growth hormone, prolactin, oxytocin, epidermal growth factor, insulin, insulinlike growth factors, adrenal corticosteroids, transforming growth factors, thyroxine, estrogen, progesterone, activin, and inhibin (Nelson and Bissell, 2006) ,However, such hormonal homeostasis can be subjected to the effects of exogenous and endogenous factors that can interfere with the

production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes, and may consequence in the presentation of breast tumors (Macon and Fenton, 2013). Breast tumors are typically heterogeneous and contain diverse subpopulations of tumor cells with different phenotypic properties; but benign and malignant breast tumors are the major categories, although the former category can be better understood under the title benign breast diseases (Guray and Sahin, 2006).

1.2.5 Malignant Breast Tumor (Breast Cancer)

Breast cancer is the most important malignancy of women, and it is caused by the presence of malignant cells in the breast, which are characterized by uncontrolled division, leading to abnormal growth (in situ carcinoma), and their ability to invade normal tissue locally. The primary tumor begins in the breast, but once it becomes invasive, may progress to the regional lymph nodes (axillary/internal mammary) or metastasize (Davies, 2012).

1.2.6Breast Cancer Origin

Human breast cancers are heterogeneous in their morphology, response to therapy and clinical course. Therefore the cascade of genetic alterations in the development of breast cancer is complex and not well known, as reviewed later in this chapter. Previously, breast cancer progression was seen as a multi-step process involving progressive changes from normal to hyperplasia with and without atypia, carcinoma *in situ*, invasive carcinoma, and metastasis , However, more recent immunohistochemical (IHC) and molecular genetic studies have shown that development of breast cancer does not follow a single pathway but is a complex series of random genetic events leading towards invasive breast cancer

(Bertolo *et al*., 2008). A further understanding has come from stem cell model investigations, which postulate that breast cancer originates from stem cells, as a consequence of dysregulation of self-renewal pathways. This implicates that breast tumors contain a subpopulation of cells with stem cell like properties, which are capable of self renewal and differentiation, and presence of these cells can promote tumorigenesis (Charafe-Jauffret *et al*., 2008). In agreement with such concept, putative cancer stem cells have been isolated from breast cancers and these cells have been shown to be resistant to chemotherapeutic drugs (Dave and Chang, 2009), and molecular genetic studies suggested that these abnormalities are due to inactivation of tumor suppressor genes and activation of oncogenes by structural alterations in these genes (Ueno *et al*., 2012).

1.2.7Types and Classification

Breast cancer, as the name implies, is defined as cancer that originates from normal breast tissue, but the progression from normal breast tissue to invasive cancer is poorly understood. Non-invasive breast cancer is called carcinoma in situ (CIS) and can arise from either ductal or lobular hyperplasia of epithelial cells. Cancer that has progressed into surrounding tissue is called invasive breast cancer and usually has the ability to metastasize (Li *et al*., 2005). Breast tumors are categorized according to type and size, histopathology, invasiveness, tumor stage and receptor expression. As molecular techniques have improved a deeper understanding of diverse breast cancer types and how they differ have been gained, and based on WHO classification, six main types are recognized; ductal, lobular, mucinous, medullary, papillary and tubular carcinoma (Tavassoli and Devilee, 2003). Histological grade is often classified according to the Nottingham Grade classification which was introduced in the 1990s and includes three different parameters; tubule formation, nuclear pleomorphism and mitotic counts. Tumor stage classification incorporates Tumor size (T), lymph Node status (N) and Metastasis (M) (usually shortened to TNM) (Benson *et al*., 2003). Expression of different receptors is also used to characterize the tumors. They are classified

according to expression of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor-2 (HER-2) (Parise *et al*., 2009).

1.2.7.1Carcinoma

This is a term used to describe a cancer that begins in the lining layer (epithelial cells) of organs like the breast. Nearly all breast cancers are carcinomas (either ductal carcinomas or lobular carcinomas) (Grenier D et al., 2011).

1.2.7.2Carcinoma in situ

This term is used for the early stage of cancer, when it is confined to the layer of cells where it began. In breast cancer, *in situ* means that the cancer cells remain confined to ducts (ductal carcinoma in situ) or lobules (lobular carcinoma in situ). They have not grown into (invaded) deeper tissues in the breast or spread to other organs in the body. Carcinoma in situ of the breast is sometimes referred to as *non-invasive* or *pre-invasive* breast cancer(Grenier D et al., 2011).

1.2.7.3Invasive (infiltrating) carcinoma

An invasive cancer is one that has already grown beyond the layer of cells where it started (as opposed to carcinoma in situ). Most breast cancers are invasive carcinomas - either invasive ductal carcinoma or invasive lobular carcinoma (Grenier *et al*., 2011).

1.2.7.4Ductal carcinoma in situ

Ductal carcinoma in situ (DCIS; also known as *intraductal carcinoma*) is the most common type of non-invasive breast cancer. DCIS means that the cancer cells are inside the ducts but have not spread through the walls of the ducts into the surrounding breast tissue. About 1 in 5 new breast cancer cases will be DCIS (Voduc *et al*., 2010). Nearly all women diagnosed at this early stage of breast

cancer can be cured. A mammogram is often the best way to find DCIS early (Sprague *et al*.,2011).

1.2.7.5Lobular carcinoma in situ

Although it is not a true cancer, lobular carcinoma in situ (LCIS; also called lobular neoplasia) is sometimes classified as a type of non-invasive breast cancer. It begins in the milk-producing glands but does not grow through the wall of the lobules. Most breast cancer specialists think that LCIS itself does not become an invasive cancer very often, but women with this condition do have a higher risk of developing an invasive breast cancer in the same breast or in the opposite breast. For this reason, women with LCIS should make sure they have regular mammograms and doctor visits(Sprague *et al*.,2011; Halpern, *et al*.,2007).

1.2.7.6Invasive (or infiltrating) ductal carcinoma

This is the most common type of breast cancer, invasive (or infiltrating) ductal carcinoma (IDC) starts in a milk passage (duct) of the breast, breaks through the wall of the duct, and grows into the fatty tissue of the breast(Sprague *et al*.,2011). At this point, it may be able to spread (metastasize) to other parts of the body through the lymphatic system and bloodstream. About 8 of 10 invasive breast cancers are infiltrating ductal carcinomas(Jatoi *et al*.,2003).

1.2.7.7 Invasive (or infiltrating) lobular carcinoma

Invasive lobular carcinoma (ILC) starts in the milk-producing glands (lobules), like IDC, it can spread (metastasize) to other parts of the body. About 1 out of 10 invasive breast cancers is an ILC. Invasive lobular carcinoma may be harder to detect by a mammogram than invasive ductal carcinoma (Hartmann *et al*.,2005).

1.2.7.8 Less common types of breast cancer

1.2.7.8.1 Inflammatory breast cancer

This uncommon type of invasive breast cancer accounts for about 1% to 3% of all breast cancers (Hartmann *et al*.,2005). Usually there is no single lump or tumor. Instead, inflammatory breast cancer (IBC) makes the skin of the breast look red and feel warm. It also gives the breast skin a thick, pitted appearance that looks a lot like an orange peel (Jatoi *et al*.,2003).The affected breast may become larger or firmer, tender, or itchy. In its early stages, inflammatory breast cancer is often mistaken for an infection in the breast (called mastitis). Often this cancer is first treated as an infection with antibiotics. If the symptoms are caused by cancer, they will not improve, and the skin may be biopsied to look for cancer cells. Because there is no actual lump, it may not show up on a mammogram, which may make it even harder to find it early. This type of breast cancer tends to have a higher chance of spreading and a worse outlook than typical invasive ductal or lobular cancer(Jatoi *et al*.,2003).

1.2.7.8.2Triple-negative breast cancer

This term is used to describe breast cancers (usually invasive ductal carcinomas) whose cells lack estrogen receptors and progesterone receptors, and do not have an excess of the HER2 protein on their surfaces (Hartmann *et al*.,2005). Breast cancers with these characteristics tend to occur more often in younger women and in African-American women. Triple-negative breast cancers tend to grow and spread more quickly than most other types of breast cancer. Because the tumor cells lack these certain receptors, neither hormone therapy nor drugs that target HER2 are effective against these cancers (Romond *et al*.,2005)

1.2.7.8.3 Mixed tumors

Mixed tumors contain a variety of cell types, such as invasive ductal cancer combined with invasive lobular breast cancer. In this situation, the tumor is treated as if it were an invasive ductal cancer (Smith et al.,2003).

1.2.7.8.4 Medullary carcinoma

This special type of infiltrating breast cancer has a rather well defined boundary between tumor tissue and normal tissue (Smith *et al*.,2003). It also has some other special features, including the large size of the cancer cells and the presence of immune system cells at the edges of the tumor. Medullary carcinoma accounts for about 3% to 5% of breast cancers(Centers for Disease Control and Prevention, 2013). The outcom (prognosis) for this kind of breast cancer is generally better than for the more common types of invasive breast cancer, Most cancer specialists think that true medullary cancer is very rare, and that cancers that are called medullary cancer should be treated as the usual invasive ductal breast cancer(Smith *et al*.,2003).

1.2.7.8.5 Metaplastic carcinoma

Metaplastic carcinoma (also known as carcinoma with metaplasia) is a very rare type of invasive ductal cancer (Fisher *et al*.,2002). These tumors include cells that are normally not found in the breast, such as cells that look like skin cells (squamous cells) or cells that make bone(Centers for Disease Control and Prevention, 2013) . These tumors are treated like invasive ductal cancer (Burstein *et al*.,2010).

1.2.7.8.6Mucinous carcinoma

Also known as colloid carcinoma, this rare type of invasive breast cancer is formed by mucus-producing cancer cells(Fisher *et al*.,2002) . The prognosis for mucinous carcinoma is usually better than for the more common types of invasive breast cancer. Still, it is treated like invasive ductal carcinoma(Burstein *et al*.,2010).

1.2.7.8.7Paget disease of the nipple

This type of breast cancer starts in the breast ducts and spreads to the skin of the nipple and then to the areola, the dark circle around the nipple. It is rare, accounting for only about 1% of all cases of breast cancer (Burstein *et al*.,2010). The skin of the nipple and areola often appears crusted, scaly, and red, with areas of bleeding or oozing (Smith *et al*.,2003) . The woman may notice burning or itching , paget disease is almost always associated with either ductal carcinoma in situ (DCIS) or, more often, with infiltrating ductal carcinoma, treatment often requires mastectomy. If only DCIS is found (with no invasive cancer) when the breast is removed, the outcome is excellent(Fisher *et al*.,2002).

1.2.7.8.8Tubular carcinoma

Tubular carcinomas are another special type of invasive ductal breast carcinoma, they are called tubular because of the way the cells are arranged when seen under the microscope (National Comprehensive Cancer Network ,2013) . Tubular carcinomas account for about 2% of all breast cancers. They are treated like invasive ductal carcinomas, but tend to have a better prognosis than most breast cancers (Reuben *et al*. ,2009).

1.2.7.8.9Papillary carcinoma

The cells of these cancers tend to be arranged in small, finger-like projections when viewed under the microscope, these tumors can be separated into noninvasive and invasive types(National Comprehensive Cancer Network ,2013). Intraductal papillary carcinoma or papillary carcinoma in situ is non-invasive (Burstein *et al*.,2010). It is often considered a subtype of ductal carcinoma in situ (DCIS), and is treated as such. In rare cases, the tumor is invasive, in which case it is treated like invasive ductal carcinoma, although the outcome is likely to be better. These cancers tend to be diagnosed in older women, and they make up no

more than 1% or 2% of all breast cancers(National Comprehensive Cancer Network ,2013).

1.2.7.8.10Adenoid cystic carcinoma (adenocystic carcinoma)

These cancers have both glandular (adenoid) and cylinder-like (cystic) features when seen under the microscope. They make up less than 1% of breast cancers. They rarely spread to the lymph nodes or distant areas, and they tend to have a very good prognosis(Reuben et al. ,2009).

1.2.7.8.11Phyllodes tumor

This very rare breast tumor develops in the stroma (connective tissue) of the breast, in contrast to carcinomas, which develop in the ducts or lobules, Other names for these tumors include *phylloides tumor* and *cystosarcoma phyllodes* (National Comprehensive Cancer Network ,2013). These tumors are usually benign but on rare occasions may be malignant. Benign phyllodes tumors are treated by removing the tumor along with a margin of normal breast tissue, a malignant phyllodes tumor is treated by removing it along with a wider margin of normal tissue, or by mastectomy. Surgery is often all that is needed, but these cancers may not respond as well to the other treatments used for more common breast cancers (Fisher *et al*.,2002). When a malignant phyllodes tumor has spread, it may be treated with the chemotherapy given for soft-tissue sarcomas (this is discussed in detail in our document, *Soft-tissue Sarcomas*(Reuben *et al*. ,2009).

1.2.7.8.12 Angiosarcoma

This is a form of cancer that starts from cells that line blood vessels or lymph vessels, it rarely occurs in the breasts(Reuben et al. ,2009) . When it does, it usually develops as a complication of previous radiation treatments. This is an extremely rare complication of breast radiation therapy that can develop about 5 to 10 years after radiation. Angiosarcoma can also occur in the arm of women

who develop lymphedema as a result of lymph node surgery or radiation therapy to treat breast cancer (Fisher *et al*.,2002).

1.2.8 Signs and symptoms of breast cancer

The first noticeable symptom of breast cancer is typically a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump (Merck Manual of Diagnosis and Therapy, 2003). The earliest breast cancers are detected by a mammogram (American Cancer Society, 2007). Lumps found in lymph nodes located in the armpits (Merck Manual of Diagnosis and Therapy 2003) can also indicate breast cancer. Indications of breast cancer other than a lump may include thickening different from the other breast tissue, one breast becoming larger or lower, a nipple changing position or shape or becoming inverted, skin puckering or dimpling, a rash on or around a nipple, discharge from nipple/s, constant pain in part of the breast or armpit, and swelling beneath the axilla or around the collarbone (Watson *et al*., 2008). Inflammatory breast cancer is a particular type of breast cancer which can pose a substantial diagnostic challenge. Symptoms may resemble a breast inflammation and may include itching, pain, swelling, nipple inversion, warmth and redness throughout the breast, as well as an orangepeel texture to the skin referred to as peaud'orange; (Merck Manual of Diagnosis and Therapy , 2003) as inflammatory breast cancer doesn't show as a lump there's sometimes a delay in diagnosis.

Another reported symptom complex of breast cancer is Paget's disease of the breast. Approximately half of women diagnosed with Paget's disease of the breast also have a lump in the breast (National Cancer Institute , 2005). Occasionally, breast cancer presents as metastatic disease-that is, cancer that has spread beyond the original organ. The symptoms caused by metastatic breast cancer will depend on the location of metastasis. Common sites of metastasis include bone, liver, lung and brain (Lacroix, 2006). Unexplained weight loss can occasionally herald an occult breast cancer, as can symptoms of fevers or chills. Bone or joint pains

can sometimes be manifestations of metastatic breast cancer, as can jaundice or neurological symptoms. These symptoms are called non-specific, meaning they could be manifestations of many other illnesses (National Cancer Institute , 2004). Most symptoms of breast disorders, including the lumps, do not turn out to represent underlying breast cancer. Fewer than 20% of lumps, for example, are cancerous, , and benign breast diseases such as mastitis and fibroadenoma of the breast are more common causes of breast disorder symptoms. Nevertheless, the appearance of a new symptom should be taken seriously by both patients and their doctors, because of the possibility of an underlying breast cancer at almost any age (Merck Manual of Diagnosis and Therapy , 2003).

1.2.9 Risk factors for breast cancer

Breast cancer is a life threatening disease with different risk factors that have a complicated role in its pathogenesis. These risk factors broadly can be classified into hormone, non-hormone factors and the genetic background of the susceptibility genes.

1.2.9.1Hormone factors

1.2.9.1.1 Endogenous hormones

It has been observed from decades of studies that the increasing rate of breast cancer with age slows dramatically after menopause (McPherson *et al*., 2000). The pattern of this indicates the role of reproductive hormones in breast cancer risk (Pike *et al*., 1993), as hormone independent cancers should not exhibit a dramatic change in incidence. Oestradiol has been found t stimulate breast cell mitosis in the athymic nude mice model , High oestrogen levels are reported to significantly increase breast cancer risk (Hankinson *et al*., 2004) and the serum oestrogen levels can be a predictive factor of increased breast cancer risk (Key *et al*., 2002). Conversely, reducing exposure was thought to be protective (Hulka, 1997). The relative risk estimated was to be 2.0 for postmenopausal breast cancer development comparing the highest with the lowest quintile of serum estradiol

concentration from nine prospective studies (Key *et al*., 2002). Furthermore, the risk for breast cancer increases while the risk substantially reduced by adjustment for serum oestrogen concentrations, showing that the higher oestrogen levels among heavier women account for breast cancer risk in obese women with increasing body-mass index (BMI) (Key *et al*., 2003). In the same way, factors that increase the number of menstrual cycles were found to be associated with an increased breast cancer risk, like early age at menarche, and late onset of menopause , Statistically significant positive association was observed between plasma level of prolactin and breast cancer risk among postmenopausal women: women in the top quartile of levels had 2-fold risk of breast cancer relative to women in the bottom quartile (Tworoger *et al*., 2004). Increased levels of serum concentrations of testosterone, androstenedione, and dehydroepiandrosterone were also reported to be associated with increased risks of breast cancer with OR (highest versus lowest quartile) of 1.73, 1.56 and 1.48 respectively. In premenopausal women, the increased levels of blood concentrations of androgens are found to be associated with an increased risk of breast cancer (Kaaks *et al*., 2005). Plasma testosterone concentration levels were found to be associated with increased risk of breast cancer among postmenopausal women, (Key *et al*., 2002).

1.2.9.1.2 Exogenous hormones

To treat the menopausal symptoms such as hot flushes and insomnia and to reduce the risk of chronic diseases such as osteoporosis, postmenopausal hormones have been prescribed for several decades. However, the duration of postmenopausal hormone use was found to be associated with increased breast cancer risk in some population. Hormone replacement therapy (HRT) was previously reported to be linked with a 10% higher breast cancer risk for each 5 years of use and the relative risk was 1.06 for oestrogen alone and 1.24 for oestrogen plus progestin (Ross *et al*., 2000). From the four studies, it is indicated that addition of a progestin to estrogen regimens increases breast cancer

risk after 5 years of use from 10% (estrogen alone) to 30% (combined HRT) (Rossouw *et al*., 2002; Ross *et al*., 2000; Schairer *et al*., 2000). In contrast, tamoxifen (a drug with function of antiestrogen) have the capacity to reduce the breast cancer incidence (Fisher *et al*., 1998). In addition to this, the increase in risk associated with hormone use was most frequent for oestrogen-receptor positive tumours (Chen *et al*., 2004).

1.2.9.2 Oral contraceptives (OCPs)

A lot of investigations have been done on the association between the use of OCs and breast cancer risk. In women taking combined OCs, independent of dose, age of first use, length of use, age of diagnosis or family history of breast cancer, a statistically significant increased risk of breast cancer has been observed (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a; Collaborative Group on Hormonal Factors in Breast Cancer, 1996b). In parous women who used OCs before their first full-term pregnancy, the risk is significantly higher (OR=1.52; 95% CI=1.26-1.82) (Kahlenborn *et al*., 2006). The risk was reported to have 24% increased risk for breast cancer development, for the current users of oral contraceptive, although the risk decreases with the years after stopping of taken OCs. However, there was no significant excess risk of breast cancer after 10 or more years of stopping OCs (relative risk: 1.01) (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a). In another phase of the study, women who began use OCs before age 20 has been found are resulting in higher relative risks of breast cancer comparing with those who began at older ages (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a).

1.2.9.3 Non-hormone factors

It is indicated from some previous studies on migrants that breast cancer incidence rates in migrants moving from low-risk to high-risk countries tend to be increased to the rates of the host countries within the migrating generation

itself. This underscores the vital role of the environmental and socio cultural factors which are estimated to play more crucial role than the genetic factors (Parkin, 2004). In the carcinogenesis of breast cancer, the role of dietary or other life style changes has been confirmed in some studies (Robert *et al*., 2004).

1.2.9.4 Lifestyle

1.2.9.4.1 Alcohol and foliate intake

Some epidemiological studies reported an increased risk of breast cancer with alcohol consumption, with an average risk of 1.6 fold compared to nondrinkers (Singletary and Gapstur, 2001). Women with non proliferative breast disease consuming ≥ 15 g/day of alcohol had a 1.67 fold risk of breast cancer in the Nurses' Health Study (Tamimi *et al*., 2005). Acetaldehyde and free radicals coming from the metabolism of ethanol are known potential carcinogenic compounds. The acetaldehyde is found to bind with DNA and proteins and destroy folate resulting in secondary hyperproliferation (Poschl and Seitz, 2004). There may have some other mechanisms that are involved with increased serum estrogen levels in both premenopausal (Coutelle *et al*., 2004) and postmenopausal (Onland-Moret *et al*., 2005) women with long term alcohol consumption. Additionally, alcohol was reported to cause increased endogenous androgens, which may be converted to estrogens by peripheral aromatization pathway (Singletary and Gapstur, 2001). Besides, alcohol can impair the immune system and make nutritional deficiencies, like folate, vitamin E, vitamin B12, vitamin D, zinc and selenium, thus impair the ability to fight carcinogen (Poschl and Seitz, 2004). An increased risk for developing estrogen receptor-negative tumors has been reported in postmenopausal women with a higheralcohol and low-folate intake (Sellers *et al*., 2002). Conversely, increased folate intake has been reported to protect chronic alcohol using women against cancer (Zhang, 2004).

1.2.9.4.2 Smoking

Cigarette smoking is associated with breast cancer risk. Pierre Band et al found that cigarette smoke can exert a dual action on the breast, with different

effects in both premenopausal and postmenopausal women. Significant increased risk for breast cancer was observed in women who had been pregnant and started to smoke within 5 years of menarche in premenopausal participants and higher risk was observed in nulliparous women who smoked 20 cigarettes daily or more (adjusted OR=7.08) and nulliparous women who had smoked for 20 cumulative pack-years or more (adjusted OR=7.48). The difference was not significant in both ever pregnant and nulliparous postmenopausal women. The discrepancy of this result may be due to the postulated "antiestrogenic" effect of cigarette smoking (Clemons and Goss, 2001), incomparison with non-smokers, women who have an earlier age at natural menopause (Baron *et al*., 1990), reduced urinary concentrations of oestrogens during the luteal phase and attenuated effects of hormone replacement therapy , in another way, investigations have proposed that increased risk of breast cancer might be due to the reason that the breast is exposed to carcinogens in smoke (Petrakis, 1993). It is demonstrated from some studies that tobacco constituents can reach breast tissue. Carcinogens in tobacco smoke have the capacity to pass through the alveolar membrane , and into the blood stream , these carcinogens are reported to be fat-soluble and can be stored in breast adipose tissue and these are metabolized and activated by human mammary epithelial cells (Morris and Seifter, 1992).

1.2.9.4.3 Diet

Various natural and chemical carcinogens and anti-carcinogens are found in our daily consumption (Sugimura, 2000). The carcinogens generating free oxygen radicals lead to DNA damage, or other deleterious components due to the production of heterocyclic aromatic hydrocarbons and other harmful compounds in the process of preparation of meat at high temperatures. Fat is postulated to be a key breast cancer risk factor from the diet and increased consumption of saturated fatty acids were reported to be associated with an increased risk of developing breast cancer (Favero *et al*., 1999). Conversely, unsaturated and polyunsaturated fatty acids, such as olive oil and fish oil, were observed to

decrease the risk of breast cancer by up to 30% (Favero *et al*., 1998). But no significant association were found between breast cancer and total fat consumption, the relationship between saturated versus unsaturated fatty acids in other two large studies (Velie *et al*., 2000). Derivatives of the vitamins A, B and E, and selenium are reported to play an important role for protection against cancer. It has been identified that intake of fruits and vegetables, rich sources of natural vitamins, have the decreased breast cancer risk in numerous studies (Van Duyn and Pivonka, 2000), and in postmenopausal women, more significant protective effects were found (Gaudet *et al*., 2004). Soy foods, which are a rich source of fiber and phytoestrogen, have been reported to protective against breast cancer development (Yuan *et al*., 1995). It was postulated based on rat models, that genistein in soy promotes more differentiated tissue in the breast causing less sensitive to later proliferative stimuli (Lamartiniere, 2000).

1.2.9.4.4 Obesity

It is observed from some previous studies that excess body mass has been implicated in approximately 5% of all cancers (Bergstrom *et al*., 2001). Obesity has little effect on the serum concentration of estrogen probably due to reduced ovarian estrogen by a negative feedback, hence contributes little change to the risk of breast cancer in premenopausal women, although obesity in these group women even has been associated with a decrease of breast cancer risk before menopause, yet the mechanism remains unclear (Lahmann *et al*., 2004). In another way, different large studies concluded that obesity and weight gain increase breast cancer risk in postmenopausal women. The risk is was more frequent among obese women who do not use hormone replacement therapy (HRT) with the relative risks up to 2 (Lahmann *et al*., 2004). In another study it is recorded that every 5 kg of weight gain since the lowest adult weight increased the breast cancer risk by 8% (Trentham-Dietz *et al*., 2000). In other study, women older than 55 with an increase in body mass of 10 kg have been found to be associated with 7% increase in breast cancer risk (Tryggvadottir *et al*., 2002). The

mechanism by which obesity increases the risk for developing breast cancer in postmenopausal may due to the unregulated estrogen level by negative feedback, and obesity is found to instigate an increase in the serum concentration of bioavailable estradiol (McTiernan *et al*., 2003). Se hormone-binding globulinis also found to be decreased with increasing body mass index (BMI) which may contribute to the increased breast cancer risk. (Verkasalo *et al*., 2001)

1.2.9.4.5 Physical activity

Physical activity is a preventive factor for breast cancer due to the non-specific immune stimulation and decreased estrogen levels during recovery (Hardman, 2001) as well as delayed onset of menarche (Hankinson *et al*., 2004). Reduced insulin resistance and hyperinsulinaemia were linked with increased physical activity (Stoll, 2000), which has been proposed to be related to breast cancer (Kaaks, 1996). Additionally, increasedphysical activities have the potentiality to control weight gain lead to reduced breast cancer risk. It has been found from the Nurses' Health Study that decreased risk for breast cancer was associated with women with 7 or more hours per week of moderate exercises (relative risk: 0.82). This association had the similarity in both preand postmenopausal women (Rockhill *et al*., 1999). Women performing physical activity during adolescence, have the reduced breast cancer risk with 3% for each one-hour increase in recreational physical activity per week (Lagerros *et al*., 2004).

1.2.9.5 Other risk factors of breast cancer

1.2.9.5.1 Reproductive factors

From some different investigations, early pregnancy has been identified to have a protective effect against breast cancer risk (Pathak *et al*., 2000). Each full term pregnancy has been reported to cause a 3% reduction in breast cancer risk diagnosed early or before menopause and the reduction attained 12% for

breast cancers diagnosed later (Clavel-Chapelon and Gerber, 2002). Another study found the result that the risk of breast cancer in women who have their first full term pregnancy after the age of 30 is about twice of women who have their first child before the age of 20. Further reduced the risk of breast cancer has been found in women second pregnancy at early age, on the other hand, women having a first child after the age of 35 have a higher risk than nulliparous women , Conversely the immediate, effect of pregnancy temporarily increases the risk, despite of having the long-term protection effect against breast cancer risk(McPherson *et al*., 2000).

1.2.9.5.2 Mammographic density

Mammographic density has been reported to be a consistent marker for breast cancer risk both in pre and postmenopausal women. A variation in mammographic density has been considered to be associated with ovarian function. In women with dense breasts compared with those having low breast density, a significantly increased risk for breast cancer was identified. Breast density has been identified for a long time as a strong and independent risk factor for breast cancer in several epidemiological studies. The odds ratio and relative risks in different studies ranging between 4 and 6 greater risks in women with dense tissue in more than 60-75% of the breast, in comparison with those with no densities. It is suggested from Estimation of attributable risk that the breast density may responsible for as many as 30% of breast cancer cases (Boyd *et al*., 1998). As nulliparous and thinner women have an increased breast density in general, they may have increased risk for breast cancer, (Biglia *et al*., 2004). Nulliparity and high breast density are postulated to act synergistically and the breast cancer risk may be 7.1 times higher (van Gils *et al*., 2000).

1.2.10 Family History and Genetics

Environmental and lifestyle factors rather than inherited genetic factors account for most cases of breast cancer, and studies of twins have allowed

estimation of the overall contribution of inherited genetic characteristics. In a population-based study of 45,000 pairs of twins in three Nordic countries, hereditary factors were estimated to contribute to around a quarter and environmental and lifestyle factors to around three-quarters of the interindividual differences in susceptibility to breast cancer (Lichtenstein *et al.*, 2000). Such observation suggests that environmental and lifestyle factors are required to develop the disease in genetically predisposed women. Accordingly, genetic testing for hereditary cancer predisposition syndromes has become integrated into the practice of medical oncology and is leading to better strategies of surveillance and prevention (Garber and Offit, 2005). This has led to the question of how to identify women at hereditary risk of breast cancer, and it has been answered by the discovery of germ-line mutations that increase breast cancer risk, in which, genetic factors that predispose to breast cancer have been divided into two categories, namely, those of high and low risk mutations (Schwartz *et al.*, 2008). For high-risk mutations, at least four germ line mutations that predispose to breast cancer have been identified or localized. These include mutations in the genes *BRCA1*, *BRCA2* and *P53* which are tumor suppressor genes, creating a protein that repairs DNA and prevents carcinogenesis. Every cell in mutation carriers has been demonstrated to lack one functional allele (i.e. the tumor-suppressor function of that gene is lost); a situation that favors cancer development (Blanco *et al.*, 2010). Because hereditary factors have been estimated to account for roughly a quarter of inter individual differences in susceptibility to breast cancer within more developed countries, and high-risk mutations account for around only 5%

of all breast cancers, a substantial component (approximately a fifth) of breast cancer risk may be determined by the combined effect of many low-risk polymorphisms that might confer a small increase in breast cancer altering the metabolism of steroid hormones or carcinogenic compounds. Three genes (*CYP19*, *GSTP1* and for postmenopausal breast cancer, *GSTM1*) have been suspected in this regard (Dumitrescu and Cotarla, 2005). However, recent

investigations suggest that an evaluation of gene environment interaction may yield new insights with respect to breast cancer etiology, because a given exposure may have different or even opposite effects on breast cancer risk or prognosis in women, depending on their genetic variants. Based on such suggestion, Milne *et al*. (2010) evaluated two-way interactions between each of age at menarche, ever having had a live birth, number of live births, age at first birth and body mass index (BMI) and each of 12 single nucleotide polymorphisms (SNPs) (10q26-rs2981582, *FGFR2*; 8q24- rs13281615 and 11p15-rs3817198, *LSP1*; 5q11-rs889312, *MAP3K1*; 16q12- rs3 803662 , *TOX3*; 2q35-rs13387042 and 5p12-rs10941679, *MRPS30*; 17q23- rs6504950, *COX11*; 3p24-rs4973768, *SLC4A7*; *CASP8* rs17468277; *TGFB1*- rs1982073 and *ESR1*- rs3020314), but their results revealed no statistical evidence of interaction. The authors observed no conclusive evidence for modification of the per-allele relative risk associated with common breast cancer susceptibility variants by age at menarche, parity, age at first birth or BMI. A further group of investigators studied interactions between certain known genetic (nine single SNPs) and phenotypic (height, BMI and hormone replacement therapy; HRT) risk factors in breast cancer patients and found one SNP (rs851987 in *ESR1*) that tended to interact with height, with an increasingly protective effect of the major allele in taller women, while rs13281615 (on 8q24) tended to confer risk only in non-users of HRT, but again there were no significant interactions after correction of probability for multiple testing (Harlid *et al*., 2012). Also, a more recent study revealed no apparent interactions between genome-wide association study-identified genetic variants and breast cancer risk factors in the etiology of this disease in Chinese patients (Li *et al*., 2013).

1.2.11 Breast Cancer Immunity

The idea that the immune system can control cancer has been a subject of debate, but recently it has become generally accepted that the immune system has the ability not only to prevent tumor growth but also to promote it through a

process called immunoediting, and this process is comprised of three phases: elimination, equilibrium and escape (Schreiber *et al*., 2011). Elimination is achieved through identification and destruction of nascent transformed cells by acute tumor-inhibiting inflammation, characterized by infiltration of effectors cells of the innate and adaptive immune system, as well as production of tumor inhibiting cytokines. The escape phase is sustained by chronic tumor-promoting inflammation, which mainly involves immunosuppressive cells and soluble factors (Vesely *et al*., 2011). Evading immune destruction has recently been recognized as a hallmark of cancer, and in general, the use of immune suppressants following organ transplantation or HIV infection has been shown to increase the risk of tumors such as skin cancer, non-Hodgkin's lymphoma or lung cancers, but not cancers of organs such as breast, brain, prostate and ovary (Jiang *et al*., 2010; Hanahan and Weinberg, 2011). These studies suggest that breast cancer cells may be less immunogenic or may take longer to develop. Historically pre-existing inflammation or infection was not considered to be an underlying risk factor for the development of breast cancer. However, it is now clear that the infiltration of leukocytes can either eliminate or promote the development of breast cancers (Coussens and Pollard, 2011).

1.2.12 Genetic risk factors

1.2.12.1 *BRCA1* and *BRCA2* structure and expression

The *BRCA1* and *BRCA2* genes have been reported not to share any obvious sequence homology despite of having common features. Both genes have been found to have an extremely large exon 11 which comprises 61% and 48% of the whole coding sequence of *BRCA1* and *BRCA2* respectively. In addition, both genes are found to have translational start sites at exon 2 and in humans, the highest levels of expression are observed in testis, thymus and ovaries (Tavtigian *et al*., 1996).

1.2.12.2 *BRCA1* structure

The *BRCA1* gene is reported to be located on chromosome 17q and spans approximately 100 kb of genomic DNA which consists of 24 exons of which 22 are encoding a 1863 amino acid protein. BRCA1 exon 1 is found as exon 4 is an Alu repeat which is not generally included in the transcript (Smith *et al*., 1996).

1.2.12.3 *BRCA1* protein

BRCA1 is a 220 kDa protein demonstrating a predominantly nuclear localization forms nuclear "dots," or foci, during S phase of the cell cycle and following DNA damage (Chen *et al*., 1995; Scully *et al*., 1997). The *BRCA1* protein has been reported to contain important functional domains that interact with a range of proteins. In some studies it is found that *BRCA1* contains a zinc-binding RING finger motif in its amino terminal region (Miki *et al*., 1994), 2 nuclear export signals near its N terminus (Thompson *et al*., 2005), 2 nuclear localization signals (NLS) (Chen *et al*., 1996), a DNA binding domain in the central region of the protein (Paull *et al*., 2001), an SQ-cluster domain (SCD) between amino acids 1280 and 1524 (Cortez *et al*., 1999) and two carboxy-terminal BRCT domains (Bork *et al*., 1997).

1.2.12.4 *BRCA2* structure

The *BRCA2* gene has been reported to be located on chromosome 13q and spans approximately 70 kb of genomic DNA. It is found to be consists of 27 exons of which 26 encode a 3418 amino acid protein (Tavtigian *et al*., 1996).

1.2.12.5*BRCA2* protein

BRCA2 protein has been reported to contain two known functional domains, the BRC-repeats motifs and the DBD binding domain. The middle region of the protein, which is encoded by exon 11, is found to contain eight BRC-repeat motifs that are conserved among mammalian species suggesting an important function. It has been reported that the BRC repeats are essential for *BRCA2* function in DNA repair by mediating direct binding to the DNA recombinase *RAD51*, a protein that is essential for DNA repair and genetic recombination. It is now postulated that in human *BRCA2*, six of the eight motifs can bind directly to *RAD51* (Chen et al., 1998). Mutations in *BRCA2* BRC repeats are found to be associated with cancer predisposition. Studies in mice have showed that deletions of all *BRCA2* BRC domains are embryonically lethal whereas deletions of several BRC repeats lead to cancer (Donoho *et al*., 2003). The BRCA2 C-terminal region has been found to contains the DBD binding domain, which interacts with DSS1 (deleted in split hand/split-foot 1), a highly conserved 70 amino-acid protein. DSS1 binding protein is essential for BRCA2 function (Gudmundsdottir *et al*., 2004).

1.2.13.6 *BRCA1* protein functions

BRCA1 has been reported to plays a key role in DNA double strand break repair and in the maintenance of genomic integrity. *BRCA1* facilitates DNA repair through its involvement in homologous recombination (HR), non-homologous end joining (NHEJ) and nucleotide excision repair (NER). *BRCA1* protein has been reported to serves as a scaffold that organizes and coordinates a number of proteins that are involved in maintaining genomic integrity (Deng and Brodie, 2000). The most deleterious form of DNA damage is found to be double strand breaks. Two main pathways have been postulated for repairing these breaks: HR and NHEJ. There is substantial evidence that *BRCA1* is implicated in both these pathways. It is now proposed that *BRCA1* protein is involved in NHEJ via its interaction with the MRN [MRE11A [meiotic recombination 11 homolog A] - *RAD50* [RAD50 homolog]- NBS1 [Nijmegen breakage syndrome 1 (nibrin)]

complex. The MRN complex has been found to plays an important role in both HR and NHEJ. The exact mechanism of *BRCA1* interaction with the MRN complex and its involvement in NHEJ is yet under investigation. There is conflicting evidence on the role of *BRCA1* in NHEJ, which is summarized in a study conducted by Bau *et al*. ,(2006). Many studies showed the evidence that BRCA1 deficient cells have decreased NHEJ fidelity. Furthermore, *BRCA1*-deficient mouse embryonic fibroblasts were reported to have significantly reduced *NHEJ* activity whereas, other studies have demonstrated that *BRCA1* can promote only specific subtypes of *NHEJ* and has no effect on others. This may be a due to the different roles of *BRCA1* in sub-pathways of *NHEJ* (Bau *et al*., 2006). *BRCA1* is also found to be involved in DNA repair by homologous recombination. The first indication that *BRCA1* participates in DNA repair forwarded the observation that it is associated and co localized with *RAD51* in sub nuclear clusters (Scully *et al*., 1997). *RAD51* is thought to be the major component of the HR pathway. The nature of interaction between *BRCA1* and *RAD51* is still unknown but it is postulated that the association is likely to be indirect and possibly mediated by *BRCA2*. In the event of DNA damage, both *RAD51* and *BRCA1* localize to the region of damage. DNA-damage dependent replication checkpoint response (Thomas *et al*., 1997). It is also further evidence that *BRCA1* is involved in DNA repair by homologous recombination comes from the observation that *BRCA1* deficiency results in decreased *RAD51* foci formation in cultured cells after γ-irradiation (Huber *et al*., 2001). Based on the fact that *BRCA2* also interacts with *RAD51*, it was suggested that a complex consisting of *BRCA1, BRCA2* and *RAD51* is formed and functions to repair damaged DNA (Chen *et al*., 1999). There is an indication that this complex functions during or after DNA replication, since the levels of *BRCA1, BRCA2* and *RAD51* expression increase in cells when they enter the S phase of the cell cycle (Venkitaraman, 2002). *BRCA1* has also been linked to a number of other DNA repair processes due to its interaction with other proteins that are involved in response to and in the repair of DNA damage. BRCA1 together with *BRCA2*,

RAD51, BARD1 and other proteins is part of the *BRCC (BRCA1-BRCA2*-Containing Complex) that constitutes an E3 ubiquitin ligase that enhances cellular survival following DNA damage (Dong *et al*., 2003). Additionally, BRCA1 forms part of the BASC (BRCA1-Associated Genome Surveillance Complex) super complex. The BASC complex includes the DNA mismatch repair proteins MLH1 , MSH2 and MSH6, the MRN complex proteins MRE11ARAD50- NBS1, the Bloom syndrome helicase ATM kinase, DNA replication factor C, RFC and *PCNA.* It is believed that this complex acts as a sensor for DNA damage and is also directly involved in repairing DNA damage by DNA replication associated repair (Wang *et al*., 2000). The involvement of *BRCA1* in repairing double strand breaks is supported by its participation in the BASC complex and its interaction with the MRN complex. The MRN complex plays a critical role in DNA damage sensing, signalling and repair mechanism, as well as in the maintenance of chromosomal integrity of the cell (Assenmacher and Hopfner, 2004). *BRCA1* also functions in signaling the response to DNA damage. Following DNA damage, *ATM* and *ATR* protein kinasesphosphorylate *BRCA1* in response to different stimuli (Okada and Ouchi, 2003) . Furthermore, in response to γ-irradiation *ATM* phosphorylates and activates *CHEK2* which in turn phosphorylates *BRCA1* (Lee *et al*., 2000). Hence, *ATM*, *ATR* and *CHEK2* kinases regulate *BRCA1* function and in turn cell cycle regulation and DNA repair by phosphorylation. There has been recent progress in elucidating the mechanism by which *BRCA1* recognizes double-strand breaks. *BRCA1*, through its C-terminal domains forms three distinct complexes with Abraxas, BACH1 and CtIP proteins. *RAP80* recruits *BRCA1*-Abraxas and BRCA1-CtIP complexes to damaged DNA (Kim et al., 2007; Sobhian *et al*., 2007). Furthermore, the BRCA1-CtIP complex interacts with the MRN complex to facilitate double-strand break resection and to activate homologous recombination mediated repair of DNA (Chen *et al*., 2008). BRCA1 also plays a role in DNA repair by NER and is involved in both transcription coupled repair and global genome repair. In detail, it has been reported that BRCA1 deficiency leads to blockage of RNA polymerase II transcription

machinery at the site of repair of oxidative 8-oxoguanine residues (Le Page *et al*., 2000).

1.2.13.7 *BRCA2* protein functions

Although the exact role of the *BRCA2* protein still unknown, it has been demonstrated that *BRCA2* plays an important role in homologous recombination, both in meiosis and in the repair of double-strand breaks. The major role of the *BRCA2* protein is found to assist in organizing *RAD51* function and facilitate homologous recombination. *BRCA2* is reported to bind *RAD51* recombinase directly and regulates recombination-mediated double strand break repair. *BRCA2* is required for the efficient nuclear localization of *RAD51* and mediates the recruitment of *RAD51* to the sites of double strand breaks. Hence it is essential for the cellular function of *RAD51* (Davies *et al*., 2001). *BRCA2*-deficient cell lines are very sensitive to DNA damaging agents and exhibit a genomic instability phenotype that includes accumulation of double-strand breaks and in turn chromosomal breaks (Kraakmanv *et al*., 2002). Furthermore, *BRCA2* has been identified as the FANCD1 gene. When both *BRCA2* alleles are inactivated, a Fanconi anemia phenotype can occur (Offit *et al*., 2003). Not long ago, a nuclear partner *of BRCA2*, namely *PALB2* wasidentified.PALB2 (partner and localizer of *BRCA2*) provides stability to the *BRCA2* protein to perform its cellular functions namely DNA repair by homologous recombination and checkpoint control. *PALB2* is also required for *BRCA2* intra nuclear localization (Xia *et al*., 2006). In recent studies, it has been demonstrated that *BRCA2* plays a critical role in meiotic recombination through its direct interaction with *DMC1* recombinase (Thorslund *et al*., 2007). Additionally, *BRCA2* controls mitotic checkpoint activity (Yu *et al*., 2000), maintains normal centrosome number and function and has been implicated in regulation of cytokinesis in the final stages of cell division (Daniels *et al*., 2004). *BRCA2* has been shown to contribute to activation of transcription (Shin and Verma, 2003), G2/M checkpoint control (Yuan *et al*., 1999), suppression of tumour development by inhibition of cancer

cell proliferation (Wang *et al*., 2002) and mammalian game to genesis (Sharan *et al*., 2004).

1.2.12.6*BRCA1* and *BRCA2* in early onset breast cancer

Genetic breast cancer has been found to occur at a considerably younger age compared to the typical age of onset in the general population (Claus *et al*., 1991). The occurrence of early onset breast cancer has been found to be associated with mutations in the *BRCA1* and *BRCA2* genes (Krainer *et al*., 1997). In the general population, women with an early age of breast cancer are more likely than others. Studies on the contribution of *BRCA1* and *BRCA2* mutations to the incidence of breast cancer were primarily focused on individuals with high-risk families and large founder effect populations. In contrast to this, few population based studies examining the prevalence of *BRCA1* and *BRCA2* mutations in women who were diagnosed with breast cancer at a young age, have been carried out. On the basis of time, the first population-based studies that were performed had concentrated on selected populations with highly recurrent founder mutations i.e. the Icelanders and the Ashkenazi Jews. Data from these studies revealed that the rates of *BRCA1* and *BRCA2* mutations amongst early onset breast cancer patients can be as high as 30% for Ashkenazi Jews , and around 25% for Icelanders (Thorlacius *et al*., 1997). This high frequency is a result of the presence of founder mutations in these two ethnic populations. In these geographical regions, the prevalence of *BRCA1* and *BRCA2* mutations among early onset breast cancer patients' ranges between 5% and 10%. Mutations in the two cancer predisposition genes make approximately equal contributions to the incidence of early onset breast cancer, with the exception of the Philippines, where *BRCA2* plays a more significant role compared to BRCA1(De Leon Matsuda *et al*., 2002). The differences observed in the mutation frequencies between various populations proposed to be explained by the different study

selection criteria as well as by differences in the sensitivity of the genotyping methods.

1.2.13 Genetic Polymorphism

Polymorphism is a term which literally can be defined as the variability of form, shape, size, structure and composition. It has a currency in a wide variety of disciplines in science and art. Genetic polymorphism is now a more specific term describing frequent variation at a specific locus in a genome. A useful practical definition indicates that a locus is polymorphic when there are two or more allelic forms in the same population and the commonest allele has a frequency of 0.99 or less (Harris, 1980). A genetic polymorphism occurs if, within a population, a single gene accountable for producing a metabolising enzyme has a variant allele with the arbitrary frequency of 1% (Meyer, 2000). Single nucleotide polymorphisms (SNP) exist and an allelic site may have more than one SNP for many such genes. Genotype gives us the detailed genestructure of an individual whereas the more commonly measured phenotype provides the outcome of metabolism of a drug in an individual. Genetic Polymorphism is termed as difference in DNA sequence among individuals, groups, or populations. Genetic polymorphisms are proposed to be the result of chance processes, or may have been induced by external agents (such as viruses or radiation). If a difference in DNA sequence among individuals has been shown to be associated with disease, it will usually be termed as a genetic mutation. Changes in DNA sequence which have been evident to be caused by external agents are also generally called "mutations" rather than "polymorphisms". Genetic Mutation is defined as alteration in the nucleotide sequence of a DNA molecule. Genetic mutations are proposed to be a kind of genetic polymorphism. The term "mutation," as opposed to "polymorphism," is generally used to refer to changes in DNA sequence which are not present in most individuals of a species and either have been associated with disease (or risk of disease) or have resulted from damage indicted by external agents (such as viruses or radiation). Recent studies have demonstrated

that the presence of sequence variants, such as pSNPs, within intronic regions could affect basic preliminarymRNA (pre-mRNA) splicing mechanisms and thereby cause altered levels of normal transcripts (Pagani *et al*., 2003). A pSNP within the 3′-untranslated region (UTR) following the coding sequence is postulated to affect the intracellular stability of the mRNA gene transcript (Quirk *et al*., 2004).

1.2.15 *P53* gene structure and origin

The *p53* tumor suppressor gene is located on chromosome 17 p13.1 its products is a nuclear protein consisting of 393 amino acid and is divided structurally and functionally into four domains, it has an important role in the regulation of growth of both normal and malignant cells. Tow separated *p53*-mediated mechanisms are known to suppress tumor genesis, *p53*-mediated cell cycle arrest and *p53*-mediated apoptosis (Moll *et al*, 2001). Although *p53* is a known nuclear protein it can also be localized by IHC to the cellular mitochondria during cell stress and *p53* mediated apoptosis in *p53* mediated cell arrest , cell are blocked near the G1\S border of the cell cycle, thus controlling cell replication (Moll *et al*, 2001).

1.2.15.1*P53* in carcinogenesis

In many human cancers, mutant forms of *p53* proteins are present and these mutant *p53* gene products no longer suppress cell division ,thirty present of breast cancer have mutant *p53* genes and gene products (Storr *et al* , 2006). In fact, *p53* mutations are the single most common genetic change to be characterized in human cancers(Vogelestin *et al* ,2000). *P53* alteration have been reported to play a pivotal role in early barest cancer ovulation, Various mutation of *p53* are possible such as a one-base deletion to tow-base deletion, a nine base deletion

point mutation and complex deletion(Kamdioler- Eckrsberger *et al*, 2000). The mutant form of *p53* are more stable than wild type and therefore has a longer half life, a second possible mechanism involved in altering *p53* breast cancer has been postulated (moll *et al*, 1992). It has been seen that in some breast cancers wild type *p53*, the *p53* which is normally located in nucleus, was accumulated in the cytoplasm, the exclusion of the *p53* protein from the cell nucleus eliminates the ability of this protein to inhibit the proliferation of cells and therefore inactivates the *p53* function independently of mutation (moll *et al*, 1992).

1.2.16 Tumor markers

A tumor marker is defined as a substance present/overexpressed in or produced by a tumor (tumor-derived), or the host (tumor-associated), that can be used for differentiating neoplastic from normal tissue in some benign disorders (Lalle *et al* ., 2000). Tumor markers are found in cells, tissues, and body fluids such as cerebrospinal fluid, serum, plasma, and milk. The ideal marker would be useful in diagnosis, staging and prognosis of cancer, provide an estimation of tumor burden, and serve for monitoring effects of therapy, detecting recurrence, localization of tumors, and screening in general populations (Pamies *et al*., 1996). Most (if not all) tumor markers do not fit the ideal profile, the reason for this can be the relative lack of sensitivity and specificity of the available tests, it should be noted, that virtually any protein or chemical has the potential to be a tumor marker. As tumor cells grow and multiply, some of their substances increase in tumor tissues and/or leak into the bloodstream or other fluids, Depending upon the tumor marker, it can be measured in blood, urine, stool or tissue. Some widely used tumor markers include: AFP, beta-HCG, CA 19.9, CA 27.29 (CA 15-3), CA 125, CEA, and PSA. Some tumor markers are associated with many types of cancer; others, with as few as one. Some tumor markers are always elevated in specific cancers; most are less predictable. However, no tumor marker is specific for cancer and most are found in low levels in healthy persons, or can be associated with non-neoplastic diseases as well as cancer. Tumor

markers have been categorized as enzymes, isoenzymes, hormones, specific cell membrane proteins, oncofetal and cell-specific antigens, carbohydrate epitopes, oncogene products, genetic changes.

There are only a handful of well-established tumor markers that are being used by physicians. Many other potential markers are still being researched, there are many studies now that are trying to find new genes involved in signaling molecules or proteins that "tell" cells to proliferate, invade or metastasize (Miyamoto *et al*.,2000). Oncofetal antigens are very non-specific and expressed by a wide number of cancer types. However, they are used both to monitor a patient's progress and their response to treatment over time (Di Bisceglie *et al*., 1988) .Researchers continue working on specific molecular pathways involved in oncogenesis, tumor response, tumor progression, etc. to discover new molecular markers that can have a potential to be routinely used in medical practices of breast cancer. Laboratory techniques for the study of potential prognostic markers are rapidly developing at both the gene and protein level (Mitas *et al*.,2001).

1.2.16.1 Tumor Markers for Breast Cancer

Second leading cause of cancer death among women if breast cancer is found early, treatment is more likely to be successful. The best way to find breast cancer early is by having regular mammograms and clinical breast examinations, and by doing breast self-examination. The tumor markers listed below have been used in breast cancer. ASCO's recommendations are included in each description (Mitas *et al*.,2001).

1.2.16.1.1 CA 15-3

Levels of CA15-3 can increase as a tumor grows. Very high levels of CA15-3 may indicate advanced disease or metastatic cancer. CA15-3 is elevated in breast carcinoma, ovarian and lung cancer, in normal pregnancy (1st trimester), benign breast disease, cirrhosis and hepatitis (Correale *et al*., 1992). For recurrent breast carcinoma, CA15-3 has a sensitivity of ~57% and a specificity of

~87% (Rodriguez *et al.,* 1995). It lacks the required sensitivity and specificity for routine detection of breast cancer and does not discriminate patients with early carcinoma from those with benign breast disease. CA15-3 is associated with the early detection of recurrent breast carcinoma, ASCO does not recommend CA15-3 as a tumor marker for screening, prognosis, or predicting recurrence of breast cancer. A rising CA15-3 level can detect recurrence after primary treatment, but it is not yet clear if using this test affects survival or quality of life for women with breast cancer. There can also be false positives (positive results in women with no cancer). CA 15-3 levels may indicate response to or failure of treatment in some women with breast cancer. Sometimes this can be helpful if other tests are not straightforward(Taback *et al*.,2001).

1.2.16.1.2 Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) is a protein found in many types of cells but associated with tumors ,cancer cells produce CEA in large amounts, but it can also be found in the blood of healthy people, CEA is a cell surface glycoprotein and it is a marker for colorectal, gastrointestinal, lung, and breast carcinomas (Bates and Longo, 1987). CEA is most useful in monitoring therapy (as declining levels correlate with tumor burden) and has utility in detecting recurrence of colorectal cancer. High CEA levels in breast cancer do not correlate with grade of tumor but are useful for monitoring therapy and detecting recurrence , the oncofetal antigens are so named because they are normally produced during embryonic development and decrease soon after birth (Miyamoto *et al*.,2000). ASCO does not recommend CEA as a tumor marker for breast cancer. Routine use of CEA for monitoring response of metastatic disease to treatment is not recommended, but if no other test is available, a rising CEA level may indicate that treatment is not working (Taback *et al*.,2001).

2. Materials and Methods

2.1 Materials

2.1.1 Equipments and Instruments

The equipments and instruments used in this study are listed in Table (2-1)

Table (2-1) Equipments and instruments with their remarks

Equipments and instruments	Remarks
Autoclave	Sturdy (Taiwan)
Beakers	AMSCO (Germany)
Cold centrifuge	Hettich (Germany)
Cylinder (100 ml)	AMSCO (Germany)
Deep freezer	GFL (Germany)
Digital camera	Sanyo (Japan)
EDTA tubes (anticoagulant tubes)	Sun (Jordan)
Eppendorf tubes	Sterilin Ltd. / UK
Flasks (different size)	AMSCO (Germany)
Gel electrophoresis system	Consort (Belgium)
Hot plate with magnetic stirrer	Heidolph (Germany)
Hp labtop	China
Incubator	Jarad (Syria)
Microcentrifuge	Lab Tech (korea)
Micropippettes (in different size)	Eppendorf (germany)
Microtiter plate reader (450 nm filter)	Bio TeK (U.S.A)
Microtiter plate shaker	KAHN (Italy)
Multichannel micropipette	SIAMED (Germany)
PCR s Sprint-Thermal-Cycler-IP20	USA
PCR tube	Sterilin Ltd. / UK
Plain tubes	AFco- Dispo (Jordan)
Printer brother	China
Rack	Sterellin Ltd. / UK.
Sensitive balance	Sartorius (Germany)
Sterile syringes	China

Tips	Sterellin Ltd. / UK.
Vortex	Stuart (UK)
Water bath	Kottermann (Germany)
Water distillatory	Lab Tech (Korea)
MAGLUMI (CLIA)	Mainland (China)

2.1.2 Polymerase chain reaction kits

In Table (2-2) chemical materials that used in DNA extraction work to this study with their companies and countries of origin are listed.

Table (2-2) The kits used in this study with their companies and countries of origin

No.	Kit	Company	Country
1	**Genomic DNA Extraction Kit**	**Geneaid**	**USA**
	GT buffer		
	GB buffer		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
	Proteinase K 10mg/ml		
2	**AccuPower™ PCR PreMix**	**Bioneer**	**Korea**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		

	Tris.HCl pH 9.0		
	KCl		
	$MgCl_2$		

2.1.3 Primers

BRCA-1, *BRCA-2* and *P53* gene polymorphisms primers were designed by (Parvin, 2015) and these primers were provided from (Bioneer company, Korea) as following Table (2-3)

Table (2-3) The Multiplex PCR primers with their sequence and amplicon size (parvin,2015).

Primer		Sequence	Amplicon
BRCA1 185delAG	F	AAAATGAAGTTGTCATTTTATAAACC	176bp
	R	CTGACTTACCAGATGGGACACT	
BRCA2-A/G	F	GGAATACAGTGATACTGAC	346bp
	R	TTGGATTACTCTTAGATTTG	
p53 intron 6 G13964C	F	GCCTCCCCTGCTTGCC	131bp
	R	CCGCCCATGCAGGAACT	

2.1.4 Restriction enzyme

Table (2-4) The restriction enzymes used in RFLP-PCR assay with their company and country of origin

Restriction enzymes	SNP	Company/Country
DdeI	**A/G**	**New England Biolabs. UK**
BspHI	**A/G**	**New England Biolabs. UK**
HhaI	**G/C**	**New England Biolabs. UK**

2.1.5 Chemicals

Table (2-5) All the chemicals materials that used in this with their company and country of origin

No.	Chemical	Company and Origin
1	Absolute Ethanol	Scharlau (Spain)
2	Agarose	BioBasic (Canada)
3	TBE buffer 10X	BioBasic (Canada)
4	Ehidium Bromide	BioBasic (Canada)
5	Ladder 100bp	Bioneer (Korea)
6	Ladder 50bp	Bioneer (Korea)
7	Free nuclease water	Bioneer (Korea)

2.1.6 Molecular weight markers

The molecular weight marker used in this work, its description and source are depicted in Table (2-6) bellow**:**

Table (2-6) Molecular weight marker with their remarks

DNA Ladder	Description	Source
KAPA Universal Ladder	The KAPA Universal Ladder kit is designed for detecting and showing the approximate size and quantity of double-stranded DNA on agarose gel. KAPA Universal Ladder kits contain eighteen DNA fragments (in base pairs): 100, 150, 200, 300, 400, **500**, 600, 800, **1000**, 1200, **1600**, 2000, 3000, **4000**, 5000, 6000, 8000, and 10000. The KAPA Universal Ladder contains four reference bands (500, 1000, 1600, and 4000) for orientation. Kits are formulated with DNA loading dye for direct loading on agarose gel.	KAPA Biosystems

2.1.7 Tumor markers kits:

Table (2-7) CEA kit components

Reagent Integral for 100 determinations	
Nano magnetic microbeads: microbeads coated with sheep	2.5ml

anti-FITC polyclonal antibody, TRIS buffer, 0.2%NaN3.	
Calibrator Low: bovine serum, 0.2%NaN3.	2.5ml
Calibrator High: bovine serum, 0.2%NaN3	2.5ml
FITC Label: anti-CEA monoclonal antibody labeled FITC, containing BSA, 0.2%NaN3.	12.5ml
ABEI Label: anti-CEA monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN3.	22.5ml
Diluents: 0.9%NaCl	25ml

Table (2-8) CA-15.3 kit components

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2 % (W/V), 0.2%NaN3 , coated with sheep anti-FITC polyclonal antibody	2.5ml

Calibrator Low: bovine serum, 0.2%NaN3.	2.5ml
Calibrator High: bovine serum, 0.2%NaN3	2.5ml
FITC Label: anti-CA-15.3 monoclonal antibody labeled FITC, containing BSA, 0.2%NaN3.	12.5ml
ABEI Label: anti-CA-15.3 monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN3.	22.5ml
Diluents: Buffer. contains BSA, 0.2%NaN3	25ml

2.2 Laboratory Methods

2.2.1 patients and controls

The case-control study was conducted on 100 females (50 patients group and 50 controls group). The patients were in post-operative stage. Both groups include females with 18-80 years old. The patients were referred to Al-Diwanya Teaching hospital , department of oncology, during the period March-July 2016. The diagnosis was made by the consultant oncologists and pathologists, all patients in after surgery stage(post-operative). Demographical and risk factor data were collected using a short structured questionnaire (Appendix I), that included information on age, marital status, family history of breast cancer or other cancers (first degree relatives). Furthermore, the patients were also followed-up after the surgical operation to define the histopathology classification of breast tumor, and on which, lymph node metastases and cancer stage at the time of testing were recorded. Another group include healthy females without any

history family of breast or other types of cancers also included in this study as a control group.

2.2.2 Included Criteria

2.2.2.1 Included Criteria of patients

1- Females only.
2- Cases after the surgical operation (post-operative stage).
3- Diagnosis of breast cancer curried out according to the treating physician
4- Taken patients more than 18 year old only

2.2.2.5 Included criteria of control

1- Females only.
2- Don't have any history of breast or other types of cancer in first and other degrees in relatives in their family .
3- No past or present breast cancer disease.

2.2.2.6 Excluded criteria of the two groups

1- Patients before surgical operation and histopathology report.
2- Presence history of cancer in all degrees in families of patients group (First –degree only).
3- Presence of history of cancer in any relatives degree among control group.
4- The individual suffering from other chronic disease, neither cancer nor non-cancer type.

2.2.2.7 Clinical assessment of patients

1- Name and age.
2- Family history

3- Physical assessment was done by physician(include, ultrasound, X-ray, MRI, etc….others) .
4- Histopathology report if present.

2.3 Collection of Samples

Ten ml of venous blood were collected from both groups patients and healthy group . Each sample was divided into two portions:

1- four ml of blood in anticoagulant (EDTA) tube for molecular study and immediately store at-20C until use.
2- Six ml of blood in sterile plain tube and allow sample to clot for few minute at room temperature then followed by separation of serum from the clot by centrifugation for 15 minute at 1000x g and store in 4 C° until use in measure tumor markers(CEA and CA-15.3).

2.4 Molecular Methods

2.4.1 Solutions preparation

All solutions and buffers were prepared according to Su *et al*.,(2008)

A.TBE(1X) buffer:

This solution was prepared by mixing 10 ml of stock TBE10X buffer with 90 ml of distilled water, then stored at 4 C until used in electrophoresis.

B.DNA loading dye:

This buffer was prepared by dissolving and mixing 40 g of sucrose and 0.25g of bromophenol blue in 100 ml of sterilized distilled water then stored in sterilized flask at room temperature until used in electrophoresis.

C. Ethidium bromide solution(0.5%):

This solution was prepared by dissolving 0.25g of ethidium bromide stain in 50 ml sterilized distilled water, stored in sterilized flask, final concentration 0.5 mg/ml. It was used in electrophoresis as specific DNA stain.

2.4.2Genomic DNA Extraction

Genomic DNA from blood samples were extracted by using Genomic DNA mini kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions as follow:

1. A 200µl of frozen blood was transferred to sterile 1.5ml microcentrifuge tube, and then added 30µl of proteinase K and mixed by vortex. And incubated at 60℃ for 15 minutes.

2. After that, 200µl of lysis buffer was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70℃ for 15 minutes, and inverted every 3 minutes through the incubation periods.

3. Two hundred µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously.

4. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow.through were discarded and placed the column in a new 2 ml collection tube.

5. Four hundred µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow.through was discarded and placed the column back in the 2 ml collection tube.

6. Six hundred µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow.through was discarded and placed the column back in the 2 ml collection tube.

7. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.

8. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix.

9. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

2.4.3Genomic DNA estimation

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of blood genomic DNA was added to measurement.

2.4.4 Primer preparation

Specific primers Table(2-3) were used for the amplification of *BRCA-1*, *BRCA-2* and *P53* genes designed by Su *et al*., (2008) , provided by Bioneer (South Korea). The primers were prepared according to manufacturer's instruction by dissolving the lyophilized primers with deionized distal water to form stock

solution with concentration of 100 pmol/µl, primers working solution with deionized water, using the equation C1V1 = C2V2 to get final working solution (10 pmol / µl) for both primers.

2.4.5 RFLP-PCR Technique

RFLP-PCR technique was performed for detection and genotyping BRCA1 (rs80357713), BRCA2 (rs11571653), and p53 intron 6 (G13964C) polymorphism in blood samples of patients and in healthy blood samples. This method was carried out according to method described by (Marsh *et al*., 2001 and Parvin, 2015) as the following steps

2.4.5.1 PCR master mix preparation

PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions as following Table(2-9):

Table (2-9) PCR master mix preparation

PCR Master mix	Volume
DNA template	5µl
Forward primer (10pmol)	1.5µl
Reveres primer (10pmol)	1.5µl
PCR water	12µl

Total volume	20µl

After that, these PCR master mix component that mentioned in Table (2-11) above placed in standard AccuPower PCR PreMix Kit that contains all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and loading dye). Then, all the PCR tubes were transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea).

2.4.5.2 PCR Thermo cycler Conditions

PCR thermo cycler conditions were done for each gene independent as described by Su *et al*., (2008) as in Table (2-10) below:

Table (2-10) Thermocycling condition for BRCA-1 gene detection

PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	45 sec.	40cycle
Annealing	56°C	30 sec.	
Extension	72°C	30 sec.	

Final extension	72°C	5min	1
Hold	4°C	5min	-

Table (2-11) Thermocycling condition for BRCA-2 gene detection

PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	30cycle
Annealing	55°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5min	1
Hold	4°C	5min	-

Table (2-12) Thermocycling condition for P53 gene detection

PCR step	Temp.	Time	repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	20 sec.	30cycle
Annealing	60°C	20 sec.	
Extension	72°C	20 sec.	

Final extension	72°C	5min	1
Hold	4°C	Forever	-

2.4.5.3 PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis as in the following steps

1- One percentage Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Three µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed carefully from the tray and 10µl of PCR product were added in to each comb well and 10ul of (100bp Ladder) in First well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- PCR product were visualized by using UV transilluminator.

2.4.5.4 RFLP-PCR mix preparation

A- RFLP-PCR mix for (BRCA1-185delAG)

RFLP-PCR mix was prepared by using *DdeI* restriction enzyme (New England Biolabs. UK) and this master mix done independent according to company instructions as the following Table:

1- RFLP-PCR master mix:

RFLP-PCR Master mix	Volume
PCR product	10µl
DdeI Restriction enzyme buffer 10X	2 µl
DdeI (10 unit)	1 µl
Free nuclease water	7 µl
Total volume	20 µl

After that, this master mix was placed in exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was subjected to 3% agarose gel electrophoresis. The genotyping of *BRCA1* gene including AA (homozygous) by two bands at (150, 26bp), GG (homozygous) as non-digested band at 176bp, A/G (heterozygous) of two bands at bp, 150bp, and 26bp.

B- RFLP-PCR mix for (*BRCA2-A/G*)

RFLP-PCR mix was prepared by using *BspHI* restriction enzyme (New England Biolabs. UK) and this master mix was done according to company instructions as following Table:

1- RFLP-PCR master mix:

RFLP-PCR Master mix	**Volume**
PCR product	10µl
BspHI Restriction enzyme buffer 10X	2 µl

BspHI (10 unit)	1 µl
Free nuclease water	7 µl
Total volume	20 µl

After that, this master mix placed in exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods. The genotyping of *BRCA2* gene including AA (homozygous) by two bands at 296bp and 50bp, GG (homozygous) three band at 235bp, 61bp, and 50bp, A/G (heterozygous) of four bands at 296bp, 235bp, 61bp, and 50bp.

C- RFLP-PCR mix for (*p53 intron 6G13964C*)

RFLP-PCR mix was prepared by using *HhaI* restriction enzyme (New England Biolabs. UK) and this master was mix done independent according to company instructions as following Table:

1- RFLP-PCR master mix:

RFLP-PCR Master mix	**Volume**
PCR product	10µl
HhaI Restriction enzyme buffer 10X	2 µl
HhaI (10 unit)	1 µl
Free nuclease water	7 µl
Total volume	20 µl

After that, this master mix was placed in exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analyzed by 3% agarose gel electrophoresis methods. The genotyping of p53 gene including GG (homozygous) by two bands at 33bp and 98bp, CC (homozygous) as non-digested band at 131bp, G/C (heterozygous) of three bands at 33bp, 98bp, and 131bp.

2.5 Tumor marker analysis

Fully-auto chemiluminescence immunoassay – CMIA

2.5.1 Principle Of The Test

Use an anti-CEA and anti-CA15.3 monoclonal antibodies to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control are mixed thoroughly with FITC Label and nano magnetic microbeads in a cuvette incubated at 37°C, then cycle washing adding for 1 time. Then added ABEI Label and incubated to form a sandwich, after sediment in a magnetic field, sucked the supernatant then cycle washing for the 2nd time. Subsequently, Starter1+2 substrates are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of CEA and CA15.3 present in samples.

2.5.2 Procedure of CEA and CA-15.3 Tests

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CMIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. refer to the Fully-auto chemiluminescence immunoassay (CMIA) analyzer MAGLUMI Operating Instructions, procedure steps below:

1-Patients specimens with a cloudy or turbid appearance centrifuge prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.

2- Specimens mix thoroughly after thawing by low speed vortexing or by gently inverting, and centrifuge prior to use to remove red blood cells or particulate matter to ensure consistency in the results.

3-Added 40 µl of serum and 100 µl of calibrator FITC label to Nano magnetic microbeads.

4-Incubate the microbead at room tempreture for 10 minute.

5-Added 400 µl of cycling washer.

6- Added 200µl of ABEI label to microbead.

7-Incubate the microbead for 10 minute.

8- Added 400 µl of cycling washer.

9- Measured the microbead directly.

2.6 Statistical analysis

Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010.Continuous random variables of age and serum concentration of immunological makers that normally distributed are described by mean, SD (standard deviation), SE (standard error), and the parametric statistical tests of significant. ANOVA test are used to analyzed the statistical significance of difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical

significance of difference in mean between each 2 groups was assessed by Bonferonni t-test (Sheskin, 2004). The statistical significance, direction and strength of linear correlation between 2 quantitative variables was measured by Spearman's rank and Pearson linear correlations coefficient as in state of serum markers. Moreover measure the strength of association between 2 categorical variables, such as the presence of certain genotype and disease status the odds ratio (OR) and Chi-square ($\chi 2$) test were used. P value calculate from different tests depend on variables and that less than the 0.05 level of significance was considered statistically significant (Sheskin, 2004; Viera, 2008).

3.Results

3.1. Demographic Features Of The Study

The present case-control study were based on the analysis of a random sample of 50 females with confirmed diagnosis of breast cancer, their ages ranged from 19 to 80 years with a mean of 46.38 (SD 14.31) and 50 (healthy) controls females their ages ranged 19 to 80 years with a mean of 45.6 (SD14.34) as in table (3-1) , that also shows not significant (p 0.05) association between mean age of cases and controls.

Table (3-1): The case-control difference in mean age

Demographic features	Case (breast cancer)	Healthy controls
Age Groups (years)	N (%)	N (%)
19-29	5(10)	6 (12)
30-39	10 (20)	9 (18)
40-50	20 (40)	23(46)
51-60	6 (12)	4(8)
61-80	9 (18)	8(16)
Total Number	50	50
Range	19-80	19-80
Mean	46.38	45.6
SD	14.31	14.34
SE	2.023	2.028
P – value	0.9369 (NS)	

❖ **NS= Not Significant (p > 0.05), SD= Standard Deviation, SE= Standard Error, N= Number**

3.2. The Association Between Breast Cancer And Selected Tumor Markers

Tables (3-2) and figure (3-1) show significant association between tumor marker (CA 15-3) levels and breast cancer ($p<0.05$). Patients have high level of CA 15-3 mean (18.396 u/ml) compared with controls mean (8.136 u/ml).

Table(3-2): The case-control difference in mean serum level of tumor marker CA 15-3

serum level of tumor antigen CA 15-3 (u/ml)	Case (breast cancer)	healthy controls	P – value
Range	3.9 - 42	0.5-28	< 0.05
Mean	18.396	8.136	
SD	8.871	6.58	
SE	1.255	0.931	
N	50	50	

- **Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number**

Tables (3-3) and figure (3-1) shows significant association between tumor markers (CEA) and breast cancer ($p< 0.05$). Patients have high level of CEA mean (11.66 ng/ml) in compared with control Mean (5.086 ng/ml).

Table(3-3): The case-control difference in mean serum level of CEA

Serum level of CEA (ng/ml)	Case (breast cancer)	Healthy controls	P – value
Range	1.9 – 24.2	0.5 – 16.5	< 0.05
Mean	11.66	5.086	
SD	5.693	4.085	
SE	0.81	0.578	

N	50	50	

❖ **Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number**

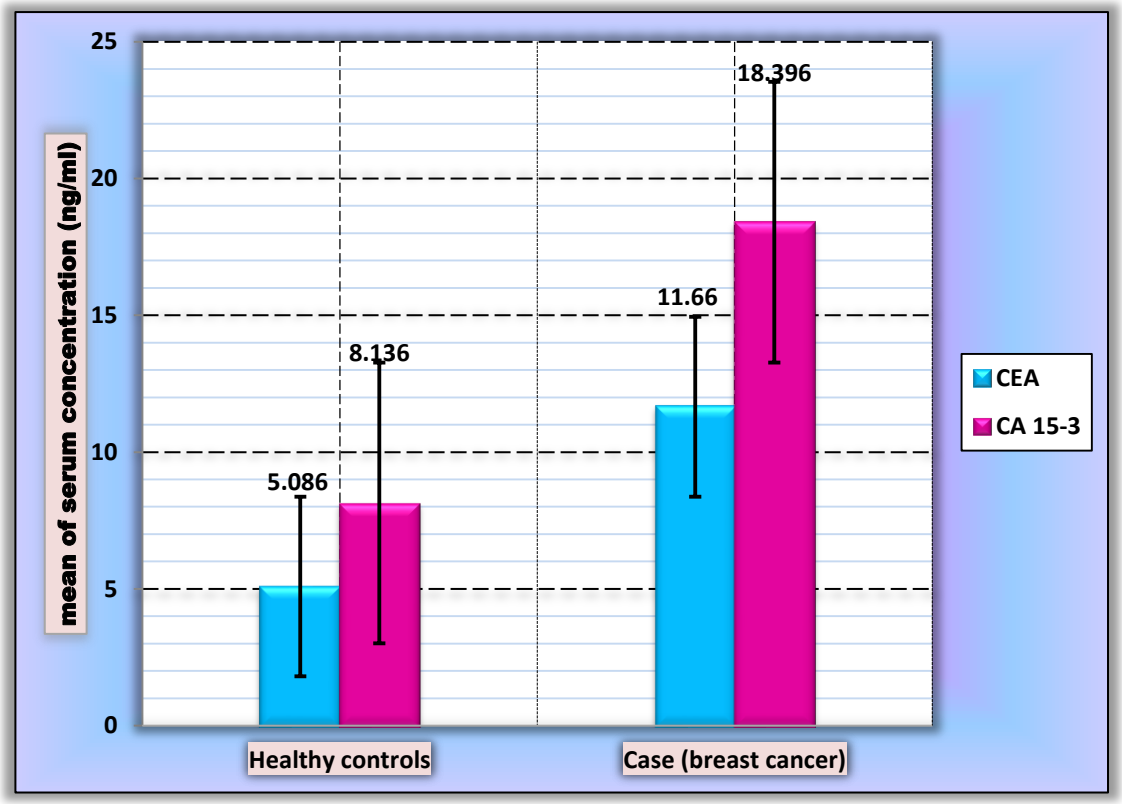


Figure (3-1): Bar chart show mean differences of CA 15-3 and CEA in patient and control

3.3 Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In present study try to found relationship between both tumor markers which included CEA and CA.15.3, figure (3-2) shows no significant association between CA 15-3 and CEA in breast cancer patients (P=0.185) ,but weak positive correlation is appeared (r = 0.2432).

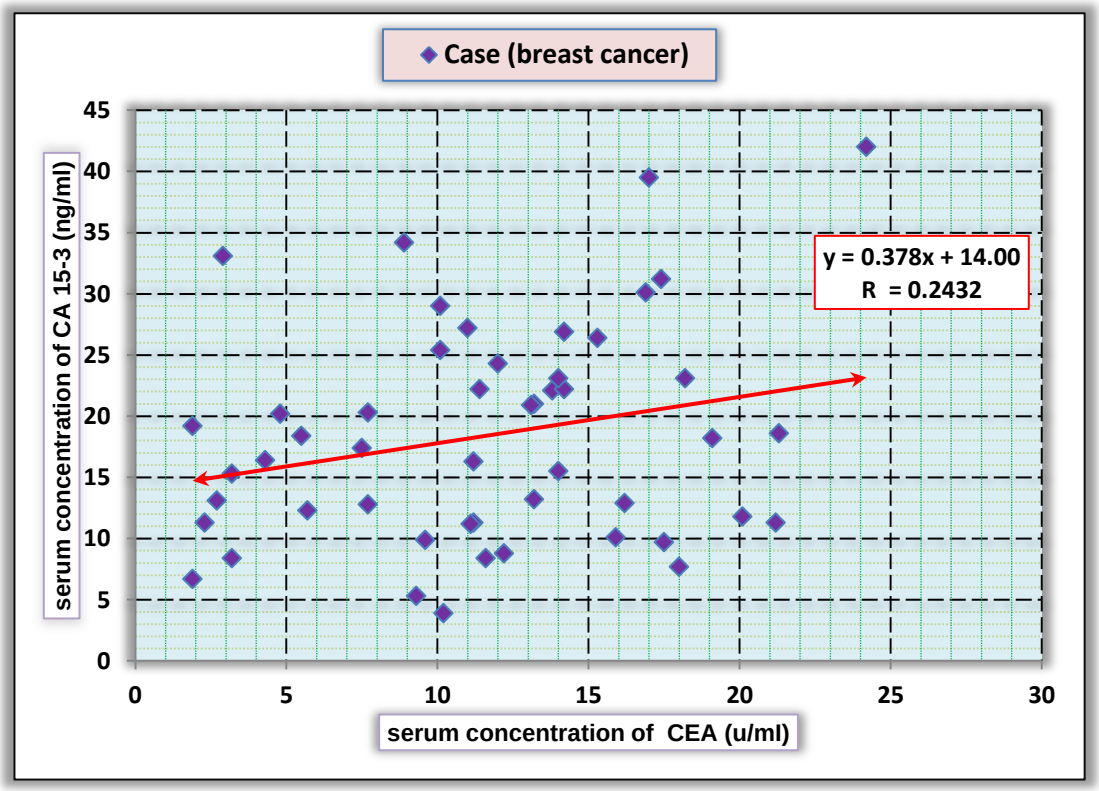


Figure (3-2): Scatter diagram showing the linear correlation between serum level of tumor antigen CA 15-3 and CEA (r= 0.2432, p= 0.185) among breast cancer patients

3.4 Family History and Tumor Markers

Table (3-4) shows significant correlation (P<0001) between breast cancer and family history ,and figure (3-3) shows 22 (44%) of patients have positive family history of breast cancer and 28 (56%) not have family history (P>0.05) . The differences of CA 15-3 and CEA in patients with and without family history are demonstrated in Figure (3-4). High serum level of tumor markers in patients with family history group when compared with negative family history patients group . Table (3-5) show serum level of CA15.3 and CEA significantly associated (p<0.05) at mean (26.45 and 16.682 respectively) compared with patients without family history mean (12.071 and 7.586 respectively) .

Table (3-4): Case-control difference in family history of tumor

Family history of breast cancer	Case-control comparison			
	Case (breast cancer) N (%)	healthy controls N (%)	X^2	P value
Negative	28 (56)	50 (100)	28.205	0.0001
Positive	22 (44)	0 (0)		
Total	50	50		

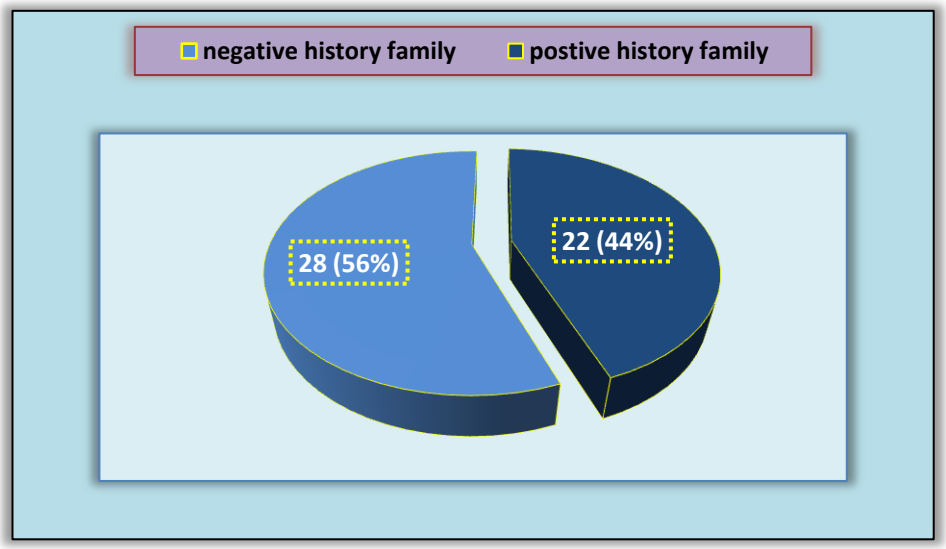


Figure (3-3): A Pie Chart showing occurrence of breast cancer among patients with and without family history for the disease (P > 0.05).

Table(3-5): Correlation between mean of serum level of tumor markers and family history in patients.

Tumor Markers	Case (breast cancer)		P value
	Positive history family	Negative history family	
CEA (ng/ml)			< 0.05

Range	**12 – 24.2**	**1.9 – 13.2**	
Mean	**16.682**	**7.586**	
SD	**3.123**	**3.678**	
SE	**0.666**	**0.695**	
N	**22**	**28**	
CA 15-3 (u/ml)			**< 0.05**
Range	**17.4 - 42**	**3.9 – 19.2**	
Mean	**26.45**	**12.071**	
SD	**6.407**	**4. 076**	
SE	**1.366**	**0.77**	
N	**22**	**28**	

❖ **Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number**

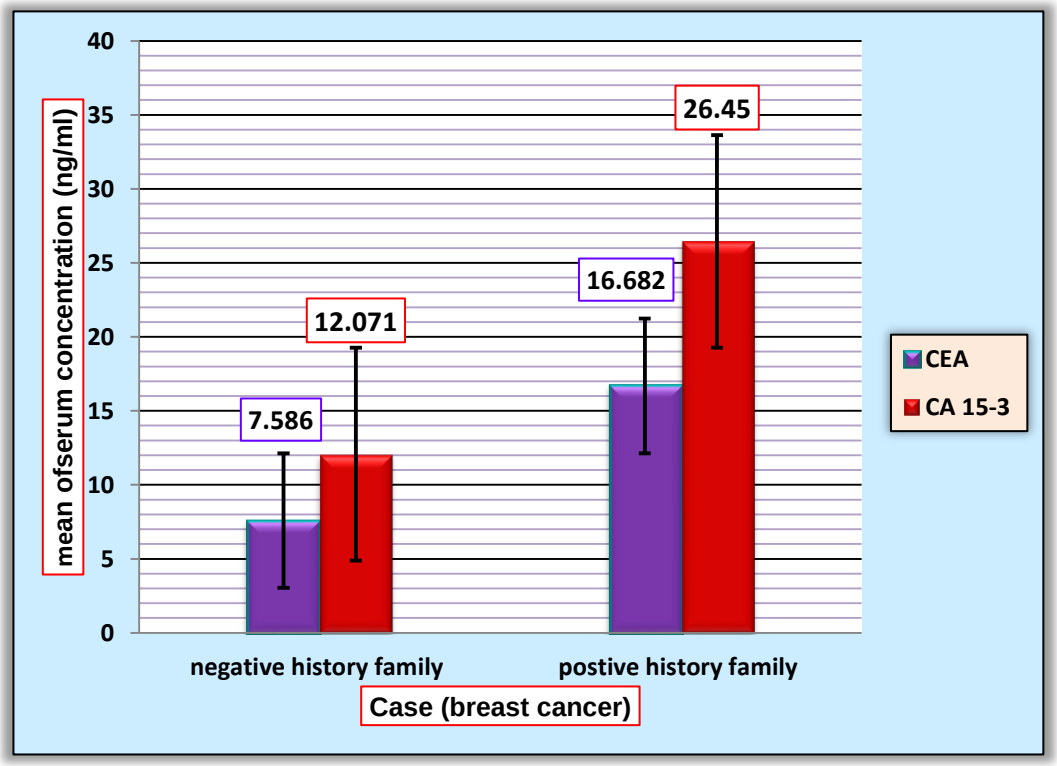


Figure (3-4): Bar chart show mean differences of CA 15-3 and CEA in patients with and without family history.

3.5.The Association Between Patients Age and Selected Tumor Markers

Serum levels of CA15-3 and CEA not effected strongly by age (r= 0.20 though not significant land r= 0.114 respectively) Figure (3-5) and (3-6). A association between tumor markers and age groups (p> 0.05) . The age group of 30-39 years have highest serum level of CA-15.3 and CEA compared with other groups (mean 24.58 and 15.74 respectively) , Table(3-6) and Figure (3-7).

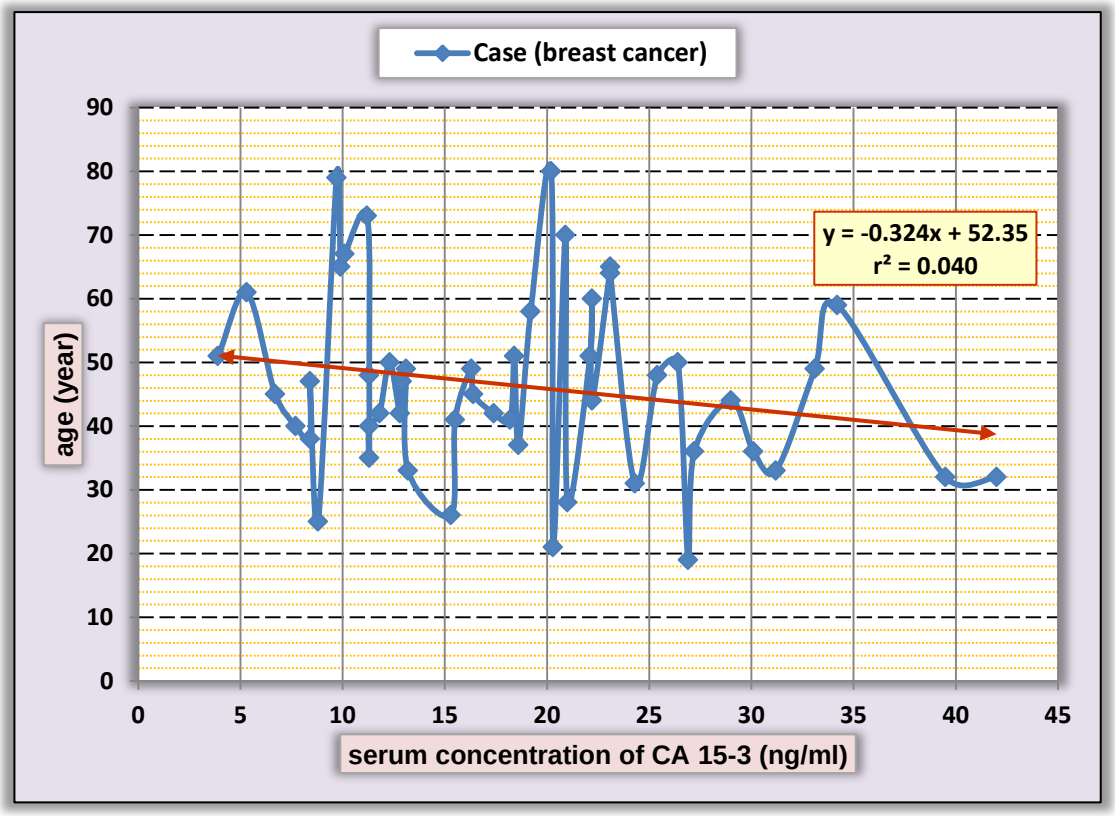


Figure (3-5): Scatter diagram showing the correlation between serum level of tumor antigen CA 15-3 and age (r= 0.20, p = 0.199).

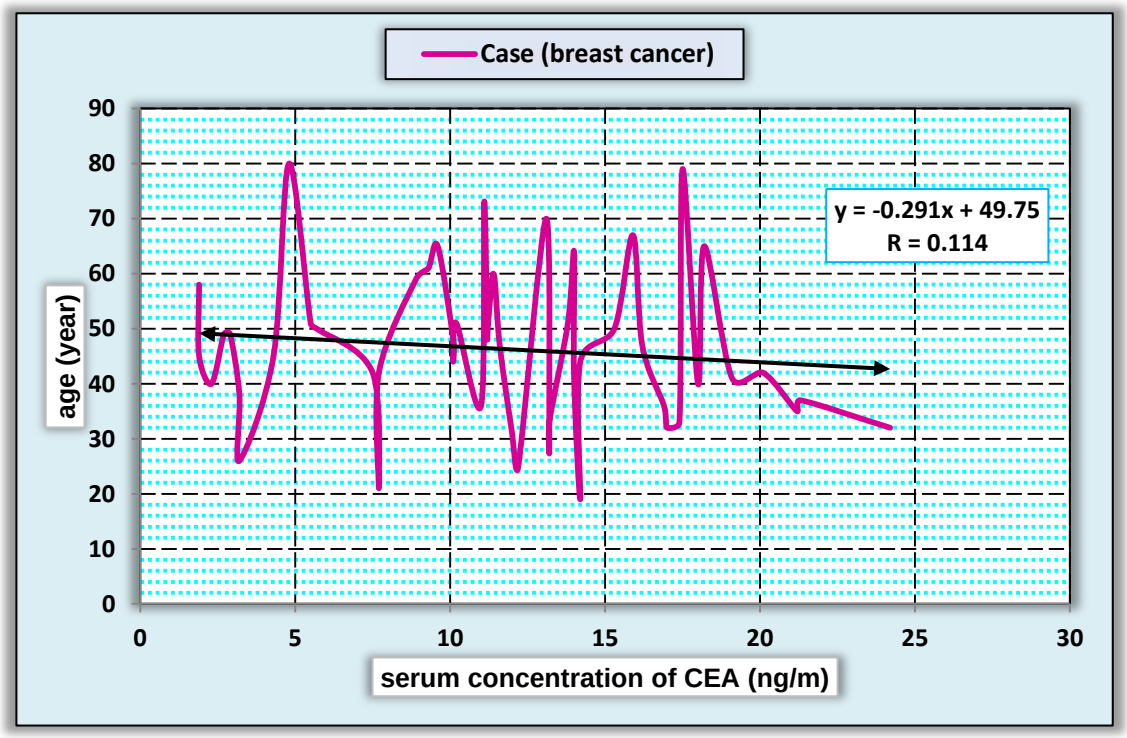


Figure (3-6): Scatter diagram showing the correlation between serum level of CEA and age (r= 0.114, p = 0.18).

Table(3-6): Showing the distribution of mean of serum level of tumor markers over different age groups.

Tumor markers	Patients Age Groups (years)					P Value
	19-29	30-39	40-50	51-60	60-80	
CEA (ng/ml)						
Range	3.2 – 14.2	3.2– 24.2	1.9-20.1	1.9-13.8	4.8-18.2	
Mean	10.1	15.74	10.305	8.617	12.611	>0.05 NS
SD	4.588	6.130	5.821	4.290	4.351	
SE	2.052	1.938	1.302	1.751	1.450	
N	5	10	20	6	9	
CA 15-3(u/ml)						> 0.05

Range	**8.8-26.9**	**8.4-42**	**6.7-33.1**	**3.9-34.2**	**5.3-23.1**	**NS**
Mean	**18.46**	**24.58**	**16.41**	**20**	**14.83**	
SD	**6.789**	**11.598**	**7.317**	**9.729**	**6.89**	
SE	**3.036**	**3.668**	**1.636**	**3.972**	**2.296**	
N	**5**	**10**	**20**	**6**	**9**	

❖ **NS= Not Significant (p > 0.05), SD= Standard Deviation, SE= Standard Error, N= Number**

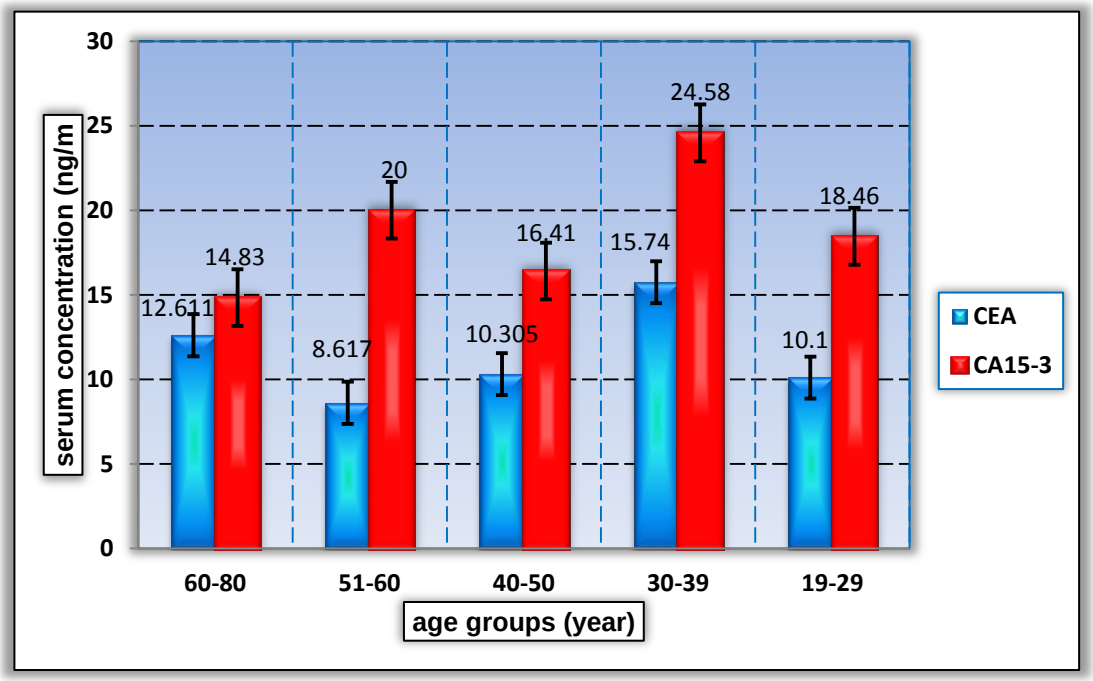


Figure (3-7): Bar chart show mean differences of CA 15-3 and CEA in patients accordant to age groups

3.6. Molecular study

3.6.1 DNA Amplification of *BRCA-1* gene

The products of successful binding between the extracted DNA and specific primers for *BRCA-1* gene were detected by gel electrophoresis analysis using DNA marker (100 bp DNA ladder) and the products size was 176bp PCR for both patients and control groups figure (3-8).

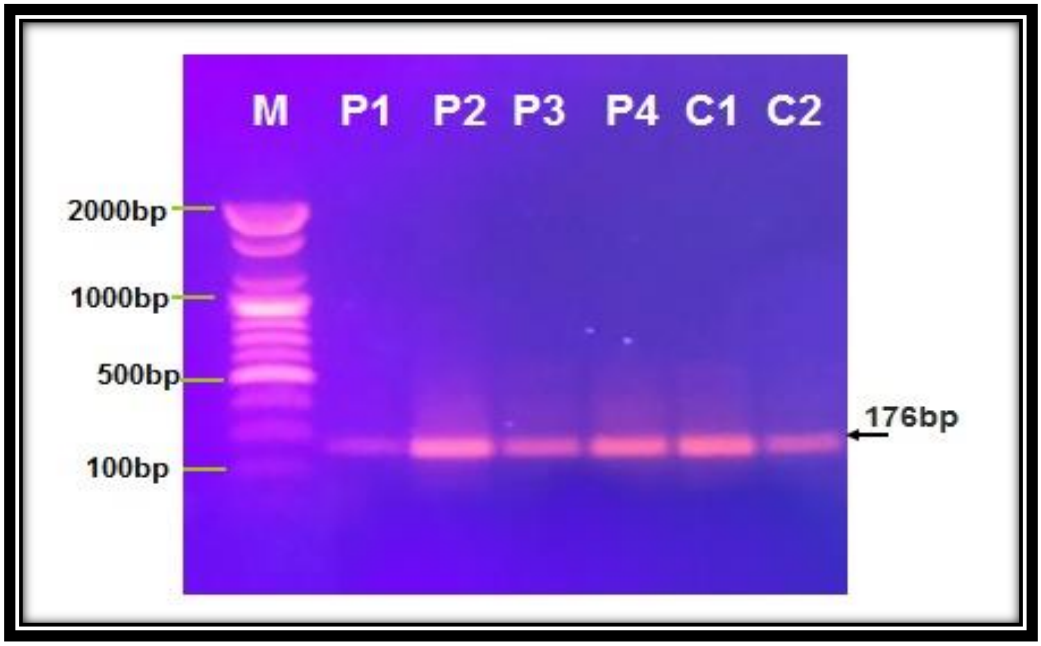
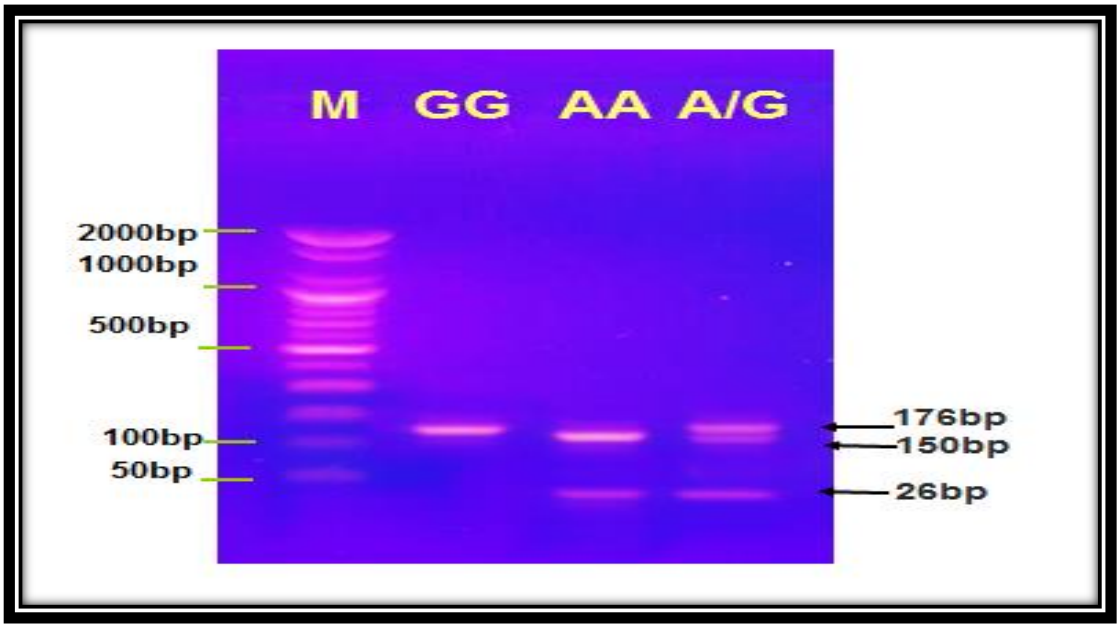


Figure (3-8): Agarose gel electrophoresis image that shows the PCR product analysis of *BRCA-1*gene from some blood patient samples and healthy control sample. Where M: marker (100-2000bp), lane (1-4) patients samples that show 176bp PCR product size.

3.6.2 Detection of *BRCA-1* Polymorphism

The distribution of *BRCA-1* polymorphism was detected by RFLP-PCR technique, at this locus there're three genotype; homozygote lane (AA) homozygous as non-digested band , lane (GG) homozygous at 150, and lane (A/G) heterozygous at 150bp and 26bp as a Figure (3-9).



Figure(3-9): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *BRCA-1*185delAG gene polymorphism by using *DdeI* restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygous at 150 and 26bp, lane (AA) homozygous as non-digested band 176bp, and lane (G/A) heterozygous at bp, 150bp, and 26bp.

In *BRCA-1* table (3-7) GG genotype has obviously suggested an etiology for tumor, as had an (OR 5.3191) and etiologic Fraction (EF 0. 065) , In contrast, the AG & AA genotypes had rather preventive role as it had protective Fraction (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively) . Figure (3-10) shows patients have 76% of AA , 8% of GG and 16% of AG compared with control show 20% of AG , 80% of AA and 0% of GG. Figure (3-11) show patient have 16% and 84% of patients have G and A alleles respectively compared with control they have 10% and 90% of G and A respectively

Table (3-7): distribution of genotypes and alleles of *BRCA-1* gene in cases & control

BRCA1 gene	Patients	Control	OR	95% CI OR	X^2	P (X^2)	EF	PF
	N (%)	N (%)						
***BRCA1* genotypes**								
AA	**38 (76)**	**40 (80)**	**0.7917**	**0.306 - 2.046**	**0.233**	**0.629**	*******	**0.1667**
GG	**4 (8)**	**0 (0)**	**5.3191**	**0.599- 47.229**	**5.233**	**0.022**	**0.065**	*******
AG	**8 (16)**	**10 (20)**	**0.7619**	**0.273 - 2.125**	**0.271**	**0.603**	*******	**0.0476**
Total number	**50**	**50**						
***BRCA1*Alleles**								
A	**84 (84)**	**90 (90)**	**0.5833**	**0.251 - 1.357**	**1.591**	**0.208**	*******	**0.3750**
G	**16 (16)**	**10 (10)**	**1.7143**	**0.737 - 3.988**	**1.59**	**0.207**	**0.0667**	*******
Total number	**100**	**100**						

❖ **OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, X^2 = chi square**

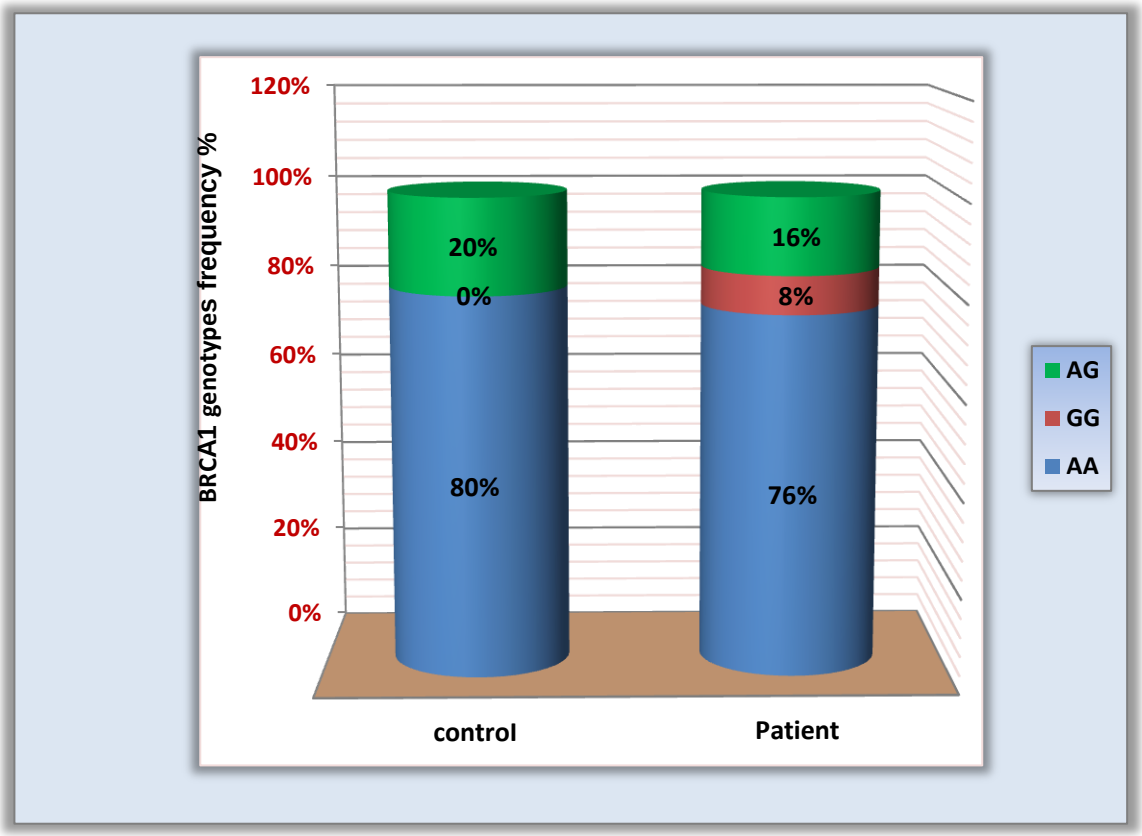


Figure (3-10): Component Bar Chart showing a case-control comparison in relative frequency of the *BRCA1* genotypes.

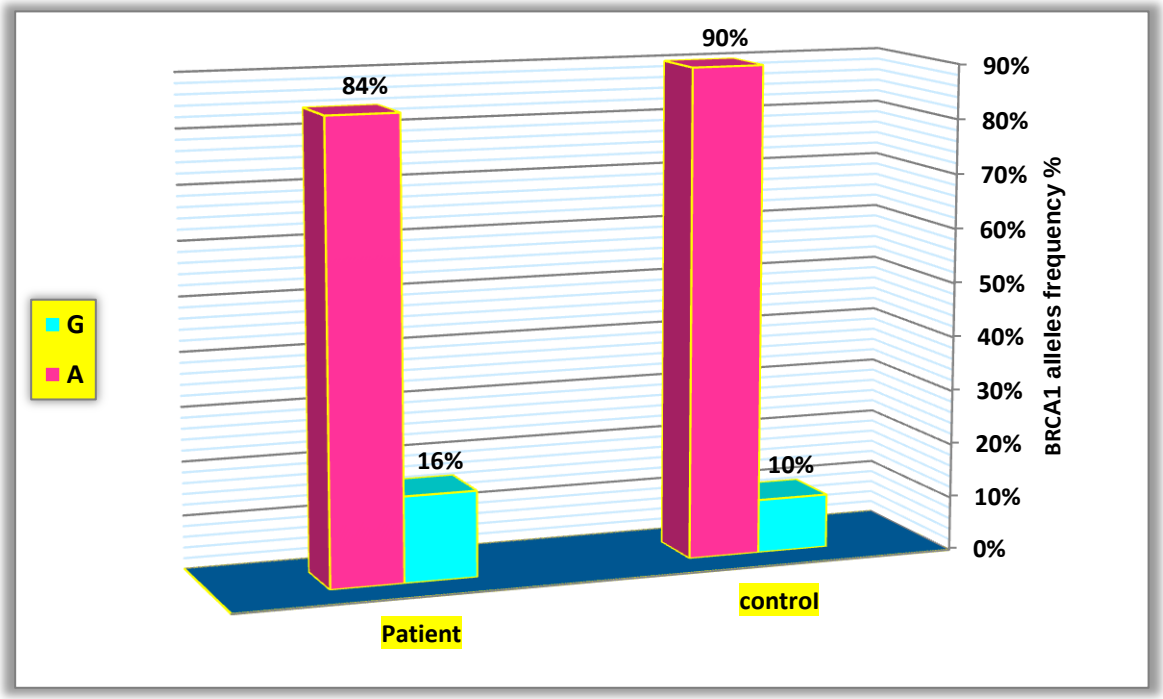
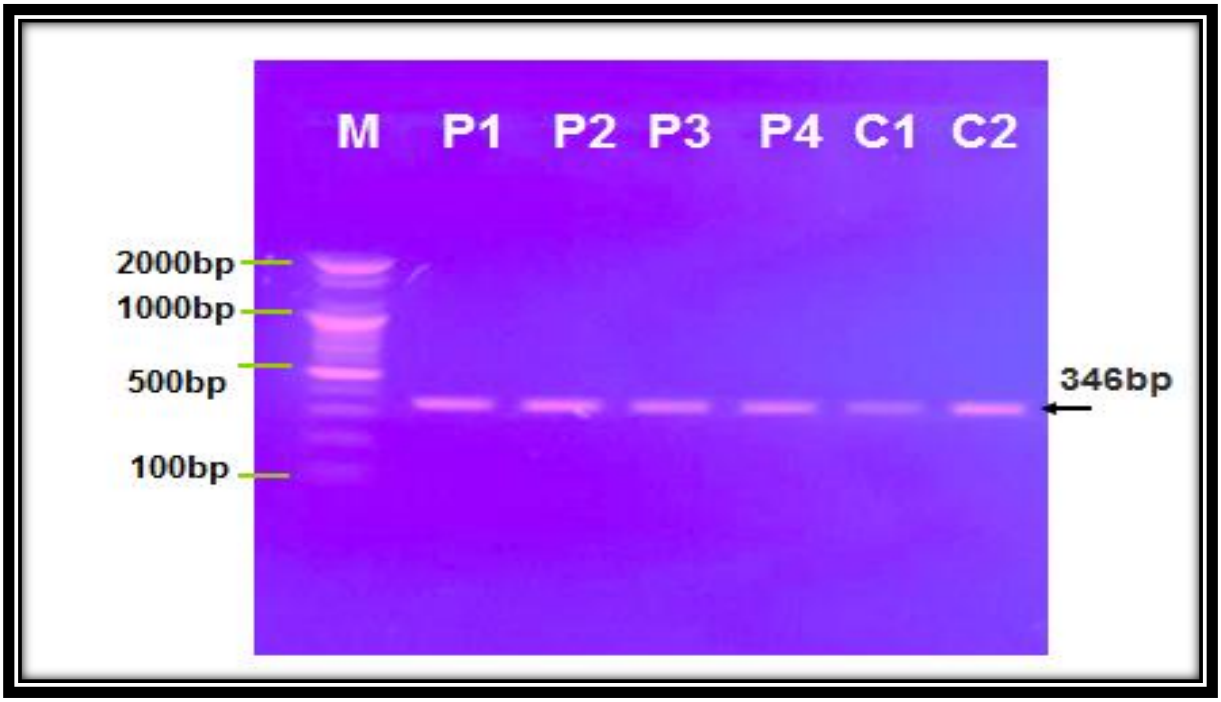


Figure (3-11): Component Bar Chart showing a case-control comparison in .es relative frequency of the *BRCA1* allel

3.6.3 DNA Amplification of *BRCA-2* gene

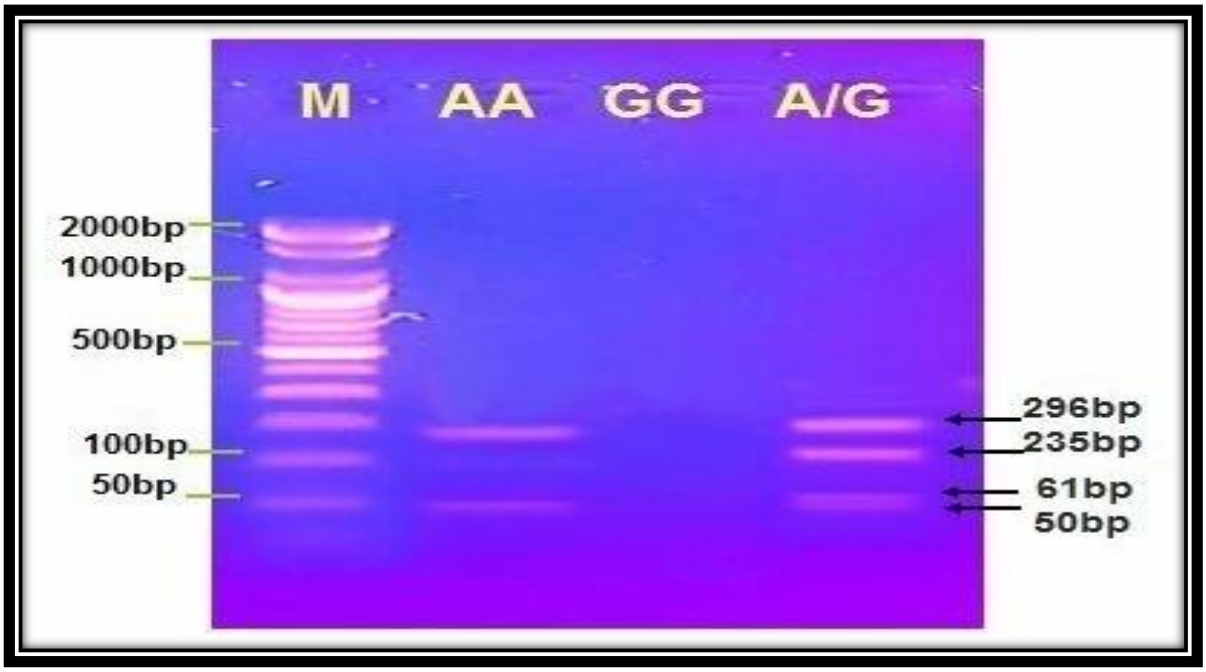
The products of successful binding between the extracted DNA and specific primers for *BRCA-2* gene were detected by gel electrophoresis analysis using DNA marker (100 bp DNA ladder) lane (1-6) patient samples that show 346bp PCR product size, figure (3-12)



Figure(3-12): Agarose gel electrophoresis image that show the PCR product analysis of *BRCA-2*gene from some blood patients samples and healthy control sample. Where M: marker (100-2000 bp), lane (1-4) patient samples that show 346bp PCR product size.

3.6.4 Detection of *BRCA-2* Polymorphism

The distribution of *BRCA-2* polymorphism was detected by RFLP-PCR technique, at this locus there're three genotype; lane (GG) non digested, lane (AA) homozygous at 235bp, 61bp, and 50bp, and lane (G/A) heterozygous at 296bp, 235bp, 61bp, and 50bp, Figure (3-13).



Figure(3-13): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *BRCA-2185delAG* gene polymorphism by using *BspHI* restriction enzyme. Where M: marker (50-2000bp), lane (GG) non digested, lane (AA) homozygous at 235bp, 61bp, and 50bp, and lane (A/G) heterozygous at 296bp, 235bp, 61bp, and 50bp.

In *BRCA-2* Table (3-8) AG genotype has obviously suggested an etiology for tumor, as had an (OR 13.4146) and Etiologic Fraction (EF 0.1851), In contrast, the AA genotype had rather preventive role as it had Protective Fraction (PF) of 0.9103 and low OR (0.0731) . Figure (3-14) show patients have 80% of AA and 20% of AG compared with control show 100% of AA and 0% of AG. Figure (3-15) show patients have 10% of G and 90% of A alleles compared with control they have 99% of A and 1% of G alleles .

Table (3-8): distribution of genotypes and alleles of *BRCA2 gene* in cases & control

BRCA2 gene	Patients N (%)	Control N (%)	OR	95% CI OR	X^2	P (x^2)	EF	PF
***BRCA2* genotype**								
AA	40 (80)	50 (100)	0.0731	0.009-0.5897	11.11	0.001	***	0.9103
GG	0 (0)	0 (0)	***	***	***	***	***	***
AG	10 (20)	0 (0)	13.4146	1.662-108.282	11.10	0.0009	0.1851	***
Total number	50	50						
***BRCA2* Alleles**								
A	90 (90)	99(99)	0.0819	0.010 - 0.647	10.50	0.0012	***	0.9098
G	10 (10)	1 (1)	12.2088	1.546 - 96.430	10.53	0.0010	0.0918	***
Total number	100	100						

❖ **OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, X^2 = chi square**

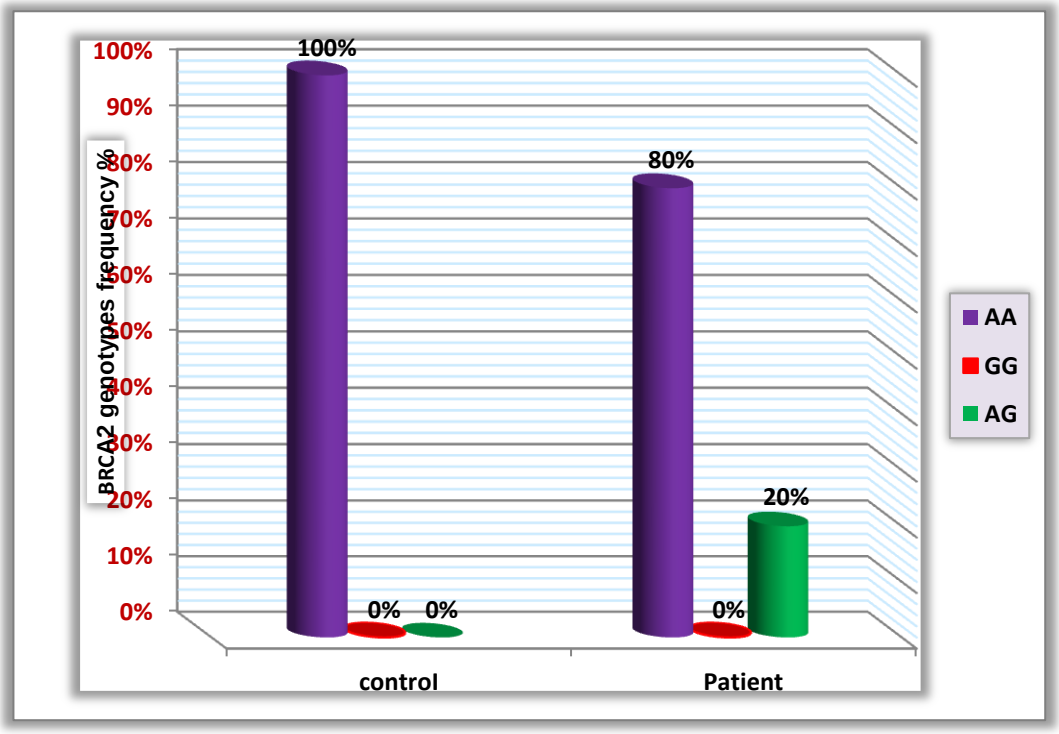


Figure (3-14): Component Bar Chart showing a case-control comparison in relative frequency of the *BRCA-2* genotypes.

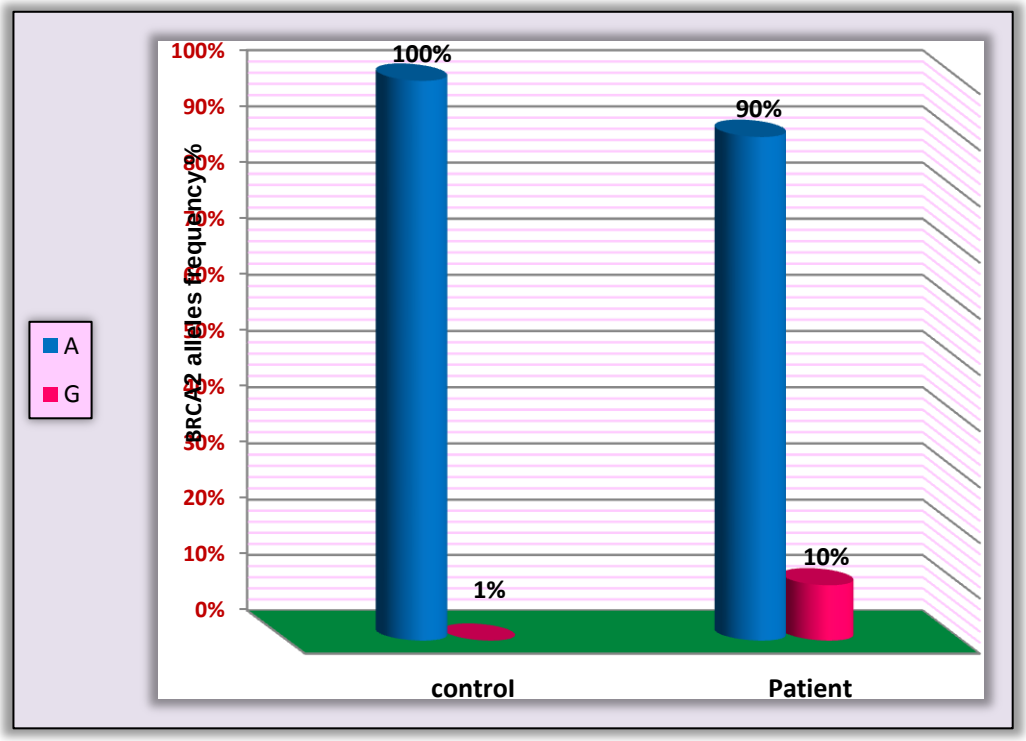
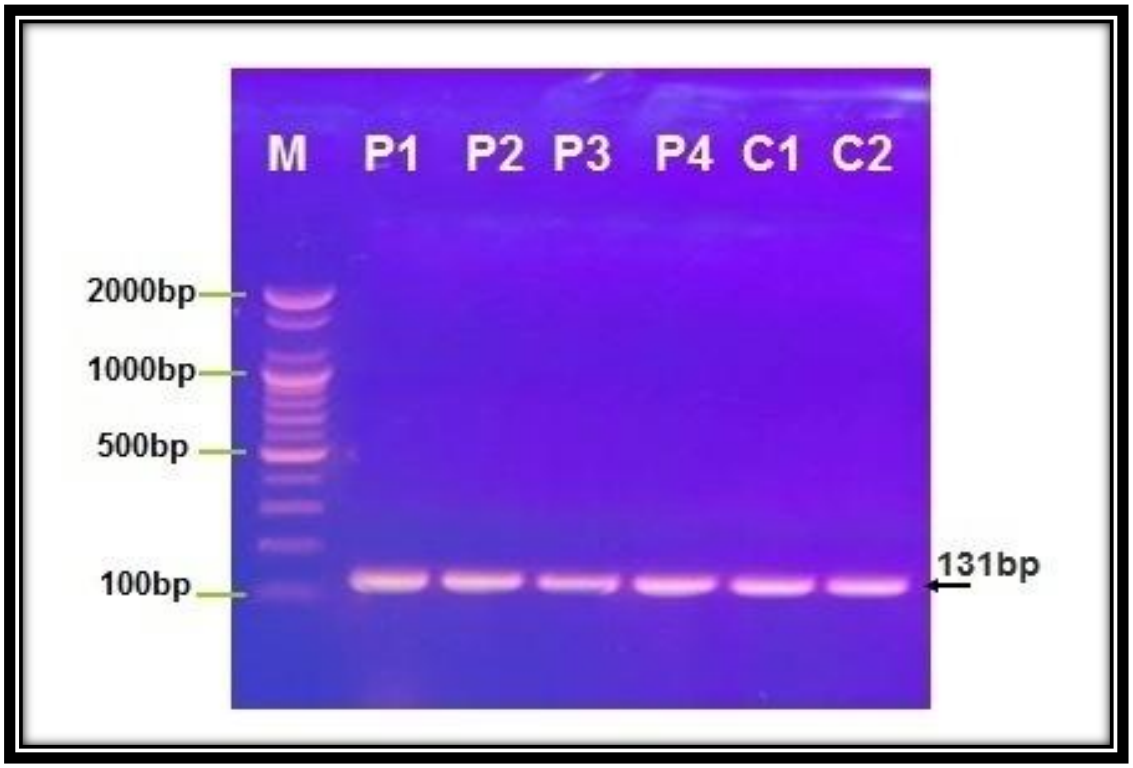


Figure (3-15): Component Bar Chart showing a case-control comparison in .es relative frequency of the *BRCA-2* allel

3.6.5 DNA Amplification of *p53* gene

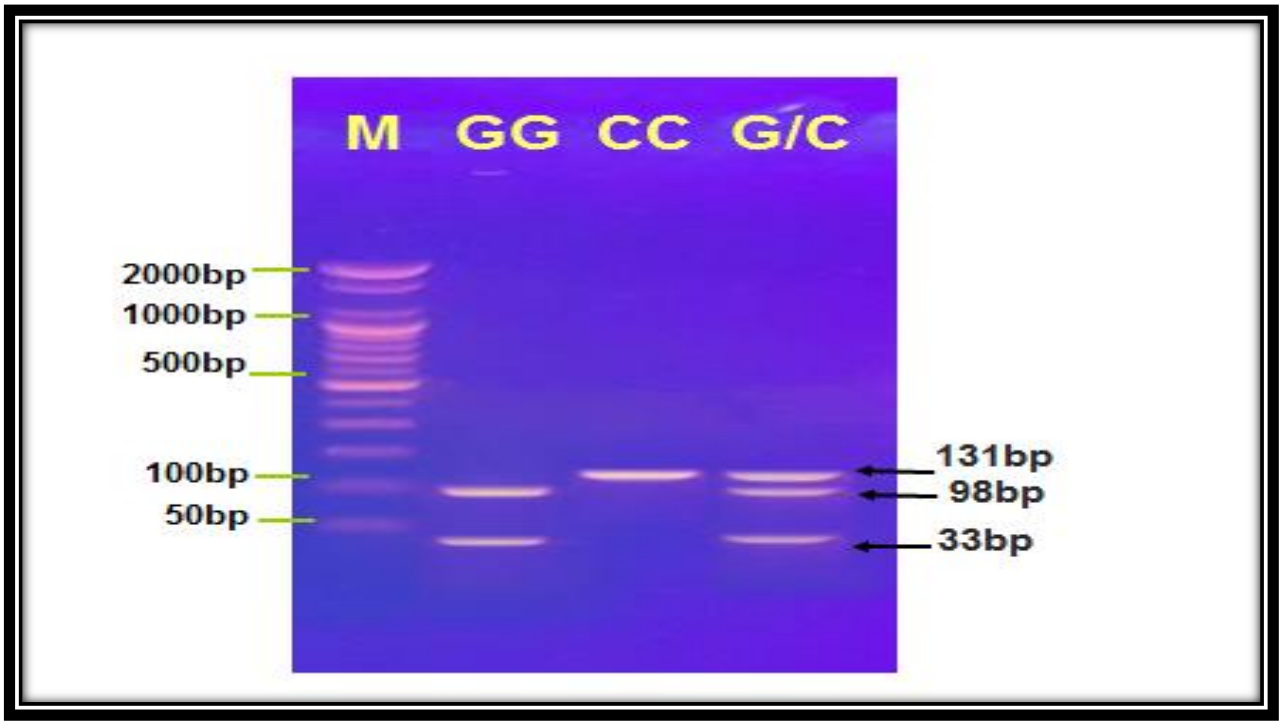
The products of successful binding between the extracted DNA and specific primers for *P53* gene were detected by gel electrophoresis analysis using DNA marker (100 bp DNA ladder) lane (1-6) patients samples that show 131bp PCR product size. Figure (3-16)



Figure(3-16): Agarose gel electrophoresis image that show the PCR product analysis of *p53* gene from some blood patient samples and healthy control sample. Where M: marker (100-2000bp), lane (1-4) patient samples that show 131bp PCR product size.

3.6.6 Detection of *p53* intron 6G13964C Polymorphism

The distribution of *P53* polymorphism was detected by RFLP-PCR technique, at this locus there're three genotype; lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygous at 131bp , and lane (G/C) heterozygous at 33bp, 98bp, and 131bp. Figure (3-17).



Figure(3-17): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *p53* intron 6G13964C gene polymorphism by using *HhaI* restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygous at 131bp, and lane (G/C) heterozygous at 33bp, 98bp, and 131bp.

In *P53* Table(3-9) CC genotype has obviously suggested an etiology for tumor, as had an (OR 1.2941) and Etiologic Fraction (EF 0.091) , In contrast, the GC genotype had rather preventive role as it had Protective Fraction (PF) of 0.087 and low OR (0.4565) . Figure (3-18) show patients have 52% of GG , 40% of CC and 8% of GC compared with control show 50% of GG , 34 % of CC and 16% of GC. Figure (3-19) show patients have 56% of G allele and 44% of C allele compared with control they have 52% of G and 48% of C.

Table (3-9): distribution of genotypes and alleles of *P 53 gene* over cases & control

P 53	Patient	Control	OR	95% CI OR	X^2	P	EF	PF

gene	N (%)	N (%)				(X^2)		
P 53 **genotype**								
GG	26 (52)	25 (50)	1	0.495 - 2.374	0.040	0.892	0	0
CC	20 (40)	17 (34)	1.2941	0.573 - 2.921	0.057	0.811	0.091	***
GC	4 (8)	8 (16)	0.4565	0.128 - 1.627	1.515	0.218	***	0.087
Total number	50	50						
P 53 **Alleles**								
G	56 (56)	58 (58)	0.9216	0.526 - 1.614	0.080	0.78	***	0.0455
C	44 (44)	42 (42)	1.0850	0.6198 -1.8996	0.082	0.775	0.0345	***
Total number	100	100						

OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, X^2 = chi square

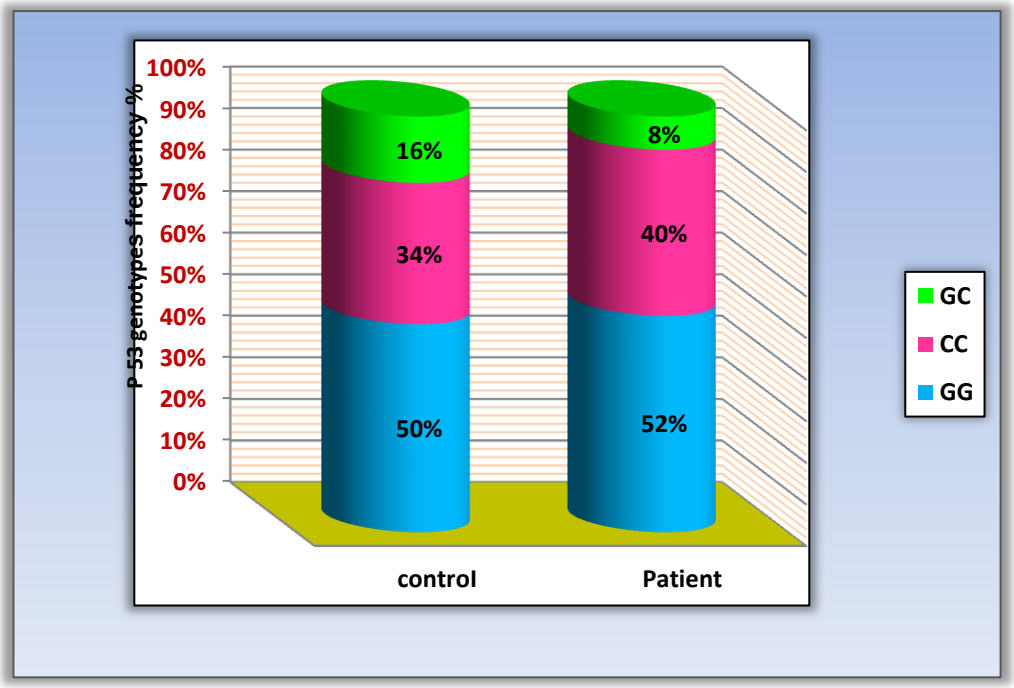


Figure (3-18): Component Bar Chart showing a case-control comparison in relative frequency of the *P 53* genotypes.

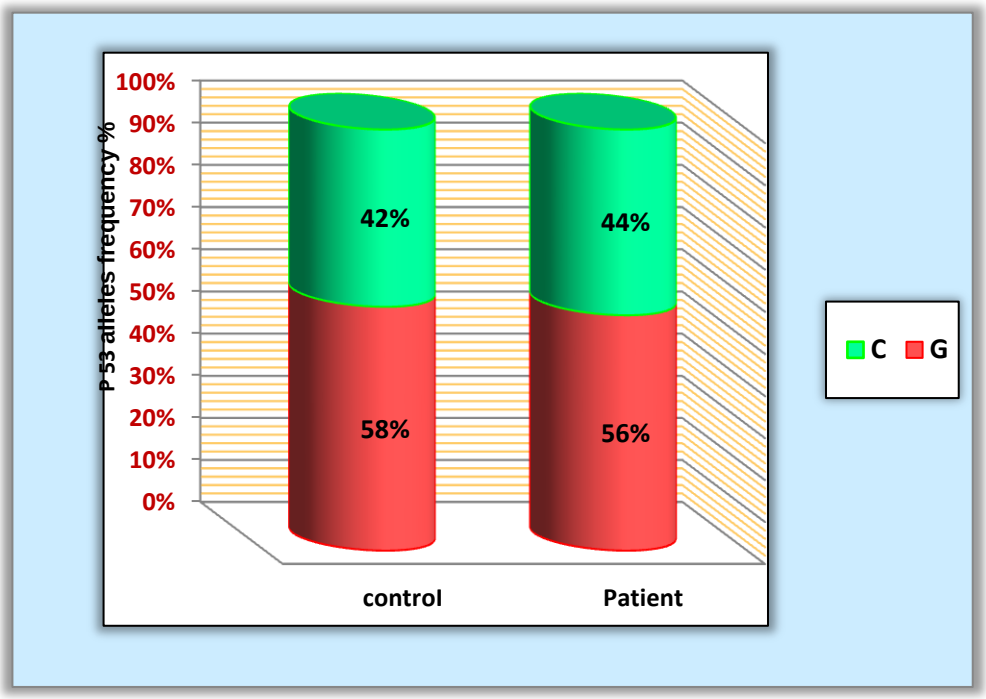


Figure (3-19): Component Bar Chart showing a case-control comparison in relative frequency of the *P 53* Alleles.

3.7Correlation Between Genes & Family History of Patients

The results of present study referred to 22 females (44%) of patients group have positive family history (First degree) , while 28 females(56%) have negative family history. Table (3-10) show significant correlation between *BRCA-1* genotype and family history (P< 0.0085) and that appear more clearly in GG genotype that have 4(100%) in patients compared (0%) in negative family history as in figure(3-20).*BRCA-2* genotype also have significant correlation with positive history family (p<0.0027) and AG genotype in patients with family history have highest frequency 90% in compared in negative history family patients 10% as in figure (3-21).*P53* genotype show significant association

with family history (P< 0.0001) and GC genotype show highest (100% compared with negative family history 0% as a figure (3-22).

Table (3-10): Association between genetic markers & history family of tumor patients

Genotypes	Case (breast cancer)				
	Positive family history	Negative family history	Df	X^2	*P* - Value
	N (%)	N (%)			
***BRCA1* genotypes**			2	9.530	0.0085
AA (N = 38)	13 (34)	25 (66)			
GG (N = 4)	4 (100)	0 (0)			
AG (N = 8)	6 (75)	2 (25)			
Total number	22	28			
***BRCA2* genotype**			2	11.823	0.0027
AA (N = 40)	12 (30)	28 (70)			
GG (N = 0)	0 (0)	0 (0)			
AG (N = 10)	9 (90)	1 (10)			
Total number	22	28			
***P 53* genotype**			2	19.218	0.0001
GG (N = 26)	4 (15)	22 (85)			
CC (N = 20)	14 (70)	6 (30)			

GC (N = 4)	4 (100)	0 (0)			
Total number	22	28			

❖ N = number, *X2* = chi square, Df = degree of freedom

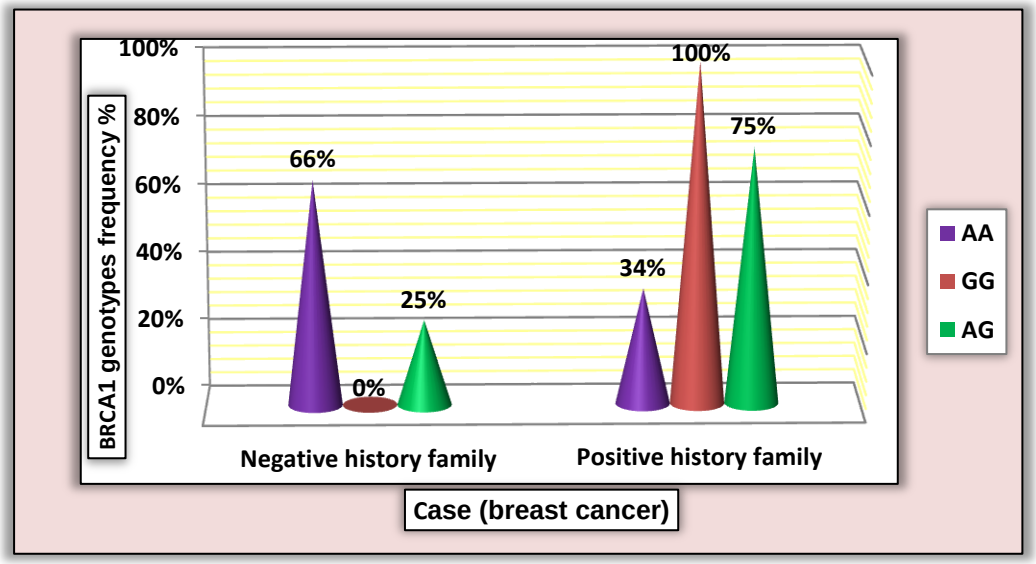


Figure (3-20):Association between *BRCA1* genotypes & history family of tumor patients.

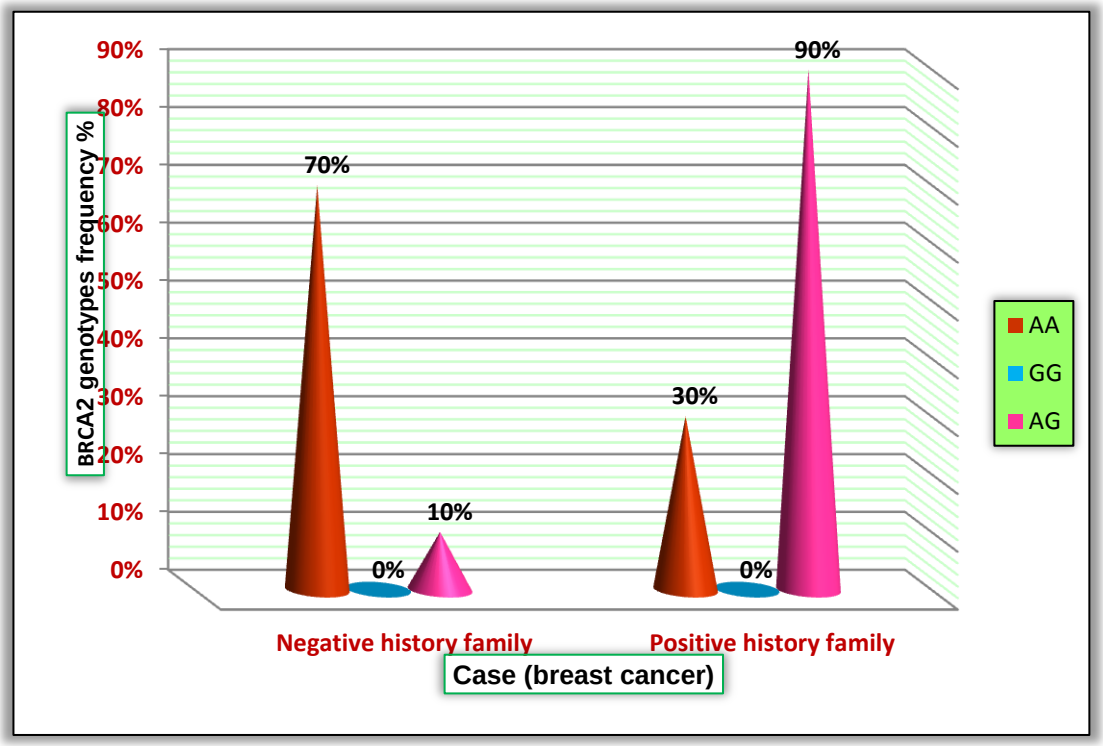


Figure (3-21):Association between *BRCA2* genotypes & family history among patients.

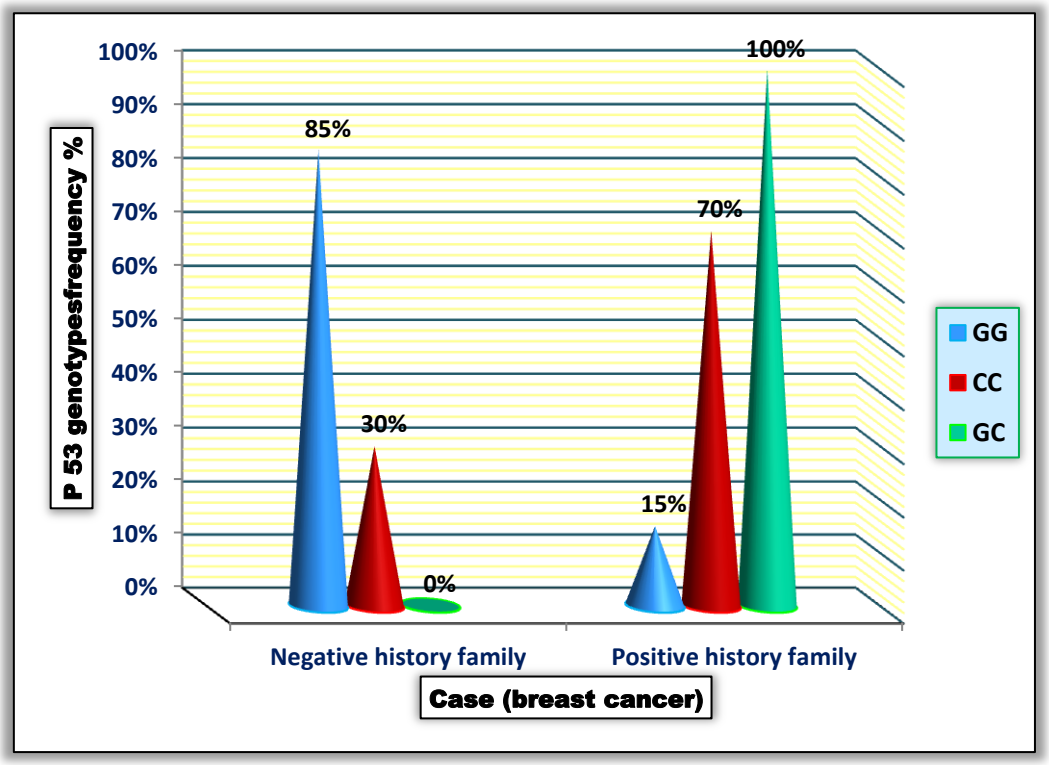


Figure (3-22):Association between *P 53* genotypes & family history among patients.

3.8 Correlation Between Genetic and Tumor Markers Of Patients

Table (3-11) show no significant between *BRCA -1* genotype and CEA levels in patients (P < 0.838) , mean 10.974 for AA, 13.8 for GG and 10.475 for AG , and no significant association between *BRCA -1* and CA15.3 in patients (p< 0.896) , mean 19.626 for AA , 17.55 for GG and 21.2 for AG .

Table(3-11): The relation between tumor markers and *BRCA-1*Gene among Cases with breast cancer.

Tumor markers	*BRCA1* Gene (N = 50)			X^2	Df	P - Value
	AA	GG	AG			
CEA (ng/ml)				0.353	2	0.838 (NS)
Range	2.9 – 20.1	10.1 –17.5	1.9 – 16. 9			

Mean	**10.974**	**13.8**	**10.475**			
SD	**5.308**	**4.272**	**5.795**			
SE	**0.861**	**2.136**	**2.049**			
N	**38**	**4**	**8**			
CA 15-3 (ng/ml)				**0.220**	**2**	**0.896 (NS)**
Range	**5.3 – 34.2**	**9.7 – 25.4**	**11.2 – 30.1**			
Mean	**19.626**	**17.55**	**21.2**			
SD	**8.222**	**9.064**	**7.422**			
SE	**1.334**	**4.532**	**2.624**			
N	**38**	**4**	**8**			

- **Ns = Not Significant (p > 0.05), SD= Standard Deviation, SE= Standard Error, N= Number, Df = degree of freedom**

Table (3-12) show no significant association between *BRCA -2* genotype and CEA in patients (P < 0.595) , mean 10.77 for AA and 8.76 for AG. No significant between BRCA -2 and CA15.3 in patients (p< 0.157) , mean 21.83 for AA and 15.5 for AG.

Table(3-12): The relation between tumor markers and *BRCA-2* Gene among Cases with breast cancer.

Tumor markers	***BRCA2* Gene (N = 50)**		X^2	**Df**	***P* - Value**
	AA	**AG**			
CEA (ng/ml)			**0.282**	**1**	**0.595 (NS)**
Range	**1.9 – 20.1**	**1.9 – 17.5**			
Mean	**10.77**	**8.76**			
SD	**5.328**	**5.993**			
SE	**0.842**	**1.895**			
N	**40**	**10**			
CA 15-3 (u/ml)			**2.008**	**1**	**0.157 (NS)**
Range	**5.3 34.2**	**8.4 - 29**			
Mean	**21.83**	**15.5**			

SD	**7.338**	**8.144**			
SE	**1.16**	**2.575**			
N	**40**	**10**			

❖ **Ns = Not Significant (p > 0.05), SD= Standard Deviation, SE= Standard Error, N= Number, Df = degree of freedom**

Table (3-13) shows no significant association between *P35* genotype and CEA in patients (P < 0.750) , mean 9.915 for GG , 12.67 for CC and 11.2 for GC. No significant between *P35* and CA15.3 in patients (p<0.619) , mean 20.762 for GG, 17.44 for CC and 24.25 for GC.

Table(3-13): The relation between tumor markers and *P53* Gene among Cases with breast cancer.

Tumor markers	***P 53* Gene**			**X^2**	**Df**	***P* - Value**
	GG	**CC**	**GC**			
CEA (ng/ml)				**0.575**	**2**	**0.750 (NS)**
Range	**2.9 - 19.1**	**1.9 - 20.1**	**5.5 – 16.9**			
Mean	**9.915**	**12.67**	**11.2**			
SD	**4.219**	**6.088**	**6.582**			
SE	**0.827**	**1.36**	**3.29**			
N	**26**	**20**	**4**			
CA 15-3 (u/ml)				**0.961**	**2**	**0.619 (NS)**
Range	**5.3 – 34.2**	**7.7 – 27.2**	**18.4 – 30.1**			
Mean	**20.762**	**17.44**	**24.25**			
SD	**8.66**	**7.044**	**6.755**			
SE	**1.70**	**1.575**	**3.377**			
N	**26**	**20**	**4**			

❖ **Ns = Not Significant (p > 0.05), SD= Standard Deviation, SE= Standard Error, N= Number,**

Df = degree of freedom

4. Discussion

4.1. Demographic characteristics

4.1.1. Age groups

The age characteristic of breast cancer patients, present study showed that the highest frequency of breast cancer disease among (40-50) years old (40%), followed by the age group of (30-39) years old (20%) , and the less frequency in the age (19-29) years (10%) , which has no significant association as compared with control group ($p > 0.05$) mean 46.38 years (SD14.34) , so breast cancer is a disease of all ages, considering the entire lifespan (United Kingdom Office for National Statistics ,2013).

In a previous study of Dodova *et al* .,(2015) which included 200 Bulgarian females with breast cancer (post operative and the age ranged from 25 to74 years) selected by the established genetic testing criteria ,the mean age of the patients at diagnosis was 49.5 years , and no significant association between patients group and control ($p >0.05$). Moreover, our findings are comparable with a study conducted an average 12% of women worldwide related breast cancer, their ages ranged between <40 - >70 years and showed 48.5 years mean of patients ages (Andrew *et al*., 2015). Other studies documented an age mean 50.3 years (Joyce *et al*., 2014). So this results that is consistence with Barthelemy *et al*., (2011) who found the mean age of breast cancer patients 45.1 years , and no significant association with control ($P = 0.903$) , another study performed by Han and Kang, (2015) stated in their study a mean age of 44.7 years of patients with breast cancer which was not different from control group ($p=0.19$) , and a similar findings was reported by Partridge *et al.*, (2013) who found 42.95 years as a mean age of breast cancer patients.

4.2The Association Between Breast Cancer And Selected Tumor Markers

As mentioned previously ,the tumor markers are tools for monitoring and for prognosis rather than for diagnosis (Harris et al.,2007). Moreover different results may be noted review different , ethnic, geographic areas regarding the

occurrence of these markers among breast cancer patients. In the present study measured two tumor markers are (CA15-3) and (CEA) on 50 females with breast cancer as a patients group and 50 healthy females as a control group, the results show a significant association between serum level of CA15-3 and breast cancer disease ($p<0.05$) , Patients have high level of CA 15-3 (mean 18.396) and this result considered as a high ratio in compare with levels of control group (mean 8.136) , compared with other studies which referred to either similar or different results, for example ; the study of Maric *et al*.,(2011) who found significant association of 150 breast cancer patients (mean 16.94) in compared with for control group (mean 7.21), so the results in present results matched with results stated by Nicolini *et al*.,(2003) who found serum CA15-3 of 100 females with breast cancer have significant association with 100 females in control group ($p<0.0001$).

Results of present study showed to presence a significant association between elevated levels of CEA and breast cancer disease ($p<0.05$) , breast cancer patients have a elevated level of CEA (mean 11.66) in compare with control group (mean 5.086), this results accepted with De Santis *et al*.,(2013) who referred to a significantly association in levels of CEA between breast cancer patients group and controls group ($p<0.05$) , and so who found elevated level of CEA in 140 post-operative breast cancer as a patients group whom visited most China hospitals (mean 13.54) compared with 280 females as a control group (mean 3.87) , So other results agreed with what we found in present study for example the mentions of Lee *et al*.,(2013) and Wu *et al*.,(2014) whose did a comparison between post-operative breast cancer females as a patients group and healthy females as a control group, and who found the levels of CEA in patients serum elevated to highest levels (means 16.43 and 19.08 respectively) compared with controls (mean 4.03 and 6.91 respectively) , and appeared a significant association between both groups ($p<0.05$).

The utility of these serum biomarkers may be served as effective prognostic indicators for post-operative breast cancer patients, further researches are needed

to determine the effectiveness of these serum biomarkers in formulating treatment strategies in clinical practice, therefore, CEA levels greater than 7.5 μg/L are associated with high probability of subclinical metastases (Molina *et al*.,2010) .Prognosis of patients whose CEA level was within the normal range at the time of diagnosis is significantly better than those with elevated CEA levels (Uehara *et al*.,2008). baseline CA 15-3 might be valuate in the identification of higher risk of relapse, where adjuvant chemotherapy must be introduced. In other hand there is a study referred to presence of an abnormal CA 15-3 pre-surgical value is associated with an increased risk of recurrence and death (Sandri *et al*.,2012).

CA-15.3 and CEA tumor markers have a low sensitivity and due to this feature , they cannot be recommended for screening or early diagnosis, but serial levels may be useful in the early diagnosis of distant metastases , European Group on tumor markers has recommended the CEA and CA15-3 levels be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer markers should be measured prior to every chemotherapy course and at least every 3 months for patients receiving hormone therapy (Moline *et al*.,2012). The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) guidelines do not currently recommend the use of serum CA 15–3 and CEA for breast cancer screening and directing treatment (Harris *et al*.,2007). On the one hand, this may partly due to the conflicting conclusions of different researches (Lee *et al*.,2013; Wu *et al*.,2014 ; Maric *et al*.,2011). CA 15-3 increase of 5-10 times above normal upper limit can predicts breast cancer, however, a low value cannot exclude metastasis making , CA 15-3 more of prognostic rather than diagnostic marker (Kumar *et al*.,2012 ; Yerushalmi *et al*.,2011)

The incidence of breast cancer has been steadily increasing in the last two decades, however, due to the early detection and increased use of more effective systemic therapy, the survival rates of breast cancer have improved in recent years, and early breast cancer accounted for a large proportion. Previous researches demonstrated that the CEA and CA15-3 levels are associated with

tumor burden indicators including tumor size and lymph node status and patients with locally advanced breast cancer exhibit significantly higher levels of CEA and CA-15.3 (Hashim , 2014; Ali *et al*.,2013; Verring *et al*.,2011).

The sensitivity of tumor markers is significantly higher in patients with advanced disease , and is related to the site of recurrence (Molina, 2012). CA 15.3 and CEA are not useful in the early diagnosis of loco regional recurrence, for which clinical examination is superior. However, abnormal CEA and CA 15.3 levels are founding 40–50 and 50–70% of patients with distant metastases respectively (Jager *et al*.,2013). Since elevated levels of CA-15.3 and CEA are related to the tumor burden and higher levels may indicate an increased likelihood of systemic metastases. Studies by Lee *et al*., (2013)showed that elevated tumor marker levels are more frequently observed in metastatic breast cancer patients than in primary breast cancer, and patients who had elevated tumor marker levels before surgery also showed more frequent elevation at recurrence. Since markers are relatively easy and inexpensive to measure, regular measurement of serum tumor marker levels could provide useful information for earlier detection of recurrence (Di Gioia *et al*.,2011).

4.3Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In present study attempt has been done to find out an possible correlation between CA-15.3 and CEA tumor markers among Iraqi CA breast patients , as they both elevated in the course of pre- and post-operative circumstance. Although weak correlation has reveled in this study (R=0.2432), it failed with gain statistical signification .This result is concordance with Mousavi *et al*.,(2011) who study on 70 Iranian women who observed a significant increased value of CA15-3 and CEA in patients group of breast cancer females as compared with healthy control females and no significant association between both biomarkers among patients group (p= 0.112).

A moderate correlation of (R=0.57) it was found in the study of Molina *et al*.,(2013) who studied on 150 females with breast cancers who visited Ramses

Medical Center in Narew . The results in present study supported by many other studies from different regional area for example the study of Yerushalmi *et al.*, (2012) who showed did not identify significant differences in CA 15-3 and CEA levels between different sites of metastasis in patients with breast cancer.

CA 15-3 in combination with CEA is also relevant tumor markers in breast cancer , and the serum level of marker CA 15-3 has superior prognostic relevance in relation to CEA, but unlike these authors, Ebsani *et al*., (2012) reported the prognostic value of CEA is higher than that of CA 15-3, which demonstrated that this marker has conflicting implications in breast carcinogenesis. Measurement of tumor markers is a tool for detection of distant metastases, and the marker CA 15-3 seems more efficient when compared to CEA and no linear relation between it . Monitoring of breast cancer patients after surgical treatment using only this tumor markers is insufficient, however, simultaneous use of both serum markers (CA 15-3 and CEA) allows the early diagnosis of metastasis in up to 60–80% of patients with breast cancer (Mendes et al.,2010 ; Bruna *et al*.,(2014). Elevated serum CA 15-3 and CEA levels at recurrence suggest increased tumor burden and may be prognostic for survival for metastatic breast cancer patients (Lee *et al*.,2013).

4.4Family History and Tumor Markers

In the context of the family history ,in present study the results showed that the presence of positive family history is an important contributory factor in breast cancer disease ,our results show a significant association between breast cancer and family history ($P< 0.0001$) in 50 females with breast cancer as a patients group, our results show to 22 (44%) of patients have positive family history of breast cancer and 28 (56%) of patients have negative family history in any degree ($P > 0.05$). These results were very similar to a study conducted by Rawaa,(2014) who reported a positive family history of breast cancer accounted for 43.3% in malignant cases ($P = 0.047$) . A comparable results was reported by (Work *et al.*, 2011) who stated a significant correlation of malignant breast tumor

with positive family history. Silvera *et al*.,(2012) reported 46.7% of breast cancer patients have a positive family history . Yamashita *et al.,* (2011) reported that 41.1% of patients with breast cancer have a positive family history of similar condition.

Family history is an important risk factor for breast cancer (Hulka &Moorman ,2001). Familial aggregation can be attributed both to shared genes and to shared physical environments and lifestyles, and it has been demonstrated that the risk of developing breast cancer is twice as high in women who have an affected first-degree relative than women in the general population (Schwartz *et al*., 2008). The majority of the genetic risk is due to low-risk or moderate-risk susceptibility alleles each of which confers only a very small increased risk in isolation, but in combination may have quite a significant effect (Murray and Davies, 2013).

The family history was found in addition with its importance on the disease frequency to have an effect on the final out-com of tumor markers among patients . Both were elevated in patients who belonged to families who suffered from breast cancer than patients didn't . The CA-15.3 and CEA were significantly differed among the two sub-groups of patients ($P<0.05$) . A comparable study was of Tomlinson *et al*.,(1995) who tested CA15.3 and CEA for 80 females with breast cancer and positive family history , are found (mean 28.11 and 17.32 respectively) compared with 160 females with breast cancer and negative family history and found (mean 10.273 and 5.711 respectively). Moreover, the study of Loomer *et al*.,(1991) and Barak *et al*.,(2000) they found mean of CEA and CA15.3 (30.12 and 19.23 respectively) at significantly association ($p<0.05$).

It is well-known that the age is among other factors that influence immunological status in general childhood and elderly who are more prone for tumors and other diseases. The results of present study regarding this context (table 4-6 and figure 4-7) told non significant effect for this factor on the tumor markers level. However, the age group of 30-39 recorded the highest levels of the two tumor markers studied. This , in our opinion , may be attributed to the small

size sample of the study. However, previous studies were in concordance with present study as Donepudi *et al*.,(2014) who found no significant association between age groups and levels of CA-15.3 and CEA ($p>0.05$) , R=0.31 and 0.15 respectively, Balch *et al*.,(2009) who study on 150 females with breast cancer in (20-90 years) and found no significant association between age and tumor markers levels (CEA and CA.15.3) in patients serums and age group ($P>0.05$) and So Duffy *et al*.,(2000) did agree the present findings.

Generally ,most studies showed no associations between age and tumor markers in breast cancer and other cancers, because the presence of high or low levels of tumor markers dependent on presence of metastasis , degree and organs which have metastasis (Harris et al.,2007).

4.5 Molecular study

4.5.1Detection of genes Polymorphism

Approximately 80% of the cases related to familial breast cancer are associated with one gene of hereditary susceptibility for breast and ovarian cancer, *BRCA1* and *BRCA2*. The *BRCA* genes have been classified as tumor-suppressor genes, because the loss of wild-type allele has been observed, currently the major genes known to influence breast cancer risk is *BRCA1* and *BRCA2* , these genes are tumor suppressor genes responsible for DNA damage repair and mutations in these genes result in a significantly increased risk of breast cancer, it is estimated that up 16% of all familial breast cancers are due to mutations in these genes and up to 5% of all breast cancer cases (Campeau *et al*., 2008), screening for *BRCA* gene mutations in high-risk patients has become a priority and scoring systems such as the Manchester scoring system provides a means to identify which patients need increased surveillance from scoring systems like this, genetic testing guidelines have recently been introduced for higher-risk patients (Evans et al.,2005).

The *BRCA-1* , *BRCA-2* and *P53* genotypes were assessed for their roles in predicting the risk of having breast cancer, each compared of control group (general population without family history for breast cancer in any degree). Present results showed *BRCA-1* genotypes, had significant predictive power, the G allele had the strongest association and significantly increases the risk of having breast cancer disease compared to general population control, to a lesser degree the A allele had a statistically significant protective, the homozygous GG genotype increase the risk of the disease EF=0.065, while the wild AA genotype showed a statistically significant protective effect 76% (PF=0.1667) . So the heterozygous AG genotype showed a statistically significant protective effect 16%(PF=0.047), compared with control group they have (0% GG, 80% AA and 20% AG) , compared with other studies which referred to similarity with results of present study , a supportive conclusion may be depicted, for example ; the study of Salma, (2015) ,(310) patients with breast cancers were recruited from different public and private hospitals of Bangladesh and as controls (250) Bangladeshi women , and found GG genotype increase the risk of malignant tumor in breast, Haytural *et al*., (2013) , have investigated 106 consecutive breast cancer patients , they found that GG responsible for risk to breast cancer . Other similar findings, the studies of Hansa *et al*., (2012); Chakraborty *et al*., (2013) they referred to G allele they have strongest association and significantly increases the risk of having breast cancer in GG genotype.

The *BRCA-2* genotypes, had significant predictive power in results of present study . The G allele had the strongest association and significantly increases the risk of having breast cancer (EF=0.0918) compared with control group . To a lesser degree the A allele had significant protective role (PF= 0.9098) . The heterozygous AG genotype increase the risk of the disease by (EF=0.1851). While the wild AA genotype showed a statistically significant protective effect (PF=0.9103) . It may be generally concluded that *BRCA-2* genotypes have higher predictive values as do *BRCA-1*. Many comparable findings support this conclusion Haytural *et al*., (2013) , have investigated *BRCA-*

2 polymorphism in 106 Turkish patients with breast cancer and they stated that AG genotype increase the risk for breast malignancies (EF=0.203) , Pilato *et al*.,(2011) they showed to (EF= 0.154) , So Rahim & Selvam, (2014) , they found AG increase risk of malignant of breast (EF= 0.106).

The *P53*, which is tumor suppressor gene , creating a protein that repairs DNA and prevents carcinogenesis. Every cell in mutation carriers has been demonstrated to lack one functional allele (i.e. the tumor-suppressor function of that gene is lost); a situation that favors cancer development , so *P53* is a tumor suppressor gene that is mutated or changed in more than 50 percent of tumors. Studying *p53* as a tumor marker helped researchers understand how tumors form, but measuring *p53* levels in cancer patients has not been shown to predict differences in survival or quality of life. *p53* was indicated as responsible for tamoxifen resistance in breast cancer suggesting that it can interfere in treatment response(Blanco *et al.*, 2010). The protein 53 (*p53*) tumor suppressor gene is the most involved genetic factor for breast cancer ,mutations in the *p53* gene are associated with more than 50% of human cancers, Breast cancer is one of the most common cancers in Iranian women. The p53gene plays a principal role in genomic stability, and its function varies according to polymorphism (Faghani *et al*.,2011).

The *p53* genotypes, and it's predictive power, regarding the C allele had the strongest association and significantly increases the risk of having breast cancer (EF=0.0345) compared with control group , to a lesser degree the G allele had significant protective role by (PF= 0.0455). The homozygous CC genotype increase the risk of the disease by (EF=0.091) , and the heterozygous GC genotype showed a statistically significant protective effect (PF=0.087). while wild type GG genotype don't have any role in increasing risk or protective effect .The previous study of Zhang *et al*., (2010) and their result referred CC genotype increase risk for breast , while GC have protective effect. In the study of Bisof *et al*.,(2010) Tunisian women , they found to increasing the risk of disease by CC

and presence of protection role for GC genotype.

4.6 Correlation Between Genes and Family History of Patients

In family history field of patients group in our present study , the positive family history of breast cancer accounted for (44%), while (56%) appeared negative family history , it seems to be in consistence with study of Jobsen *et al*.,2015, who show 42% of breast cancer patients have first degree of family history (mothers and sisters), and 58% have negative family history in any degrees, so agreed with Malone *et al*.,(2011) who studied on women with breast cancer was ascertained through the Cancer Surveillance System (CSS) of Western Washington, a participant in the SEER Cancer Registry Program of the National Cancer Institute, and found 40% of patients have positive first degree family history, and 60% with negative history family.

Other hand, study of mutations in predisposing genes (*BRCA-1*, *BRCA-2* and *P53*) by study genetic polymorphisms of these genes among patients group (positive and negative family history) , our findings show significant correlation between *BRCA-1* genotype and family history (P< 0.0085) and the mutation in *BRCA-1* gene appear more clearly in GG genotype that have (100%) in patient compared 0 (0%) in negative history family. This results were agreed with Pavel *et al*.,(2016) their result showed to 90% of patient with positive history family have mutation in *BRCA-1*gene, So agreed with Ibrahim *et al*.,(2010) in their study was applied on Egyptian breast cancer patients with first degree relatives had similar findings . Cherbal *et al*.,(2012) who studied on Anti Cancer Center of Blida, the Central Hospital of Algiers, and five private medical clinics which provide oncology services throughout Algeria patients with breast cancer and who have positive family history,these two studies showed mutation in *BRCA-1* gene belong to this group of patients .

In *BRCA-2* genotype also have significant correlation with positive family history patients group (p<0.0027) and the mutation in *BRCA-2* appeared in AG

genotype in patients have highest frequency (90%) in compared in negative patients 10%. The previous study of Yiannakopoulou,(2013) has similar findings, he show recessive *BRCA-2* gene mutations associated with horizontal inheritance patterns (sister-sister), and polygenic inheritance where susceptibility to familial breast cancer is thought to be conferred by a large number of low risk alleles.

The *P53* genotype show significant with positive family history (P<0.0001) and the mutation in *p53* appeared in GC genotype show highest (100%) compared with negative family history 0%. The present results very similar with Hussani *et al*.,(2014) , genotyping on their study was performed for 288 breast cancer Pakistani women and 188 controls , and their results showed to differ percentages and significant association between *P53* mutation polymorphisms of positive family history patients compared with negative family history patients with breast cancer.

Cloning of the *BRCA*1 and *BRCA*2 genes, the major genes known to confer high risk of breast and ovarian cancer, has resulted in the characterization of a large number of mutations in both genes for high-risk mutations, at least four germ line mutations that predispose to breast cancer have been identified or localized. These include mutations in the genes *BRCA1*, *BRCA2, P53* and *PTEN*, which are tumor suppressor genes, creating a protein that repairs DNA and prevents carcinogenesis. Every cell in mutation carriers has been demonstrated to lack one functional allele (i.e. the tumor-suppressor function of that gene is lost); a situation that favors cancer development (Blanco *et al.,* 2010).

Approximately 5–10% of all newly diagnosed breast cancers in Western nations are hereditary, attributable primarily to hereditary mutations in the *BRCA1* and *BRCA2* (*BRCA1/2*) genes approximately 15% of breast cancers arise in women with a history of the disease in first-degree relatives (i.e., mothers, sisters, or daughters) ,and approximately 5–10% of breast cancers may be directly

attributable to heredity (Chen *et al*.,2007). While the heredity of breast cancer susceptibility is not fully understood, it is assumed that the majority of familial breast cancers are attributable to a small number of high penetrance susceptibility genes. To date, two breast cancer susceptibility genes have been well described: *BRCA1* and *BRCA2* (Gerdes *et al* .,2006). Familial breast cancers in general, and *BRCA-1*associated breast cancers in particular, are characterized by an epidemiologic, phenotypic, and clinic profile that distinguishes them from sporadic breast tumors; (Diez *et al*.,2003).

The *P53* mutation is not the relevant source of breast cancer susceptibility in history family, but this would need to be confirmed by testing the germline DNA, which was unavailable, germline mutations within evolutionary *p53* gene predispose to tumor development in several familial cancer syndromes, mutations in families with early-onset hereditary breast and breast-ovarian cancers not linked to the *BRCA1* gene on chromosome 17q. We propose that the term *p53* familial cancer syndrome' be applied to clusters of tumors in families with documented germline *p53* mutations, regardless of the histopathology findings or pattern of tumor development (Blanco *et al.*, 2010).

4.7Correlation Between Genetic and Tumor Markers Of Patients

In other side ,the relationship between presence or absence mutations in genetic markers *(BRCA-1, BRCA2* and *P53* genotypes) in breast cancer patients group and elevation of tumor markers (CA-15.3 and CEA) levels in serums of same patients group , have been also detected in this study, Neither , present results show no significant association between *BRCA-1* mutation genotype and elevation of CEA and CA-15.3 levels in patients serum ($P<0.838$ and $P<0.896$ respectively). The same findings found regarding *BRCA-2* mutation genotype and elevation of CEA and CA-15.3 levels in serum of patients ($P<0.595$ and $P<0.157$ respectively. Moreover , the results of present study show

no significant between *P35* mutation genotype and levels of tumor markers in among present study (CEA and CA-15.3) in serums of patients with breast cancer group (P < 0.750 and p< 0.619 respectively) .

Conclusions

Based on the findings of present study, it is possible to reach the following

1. The present study confirmed the independent prognostic value of elevated serum CEA and CA 15–3 levels for breast cancer, when considering one or the combination of both markers. Elevated post-operative serum tumor markers could be useful in determining the risk of recurrence and metastasis of breast cancer after operation.
2. Tumor markers, when well defined, can play a significant role in prediction and prognosis for breast cancer patients. Because of the abundance of poorly designed tumor marker studies to date, however, very few markers have been accepted for routine use.
3. Patients how have a history family considered a risk for breast cancer disease because presence of mutations in *BRCA*-1 and *BRCA-2* genes.

Recommendations

1- These recommendations would be better evaluated with larger numbers of breast cancer patients and controls by Increasing the sample size of

patients and controls , and interpreting the results in the light of histopathology types, grade and stage of tumor.

2- Studying demographic parameters and including family history in all relative degrees of breast cancer patients.
3- Genotyping study for CA 15.3 and CEA coding genes .
4- Use of tumor markers as a routine tests in breast cancer have been conservative.
5- Work on making genotyping for *BRCA* genes as a routine tests for breast cancer checking.
6- Make inclusive study for breast cancer patients in pre and post- operative stages.

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Index 1: Date Collection Form

1.2 Name

1.2 Father’s Name

1.3 Marital Status

Married		Un married	

1.4Age (yr)

1.5 Permanent address

1.6 Telephone No.

1.7 Family History of breast or ovarian cancer

Yes		No	

1.8 Date of mastectomy operation

D	M	Y

Index 2: The Result of tumor markers

patient	CEA	CA-15.3	Control	CEA	CA-15.3

1	7.7	20.3	1	4.3	11.2
2	14.2	26.9	2	2.3	3.9
3	16.9	30.1	3	1.6	26
4	19.1	18.2	4	12.2	20.1
5	11.2	11.3	5	1.2	10.2
6	5.5	18.4	6	7	7.5
7	3.2	8.4	7	5.5	1.9
8	18	7.7	8	3.3	0.6
9	11	27.2	9	15.3	0.9
10	10.1	25.4	10	11.1	6.4
11	1.9	19.2	11	10.1	3.5
12	9.3	5.3	12	6.6	2.2
13	17.5	9.7	13	5.2	2.1
14	20.1	11.8	14	2.3	19.1
15	13.2	21	15	1.9	11.2
16	12	24.3	16	0.6	5.5
17	10.1	29	17	0.9	3.2
18	11.4	22.2	18	6.4	18
19	18.2	23.1	19	3.5	11
20	11.1	11.2	20	2.2	10.1
21	7.5	17.4	21	2.1	11.4
22	3.2	15.3	22	3	18.2
23	2.9	33.1	23	3.5	11.1
24	13.8	22.1	24	4.7	7.5
25	8.9	34.2	25	5.9	3.2
26	17	39.5	26	9	2.2
27	21.2	11.3	27	11.4	2.1
28	11.6	8.4	28	5.5	3
29	15.9	10.1	29	5.3	3.5

30	10.2	3.9	30	2.8	4.7
31	9.6	9.9	31	3.9	5.9
32	4.3	16.4	32	2.5	9
33	2.3	11.3	33	1.1	28
34	21.3	18.6	34	0.8	5.7
35	11.2	16.3	35	1.5	7.7
36	24.2	42	36	2.3	4.8
37	13.2	13.2	37	11.2	13.1
38	1.9	6.7	38	16.5	5.5
39	2.7	13.1	39	1.8	1.3
40	12.2	8.8	40	0.5	11.6
41	14.2	22.2	41	2.7	15.9
42	14	23.1	42	6.1	10.2
43	5.7	12.3	43	2.4	9.6
44	7.7	12.8	44	13.2	4.3
45	4.8	20.2	45	11	2.3
46	13.1	20.9	46	5.8	8.9
47	16.2	12.9	47	6.5	17
48	14	15.5	48	5.5	2.1
49	17.4	31.2	49	1.3	0.5
50	15.3	26.4	50	1	1.9

*Units of CEA= (ng\ml) and CA-15.3=(u/ml)

Index 3: The Ages and History Family of Patients and Control

patient	H. family	age	Control	H.family	age
1	no	21	1	no	32
2	yes	19	2	no	33

3	yes	36	3	no	45
4	yes	41	4	no	49
5	no	48	5	no	25
6	yes	51	6	no	44
7	no	38	7	no	64
8	no	40	8	no	50
9	yes	36	9	no	42
10	no	48	10	no	80
11	yes	58	11	no	70
12	no	61	12	no	47
13	no	79	13	no	41
14	no	42	14	no	33
15	yes	28	15	no	50
16	yes	31	16	no	41
17	no	44	17	no	48
18	yes	60	18	n	51
19	yes	65	19	no	38
20	no	73	20	no	79
21	no	42	21	no	42
22	no	26	22	no	28
23	no	49	23	no	31
24	yes	51	24	no	44
25	yes	59	25	no	60
26	no	32	26	no	65
27	yes	35	27	no	73
28	no	47	28	no	47
29	no	67	29	no	67
30	no	51	30	no	51
31	yes	65	31	no	65

32	no	45	32	no	45
33	yes	40	33	no	40
34	yes	37	34	no	37
35	yes	49	35	no	49
36	no	32	36	no	49
37	no	33	37	no	32
38	no	45	38	no	33
39	yes	49	39	no	45
40	no	25	40	no	19
41	yes	44	41	no	22
42	no	64	42	no	30
43	no	50	43	no	45
44	no	42	44	no	40
45	yes	80	45	no	32
46	yes	70	46	no	45
47	no	47	47	no	49
48	yes	41	48	no	25
49	no	33	49	no	44
50	no	50	50	no	64

الخلاصة

إن ثقل مرض سرطان الثدي يزداد في جميع دول العالم حيث يعتبر هو أكثر مرض تتعرض إليه الإناث من بين بقية أنواع السرطانات حيث يشكل حوالي 18% من بين السرطانات التي تصيب الإناث ويشكل حوالي خمس حالات الوفيات الناجمة عن أمراض السرطان حول العالم.

ترتبت الدراسة الحالية على التحقق من دور بعض الجينات و المعلمات السرطانية في عينات عشوائية للمرضى الوافدين إلى مستشفى الديوانية التعليمي في محافظة الديوانية . تم جمع 10 ملي لتر دم من 50 مريضة مصابة بسرطان الثدي في مرحلة ما بعد العملية الجراحية كمجموعة مرضى و 50 أنثى لا تعاني من أي نوع من أنواع السرطانات كمجموعة سيرة في الدراسة الحالية, تراوحت أعمار كلا المجموعتين بين 18 إلى 80 سنة.

تم استخدام 6 ملي لتر من عينات الدم المأخوذة من مجموعة المرضى ومجموعة السيطرة لقياس المعلمات السرطانية (CA-15.3 و CEA) باستخدام تقنية Fully-auto chemiluminescence immunoassay – CMIA , ولاستخلاص ألدنا تم استخدام 4 ملي لتر من عينات الدم لأفراد كلتا المجموعتين للتحري عن الجينات *BRCA-1, BRCA-2* و *P53* باستخدام تقنية RFLP-PCR .

أظهرت نتائج الدراسة الحالية عدم وجود علاقة إحصائية بين الفئات العمرية والإصابة بسرطان الثدي ولكن تكررت الإصابات في الفئة العمرية 40- 50 سنة (50%). في مجال العلاقة بين مرض سرطان الثدي وبعض المعلمات السرطانية أظهرت نتائج دراستنا وجود علاقة إحصائية ($p<0.05$) بين الإصابة بسرطان الثدي وارتفاع تركيز CA-15.3 و CEA في مصل المرضى إلى مستويات عالية بالمقارنة مع مجموعة السيطرة $P <0.0001$. كذلك أظهرت نتائج دراستنا عدم وجود علاقة إحصائية بين CA-15.3 و CEA ($p=0.185$) لكن هنالك علاقة موجبة طفيفة ($r = 0.2432$) . من ناحية أخرى بينت نتائج دراستنا الحالية عدم وجود علاقة إحصائية بين ارتفاع تركيز المعلمات السرطانية والعمر حيث إن تركيز CA-15.3 و CEA لا تتأثر بالعمر(CA-15.3 r = 0.20 and CEA r = 0.114) .

أما في حقل التاريخ العائلي للإصابة بالمرض أظهرت نتائج دراستنا وجود علاقة إحصائية بين الإصابة بسرطان الثدي وجود تاريخ عائلي للإصابة في مجموعة المرضى ($P< 0.0001$), حيث بينت 22 (44%) من المرضى يوجد لديهم تاريخ عائلي للإصابة و(56%) 28 من المرضى لا يوجد لديهم تاريخ عائلي للإصابة بسرطان الثدي ($P > 0.05$).

خلال دراسة التغاير الجيني لجينات *BRCA-1, BRCA-2* و *P53* ضمن حقل الدراسة الجزيئية لدينا , أظهرت نتائج الدراسة وجود علاقة إحصائية بين وجود الطفرات في هذه الجينات والإصابة

بسرطان الثدي. في جين BRCA-1 أظهرت نتائج الدراسة الحالية إن الطراز الوراثي GG له دور كعامل مسبب للورم حيث كان OR (5.3191) و(EF 0. 065) , في المقابل أظهرت الطرز الوراثية AG و AA بدلا من ذلك دور وقائي ضد الورم حيث PF(0.0476 و0.1667 على التوالي) كذلك أظهرت OR نسب منخفضة معنويا حيث كانت (0.7619 و0.7917 على التوالي) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و A حيث كانت 16% و84% على التوالي. في جين BRCA-2 أظهرت نتائج الدراسة الحالية إن الطراز الوراثي AG له دور كعامل مسبب للورم حيث كان OR (13.4146) و(EF 0.1851) , في المقابل أظهر الطراز الوراثي AA بدلا من ذلك دور وقائي ضد الورم حيث PF(0.9103) كذلك أظهرت OR نسب منخفضة معنويا حيث كانت (0.0731) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و A حيث كانت10 % و90% على التوالي . في جين P53 أظهرت نتائج الدراسة الحالية إن الطراز الوراثي CC له دور كعامل مسبب للورم حيث كان OR (1.2941) و EF) (0.091 , في المقابل أظهرت الطراز الوراثي GC دور وقائي ضد الورم حيث PF (0.087) كذلك أظهرت OR نسب منخفضة معنويا حيث كانت (0.4565) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و C حيث كانت 56% و44% على التوالي.

في مجال دراسة التاريخ العائلي أظهرت نتائج دراستنا إن هنالك علاقة إحصائية بين وجود طفرة في جين *BRCA-1* و وجود تاريخ عائلي للإصابة بمرض سرطان الثدي (P<0.0085) , حيث ظهر أكثر وضوحا في الطراز الوراثي GG حيث بلغ عدد المرضى 4 (100%) ضمن المرضى الذين يمتلكون تاريخ عائلة للإصابة مقابل 0(0%) من المرضى الذين لا يمتلكون أي تاريخ عائلي للإصابة . كذلك أظهرت نتائج دراستنا وجود علاقة إحصائية بين وجود طفرة في جين *BRCA-2* والتاريخ العائلي للمرضى المصابين بسرطان الثدي(P<0.0027) , حيث ظهر الطراز الوراثي AG نسبة عالية بالنسبة لوجوده لدى المرضى الذين يمتلكون تاريخ عائلي حيث إذ بلغت النسبة 90% بالمقارنة مع 10% لدى المرضى الذين لا يمتلكون أي تاريخ عائلي للإصابة بالمرض, كذلك الحال بالنسبة للجين أيضا أظهرت نتائج دراستنا وجود علاقة إحصائية بين جين وجود طفرة في جين *P53* والتاريخ العائلي ضمن المرضى المصابين بسرطان الثدي, حيث كان ذلك واضحا في الطراز الوراثي (P<0.0001), اظهر الطراز الوراثي GC نسبة عالية 100% ضمن المرضى المصابين والذين يمتلكون تاريخ عائلي للإصابة بمرض سرطان الثدي مقابل 0% من المرضى المصابين بسرطان الثدي والذين لا يمتلكون تاريخ عائلي للإصابة بسرطان الثدي.

خلال محاولاتنا في الدراسة الحالية للربط بين التغاير الجيني للجينات (*BRCA-1,BRCA-2*و*P53*) والمعلمات السرطانية(CA-15.3 وCEA) , أظهرت نتائج دراستنا انه لا يوجد علاقة إحصائية بين

وجود تغاير جيني من لجين BRCA-1 وكل من المعلمات السرطانيةCEA وCA-15.3 (P< 0.838& p<0.896 على التوالي), كذلك الحال بالنسبة لجين BRCA-2 حيث أظهرت النتائج أيضا انه لا توجد علاقة إحصائية بين هذا ألجين والمعلمات السرطانية CEAو CA-15.3 (P < 0.595 & p< 0.157 على التوالي), أيضا أظهرت نتائجنا انه لا يوجد ارتباط إحصائي بين جين P53 والمعلمات السرطانية ضمن دراستنا " CEA وCA-15.3" (P < 0.750& p< 0.619 على التوالي).

وزارة التعليم العالي والبحث العلمي

جامعة القادسية

كلية الطب

فرع الإحياء المجهرية

تقييم ألتغاير ألجيني لجينات *BRCA-1, BRCA-2* و*P53* وعلاقتها بالمعلمات السرطانية CEAو CA 15-3 بين النساء المصابات بسرطان الثدي في محافظة الديوانية

أطروحة

مقدمة إلى مجلس كلية الطب/جامعة القادسية

وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الأحياء المجهرية الطبية

من قبل

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بكالوريوس\ماجستير أحياء مجهرية(2014)

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