Islamic University – Gaza
Deanship of Graduate Study
Biological Sciences Master Program

Reproductive factors and common genetic mutations associated with breast cancer risk in Gaza Strip.

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Submitted in Order to Fulfill the Master Degree of Science in Biological Sciences – Zoology Department.

May, 2011

بسم الله الرحمن الرحيم

الجامعة الإسلامية – غزة
The Islamic University - Gaza

عمادة الدراسات العليا
هاتف داخلي: 1150

الرقم Ref ج س غ/35/
التاريخ Date 2011/05/09

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة الدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **ميسرة سليم عبد الحميد صالح** لنيل درجة الماجستير في **كلية *العلوم*/ قسم العلوم الحياتية/ علم الحيوان** وموضوعها:

Reproductive Factors and Common genetic mutation associated with breast cancer risk in Gaza Strip

وبعد المناقشة العلنية التي تمت اليوم الاثنين 05 جماد الثاني 1432هـ، الموافق 2011/05/09م الساعة الواحدة ظهراً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another nor material which to a substantial extent has been accepted for the award of any other higher degree or diploma of the university or institute of higher learning, except where due acknowledgement is made in the text.

Author

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April 2011

Abstract

Context and objective: *BRCA1* and *BRCA2* are the two principal hereditary breast cancer susceptibility genes, and the incidence of their mutations among breast cancer patients in Gaza Strip is unknown. The objective was to study the incidence of the most common *BRCA1* and *BRCA2* mutations in Gaza Strip patients with breast cancer, to establish genetic profiles.

Design and setting: The study is a cross sectional. The patients group size was 122 patients and was selected from El-Shifa Hospital and The European Hospital in Gaza Strip. The control sample size was 55 healthy married women.

Methods: One hundred and seventy seven participants were interviewed face to face using a validated questionnaire. In addition to their demographic characteristics, ninety four participants were manage to genetic testing for *BRCA1* and *BRCA2* mutations; Peripheral blood samples were collected, genomic DNA was extracted for the detection of 185delAG and 5382insC mutations in*BRCA1*, and 6174delT mutation in *BRCA2*, a multiplex polymerase chain reaction (PCR) was performed with allele-specific oligonucleotide primers. In this method, two primers (one specific for the mutant, and one specific for the wild-type allele) were designed. All data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc.) version 16.0.

Results: There were statistically significant differences among study population with respect to age of women (P=0.00), BMI (P=0.03), number of pregnancies (P=0.00), duration of breast feeding (P=0.00), using oral contraceptives (P=0.01), and relatives with cancer (P= 0.00). In contrast the results showed no statistically significant differences among the study population related to age at menarche (P=0.46), age at first birth (P=0.37), parents consanguinity (P =0.40), and relatives with cancer (P=1.0). Moreover, within breast cancer patients (n=122) there were statistically significant differences related to age of breast cancer women (P=0.04), age at first birth (P=0.03), and age of onset (P=0.00).

But there were no statistically significant differences among the patients related to the age at menarche (P= 0.68), number of pregnancies (P=1.0), duration of breast feeding (P=0.65), and using of contraceptives (P=0.25). Finally, our study results indicated that *BRCA1* mutation (185AG and 5382C mutations) incidence (49%) was more than *BRCA2* mutations (6174T mutation) incidence (26.5%), and there were patients with both mutations (24.5%).

Conclusions: Age is associated with increasing breast cancer risk. Remarkably most risk increase occur during the reproductive years as breast cancer incidence is very low before 22 years. Risk of breast cancer decreased with an increase age at menarche, an increasing parity, a low age at first birth, and breast feeding. The incidence of *BRCA1* mutations (185AG and 5382C mutations) was more than *BRCA2* mutation (6174T mutation).

Keywords: Breast cancer, genetic mutations, *BRCA1* and *BRCA2,* PCR, SPSS, Gaza Strip, BMI, menarche, incidence.

العوامل التكاثرية و الطفرات الجينية الشائعة المتعلقة بمرض سرطان الثدي في قطاع غزة.

ملخص الدراسة:

الهدف:

تقييم العوامل التكاثرية و الطفرات الأكثر شيوعاً المتعلقة بسرطان الثدي في قطاع غزة.

منهجية الدراسة:

تقارن الدراسة بين مجموعة تجريبية من النساء اللاتي أصبن بسرطان الثدي عدد (١٢٢ مريضة) تتراوح أعمارهن ما بين (٢٢-٥٠) سنة من عيادة الأورام بمستشفى الشفاء في غزة و المستشفى الأوروبي في خانيونس، و العينة الضابطة من النساء الأصحاء عدد (٥٥) .

تشترك العينة التجريبية و الضابطة في العمر، والعمر عند الإنجاب، والرضاعة، وعدد مرات الحمل، ووجود أقارب مصابين بسرطان الثدي. وقد تم الحصول على البيانات المستخدمة من خلال استبانه تشتمل العمر و الوزن و العمر عند أول دورة شهرية و العمر عند الزواج و عدد الولادات وسنة اكتشاف المرض ..... إلخ.

وقد تم تنفيذ فحص جيني للطفرات الشائعة المسببة لسرطان الثدي BRCA1 and BRCA2 للمريضات عدد (٩٤) من المجموعة التجريبية. وقد تم استخدام برنامج SPSS لعملية التحليل الإحصائي.

النتائج:

أوضحت النتائج أن هناك فروق ذات دلالة إحصائية بين المجموعة التجريبية و المجموعة الضابطة من حيث العمر و دليل الوزن و عدد الولادات و الرضاعة و استخدام حبوب منع الحمل و وجود أقارب مصابين بالسرطان.

على العكس من ذلك أوضحت النتائج أنه لا توجد فروقاً ذات دلالة إحصائية بين المجموعتين من حيث العمر عند أول دورة شهرية و العمر عند أول ولادة و قرابة الآباء.

و زيادة على ذلك بينت الدراسة وجود فروق ذات دلالة إحصائية بين أفراد المجموعة التجريبية من حيث العمر و العمر عند أول ولادة و العمر عند اكتشاف المرض.

أخيراً أثبتت الدراسة أن انتشار الطفرات المسببة لسرطان الثدي (5382Cand185AG) للجين BRCA1 أكتر من انتشار الطفرة (6174T) للجين BRCA2 في المجموعة التجريبية.

الكلمات المفتاحية: جين سرطان الثدي، حبوب منع الحمل، النساء الأصحاء، قطاع غزة.

Dedication

This thesis is dedicated to

The spirit of all Palestinian martyrs who sacrificed themselves to enlighten the way of freedom for us.

My great parents who encouraged me accomplish this work successfully.

My husband who have given me love and support through the years.

My sons, daughters, the essence of my immortal delight .

My dear uncle, aunt, brothers, sisters, and my friends who have given me the will to overcome difficulties.

Acknowledgement

***All thanks be to Allah . " وما توفيقي إلا بالله . "**

*My special thanks are to the Master Degree program coordinators at the Islamic University-Gaza, especially Dr. Abode Al Kishawi, Dr. Majed Yasin, Mohammed Abu Odah, and I owe my thanks to Mazin Abu Zayid, Yousif Abu Selmeia, and Waal Shehadah to the creative staff of the Genetics laboratory at the Islamic University- Gaza.

*And I would like to take opportunity to express my gratitude and thanks to those who have played a role in the process of this work.

*First, I am greatly and deeply indebted to Dr. Abdullah Abed whose foresight, efforts and supervision helped me to produce this thesis.

*I am deeply indebted to Dr. Khalil Hamdan for his support, recruitment, and enrollment to this work and my sincere thanks toDr. Ziad Alkhuzondar, Dr. Mohammed Khalifah, Dr. Rami Meghdad, Dr. Zaki Zakzook, Dr. Anwar Jadallah, Awny shaqurah, Ali Jodah and all people who helped me in my work in El Shifa Hospital and The European Hospital.

*My sincere thanks to all of the patients that agreed to participate in my study without them this study would not have been possible.

*I would like to express my thanks to Eng. Hammam Al Rayes and Masoud Abu Halima who supplied us with the materials for this work.

* I owe my thanks to Dr. Izz Eldeen Abu Aleish and Abdullah Al- Hamadany (Assistant of statistics) for teaching and helping me in the statistical analysis.

*I am very grateful to my husband Fadel Saleh, my brothers Yaser and Mohammed, my sisters, my cousin Eng. Majed Saleh and all of my family in Gaza and Jordan for the support and encouragement.

*Finally, I would like to thank my friends and every one for their ongoing support and encouragement.

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Abbreviations

ASA	Allele-specific amplification
ASR	Age standardized rates
ATM	Ataxia telangiectasia-mutated gene
BARD1	BRCA1-associated RING domain
BC	Breast cancer
BCT	Breast-conserving therapy
BIC	Breast Cancer Information Core
BRAF35	BRCA2-associated factor 35
BRCA1	Breast cancer gene 1
BRCA1, BRCA2	Breast cancer susceptibility genes 1, 2
BRCA2	Breast cancer gene 2
BRCT	Breast cancer terminus domain
BSE	Breast self-examination
CBE	Clinical breast examination
CHEK2	Cell cycle checkpoint kinase gene
CI	Confidence interval
CK5/6	Pluripotent positive stem cell
CSGE	Conformation-Sensitive Gel Electrophoresis
DCIS	Ductal carcinoma in situ
DES	Diethylstilbestro
DHPLC	Denaturing high performance liquid chromatography
DNA	Deoxyribonucleic Acid
DSBs	Double strand breaks
EDETA	Ethylene diaminetetraaceticacid
ER	Estrogen receptor
FDRS	First-degree relatives
H3	Histone
HR	Homologous recombination
HR	Hazard ratio
HRT	Hormone replacement therapy

HSD	Honestly Significant Difference
Kb	Kilo base pair
LCIS	Lobular carcinoma in situ
LOH	Loss of heterozygosity
MCF-7	Breast cancer cell line
µl	Microliter
NIH	National Institutes of Health
OC	Ovarian cancer
OR	Odds ratio
P/CAF(p300/CBP-associated factor)	Transcriptional co-activator protein
p21	Promoter
P21 and GADD45	Stress-response factors
p27KIP1	Cyclin-dependent kinase inhibitor
PA	Palestinian
PCR	Polymerase chain reaction
PTEN	Phosphoprotein tumor suppressor gene
q	The short arm of chromosome
RAD4, RAP1, Ect2	Proteins involved in cell cycle
53BPI, RAD9, XRCC1	regulation or DNA repair
Rad51	Eukaryotic proteins
RecA	Bacterial proteins
RNA	Ribonucleic Acid
RR	Relative risk
SIRS	Standardized incidence ratios
SPSS	Statistical Package for the Social Sciences
TP53	Tumor suppressor gene
WHI	Women's Health Initiative
WHO	World Health Organization

Chapter One

Introduction

1.1 Overview

Cancer is a common disease. All of us start with about a 1 in 3 (30%) chance of developing cancer (excluding skin cancer) over the course of our lives. When mutations occur in genes controlling cell growth and division, the cell's normal growth-regulation breaks down, allowing it to progress towards malignancy. An inherited factor, or altered gene, is responsible for the pattern of cancer in the family [1]. Cancer has emerged beyond heart disease to become the most common cause of death, making the risk of contracting some type of malignancy during extended lifetimes more likely. Most cancers are triggered by environmental and lifestyle or unknown causes. Most malignancies are not due to heredity. In general, about 10% of breast cancer and 5% of ovarian cancer cases are contracted by carriers of defective genes [2].

1.1.1 Breast cancer

1.1.1.1 Epidemiology of breast cancer

Breast cancer (BC) is the most common malignancy in women, accounting for 31% of all female cancers, and responsible for 15% of cancer deaths in women [3]. One million females worldwide are diagnosed with BC every year. Making early detection have a high priority in medical management of the disease [4]. Breast cancer, one of the most common serious malignancies affecting women, occurs in hereditary and sporadic forms. Hereditary breast cancer accounts for 5–10% of all cases and has some distinctive clinical features compared with sporadic breast cancer [5].These percentages of all breast cancer cases are associated with a strong genetic predisposition and highly penetrate autosomal dominant trait. Two major breast cancer susceptibility genes, BRCA1 and BRCA2, have been identified [6]. The cumulative lifetime risk for the development of the disease in the general population is estimated to be 10% [7]. It is the most common cancer among women in developed countries, each year more than 1.15 million new cases of BC are diagnosed worldwide [8]. Breast cancer is the second leading cause of cancer deaths in women today (after lung cancer) [9].

1.1.1.2 The multi-step progression model of breast cancer

Breast cancer tumorigenesis can be described as a multi-step process in which each step is thought to correlate with one or more distinct mutations in major or minor regulatory genes [10].

Breast development begins in the embryonic period. In the adult breast two major cell types can be distinguished: the myoepithelial cell and the luminal secretory cell. Clinically and histopathologically, various morphologically definable steps can be identified during progression to malignancy [11].

1.1.2 Genetic aspects of hereditary breast cancer

Approximately 5% of breast cancers show a familial pattern of occurrence. This was related to germline mutations in different (tumor suppressor) genes of which the proteins have a crucial function in the breast. In patients with a germline mutation, loss of expression of the other allele by point mutations or deletions will lead to a significant or complete loss of protein function. In familial breast cancer patients, germline mutations have been described in *BRCA1*, *BRCA2*, *PTEN*, *p53*, *ATM* and *CHEK2*. Together, these account for most but certainly not all hereditary cases [12].

1.1.2.1 Breast cancer genes BRCA1 and BRCA2

In 1990, genetic studies provided initial evidence that the risk of breast cancer in some families is linked to chromosome 17q21 [13]. This 17q-associated syndrome was characterized by autosomal dominant inheritance with incomplete penetrance. In fact, loss of heterozygosity (LOH) at 17q was found in most familial breast and ovarian tumors, suggesting the involvement of tumor suppressor gene(s) [14, 15]. In 1994, the breast-cancer susceptibility gene, *BRCA1*, was identified by positional cloning [16].

In 1994, a second breast cancer susceptibility gene, *BRCA2*, was localized by linkage analysis to a 6 - centimorgan interval on chromosome 13q12-13.*BRCA2* accounts for a proportion of breast cancer roughly equal to that attributable to *BRCA1*. Like *BRCA1*, *BRCA2* appears to confer a high risk of early-onset breast cancer in females [17]. The *BRCA2* gene is composed of 27 exons distributed over roughly 70 kb of genomic DNA. The known sequence of 3418 amino acids encoded by the *BRCA2* gene does not show strong homology to sequences of other proteins [18]. In sporadic breast cancer, mutational inactivation of *BRCA2* is rare as inactivation requires both gene copies to be mutated or totally lost [10, 19- 21]. Surprisingly, despite the inherited predisposition to cancer associated with *BRCA1* and *BRCA2*, somatic disease-causing mutations in either of these genes are extremely rare in sporadic breast cancers [11,22]. Because only 45% of familial breast cancers showed evidence of linkage to *BRCA1*, the search for a second breast cancer susceptibility gene continued [23- 25].

1.1.2.2 Structure of BRCA1 and BRCA2

Although there is no sequence similarity between the two genes, many structural and functional features of *BRCA1* and *BRCA2* are similar. Both genes have complex genomic structures (*BRCA1* is composed of 24 exons and *BRCA2* of 27 exons), and they both encode large proteins (*BRCA1* 1863 amino acids and *BRCA2* 3418 amino acids) [16, 18, 23]. *BRCA1* and *BRCA2* are expressed in a wide range of tissues and show remarkably similar temporal and spatial patterns of expression [26, 27].

In *BRCA1*, a highly conserved zinc-binding RING finger domain is located close to the amino-terminus (residues 20–68) [28]. Indeed, a search for interacting proteins, using the *BRCA1* RING finger domain as bait, uncovered a further RING-domain protein designated BARD1 (*BRCA1*-associated RING domain), which binds to *BRCA1* [29]. They are located within the region of *BRCA1* that is reported to activate transcription when fused to a DNA-binding domain [30, 31]. RING finger or BRCT domains are not present in *BRCA2*, nor does it bear substantial similarity to any other sequence presently registered in the databases. [32].

1.1.2.3 Function of BRCA1 and BRCA2

1.A. DNA repair

Subsequent studies demonstrated the involvement of *BRCA1* and *BRCA2* in complexes that activate the repair of double strand breaks (DSBs) and initiate homologous recombination (HR), linking the maintenance of genomic integrity to tumor suppression. *BRCA1* and *BRCA2* co-localize with Rad51 to form complexes [33, 34]. Eukaryotic Rad51 proteins are homologues of bacterial RecA and are required for recombination during mitosis and meiosis, as well as for HR repair of DSBs [35]. Rad51 coats single-stranded DNA to form a nucleoprotein filament [36, 37]. Co-localization of BRCAs with Rad51 at sites of recombination and DNA damage-induced foci strongly suggests that BRCAs have a role in both the detection and the repair of DSBs [36]. In this regard, focus formation of Rad51 is reduced after treatment with DNA-damaging agents and is deficient during repair of DSBs by HR in *BRCA1*-deficient cells [30, 38].

1.B. Transcriptional response to DNA damage

BRCA1 has been implicated in the transcriptional regulation of several genes activated in response to DNA damage [39, 40]. A subsequent series of studies demonstrated that the C-terminus of human *BRCA1* (amino acids 1528–1863) complexes with RNA polymerase II through RNA helicase A [41]. This interaction appears to involve several proteins associated with the core polymerase complex.

In fact, *BRCA1* protein is a component of the RNA polymerase II holoenzyme, and deletion of the C-terminal 11 amino acids of *BRCA1* attenuates the association with this holoenzyme [42]. Subsequent investigations have revealed that *BRCA1* serves as a co-activator for p53 [43]. Available evidence suggests that the product of *BRCA2* exon 3 (amino acids 23–105) activates transcription [44].

1.C. DNA damage-responsive cell cycle checkpoints

Recent studies using cells defective for different DNA damage-responsive proteins have demonstrated that both ATM and BRCA1 are required for effective S-phase and G2/M-phase checkpoints [45]. Other work has indicated that BRCA1 regulates G2/M DNA damage induced checkpoints through its ability to activate Chk1 kinase and thereby induce signaling cascades downstream of Chk1 [46]. As mentioned above, BRCA1 functions as a co-activator of p53-mediated gene transcription. Importantly, cancer-associated mutant BRCA1 failed to activate the p21 promoter. BRCA1 has also been found to transactivate the cyclin-dependent kinase inhibitor p27KIP1 [47]. Available evidence suggested that BRCA2 mediates G2/M-phase control by interacting with a novel protein, BRCA2-associated factor 35 (BRAF35), which binds to branched DNA structures [48].

1.1.3 Etiology of breast cancer

The etiology of breast cancer is still poorly understood. Several known risk factors can only explain a small proportion of breast cancer cases [49]. This is most strongly seen in families with a germline mutation in a breast cancer susceptibility gene such as *BRCA1* and *BRCA2* [50,51]. However, because of their low allele frequencies in the general population, it is believed that only 5 to 10% of all breast cancers are associated with the presence a specific germline mutation. Besides the above mentioned well known genetic alterations, epigenetic alterations are among the most common molecular alterations in human neoplasia [52-54].

1.1.3.1 Factors increase or decrease the chance of developing breast cancer

The following factors have been associated with increased or decreased risk of developing breast in the general population. It is not yet known exactly how these factors influence risk in people with *BRCA1* or *BRCA2* mutations. In addition, a significant portion of hereditary breast cancers are not associated with *BRCA1* or *BRCA2* mutations [55].

Age and gender [56], **Family history** [56], **Genes** [56], **Pregnancies, and breast feeding** [56, 57], **Medical history** [56], **Radiation** [56]**, Hormonal influences** [58]**, Alcohol** [58],

Physical activity [58]**, Birth control pills (Oral contraceptives)** [58-60], **Hormone replacement therapy (HRT)** [61- 63], **Obesity** [57,64], and **Dietary fat** [65]**.**

1.2 Significance

As many as 5%–10% of diagnosed women carried an inherited mutation in the hereditary breast and ovarian cancer genes, *BRCA1* and *BRCA2*. The presence of these mutations confers elevated risks of several adverse health outcomes. The general female population has a lifetime risk of breast cancer but women with *BRCA1/2* mutations face much higher risks of this malignancy. Carriers of these mutations have a lifetime risk, and the diagnosis is often made at a younger age (i.e., before menopause). Secondly, women in the general population with breast cancer have an estimated 20-year cumulative risk of developing a contralateral breast cancer. In contrast, *BRCA1/2* carriers have a lifetime risk of up to 65% of developing a contralateral breast cancer[47].

It is the first study in Gaza Strip about cancer and specialized in breast cancer. The study of correlation between the different risk factors of breast cancer mutations in BRCA1 and BRCA2 will give indication about future incidence of the disease. As the breast cancer is the most common type of female malignancies (39%) in Gaza Strip.

1.3 Objectives

The general objective of the study is to investigate the incidence of *BRCA1* and *BRCA2* mutations in Gaza familial and sporadic cases of breast cancer in an attempt to establish a genetic profile for this population. This information will facilitate *BRCA*1 and *BRCA*2 mutational screening in the Gaza Strip population and identify individuals at high risk, who will then be able to seek genetic counseling.

The specific objectives were

1. To assess the incidence of breast cancer in Gaza Strip.
2. To estimate the incidence of the most common BRCA1, BRCA2 mutations (185delAG and 5382insC of BRCA1, and 6174delT of BRCA2) in breast cancer patients in Gaza Strip.
3. To investigate the effect of reproductive risk factors associated with breast cancer of patients in Gaza Strip.

Chapter Two

Literature Review

2.1 Breast cancer gremline mutations

Breast Cancer is a disease in which the uncontrollable growth and division of malignant cells in the tissue of the breast lead to the formation of a "lump". These cells can invade the healthy surrounding tissues through the lymph or blood vessels and result in the formation of secondary metastases. [66]

Breast carcinoma is the most common malignancy among females worldwide, and more than 1,000,000 new cases are diagnosed every year. Breast cancer (BC) may be of early or late onset, depending on age of onset. However, the cutoff value ranging from 35 to 50 years for early onset BC varies among investigators [67]. Breast cancer is the most prevalent malignancy and primary cause of cancer death in women worldwide. It accounts for 23% of all cancers among women, and is the second most common cancer overall when both sexes are considered together [68].

To date, two major genes, *BRCA1* and *BRCA2*, germline mutations of which predispose to both breast and ovarian carcinoma, have been identified. More than 1000 distinct germline alterations have been identified in each gene, most of them appearing uniquely in a single family [the Breast Cancer Information Core (BIC) Database]. [16, 24].

2.2 Types of breast cancer

Breast cancer is a cancer that propagates in different tissues of the breast. Based on type of tissue breast cancer is divided into:

1. Ductal carcinoma,
2. Lobular carcinoma.

Breast cancer may be invasive or noninvasive. Invasive means it has spread to other tissues. Noninvasive means it has not yet spread. Noninvasive breast cancer is referred to as "in situ."

1. Ductal carcinoma in situ (DCIS), or intraductal carcinoma
2. Lobular carcinoma in situ (LCIS) [56].

2.3 Symptoms of breast cancer

Symptoms of advanced breast cancer may include bone pain, breast pain or discomfort, skin ulcers, swelling of one arm (next to breast with cancer), weight loss.

2.4 Breast cancer treatment

In general, cancer treatments may include:

- Chemotherapy to kill cancer cells
- Radiation therapy to destroy cancerous tissue
- Surgery to remove cancerous tissue [56].

2.5 Breast cancer incidence

The incidence of breast cancer in Arab women is low compared to the incidence in the Jewish population in Israel; still, it is the most common malignancy among Arab women. There is a steep rise in breast cancer incidence in the Arab population in Israel over the last 10 years that can be attributed to life style changes. But, the younger age of BC onset in Arab women compared with that of the Jewish population is suggestive of a genetic component in BC occurrence in that population. The family history of 31 women of Palestinian Arab (PA) origin affected with breast (n=28), ovarian (n=3) cancer was studied. Using denaturing high performance liquid chromatography (DHPLC) to screen for mutations of *BRCA1/2* in 4 women with a personal and family history highly suggestive of genetic predisposition. A novel *BRCA1* mutation, E1373X in exon 12, was found in a patient affected with ovarian cancer. They found a novel BRCA1 mutation in a family of PA origin with a history highly compatible with *BRCA1* phenotype. This mutation was not found in additional 30 PA women affected with BC or ovarian cancer. However, it is likely that the E1373X mutation is not a founder frequent mutation in the PA population [69].

Relatives of breast cancer cases have an increased risk of the disease. The risk increases with increasing numbers and decreasing age of onset of affected relatives. In families with a *BRCA1* or a *BRCA2* mutation, individual carrier status predicts the risk of breast cancer. In relatives of cases where both *BRCA1* and *BRCA2* mutations are excluded, the risk remains undetermined. Standardized incidence ratios (SIRs) and cumulative cancer incidences were calculated for relatives of a population based set of early-onset breast cancer index cases (younger than age 41 years) with a defined BRCA mutation status (n=203).

In first-degree relatives (FDRs) of mutation-negative cases, breast cancer incidences (SIR=2.3) were increased. In high-risk individuals with at least one relative with breast cancer apart from the index case, but no BRCA mutation in the family, breast cancer incidence was increased (SIR=5.3). The cumulative incidence of breast cancer at ages 50 and 70 years for FDRs of index cases without a BRCA mutation was 3.6% and 12.8%, respectively. Similarly, the cumulative incidence of breast cancer for high-risk women was 6.3% and 21.1% at ages 50 and 70 years, and that for FDRs of BRCA mutation carriers was 17.2% and 27.7% at the same ages. So that the incidence of breast cancer was increased for FDRs of women (with early-onset breast cancer irrespective of the *BRCA*) status in the family. Risk increases with decreasing age and with increasing number of affected relatives [70].

There has been contradictory evidence as to whether *BRCA1* associated breast cancers have a poorer prognosis than non-*BRCA1* cancers. In an issue of breast cancer researcher Robson and colleagues provide further evidence for poorer survival in *BRCA1* carriers and show that it could be attributed to failure to treat small node-negative grade 3 breast cancers with chemotherapy. There still remains little evidence for a survival difference for *BRCA2* related breast cancers. Although the high contralateral breast cancer risk is confirmed by this study there is no real evidence for an increase in recurrence or new primary breast cancers in mutation carriers up to the 10-year point[71].

2.6 Mutations in BRCA gene

Linkage analyses of more than 200 families have demonstrated BRCA1 to be involved in 80–90% of the hereditary breast-ovarian cancer families and in 40– 45% of the site-specific breast cancer families [72]. The percentage of breast cancer-only families attributed to BRCA1 mutations rises to almost 70% if the median age of onset of breast cancer in families is less than 45 years [73]. Epigenetic changes differ from genetic changes mainly in that they occur at a higher frequency than genetic changes, are reversible upon treatment with pharmacological agents and occur at defined regions in a gene [53]. Breast cancer in males is sometimes hereditary but does not seem to be a feature of BRCA1 families [74]. In a recent genetic analysis study using a sample of hereditary breast cancers from Northern American women, the pattern of hereditary cancer in 14 (61%) of the 23 families studied was attributed to BRCA1 by a combination of linkage and mutation analyses [75]. In the Polish population, the most important mutation was a very high frequency of single BRCA1 5382insC mutation.

Mutations 185delAG and C61G were less frequent. A recurrent mutation was earlier described also in the BRCA2 gene–9631delC [76].

A study was conducted to delineate the genetic component of BC/ OC among the PA population [77]. Only full sequencing of the *BRCA1/2* genes and study of the particular *BRCA1* mutation that they identified in a larger population may provide complete picture regarding the role of *BRCA1/2* mutations in the studied population. Based on their study full *BRCA1/2* screening should be offered to families with a history highly suggestive of genetic predisposition. It is likely that the E1373X mutation is not a founder frequent mutation in the PA population.

BRCA1 could probably explain the majority of hereditary breast cancer that exists in the North American population, but one or more additional genes may yet be found that explain some proportion of the rest. Germline mutations in *BRCA1* were initially detected in five of eight families that demonstrated linkage to *BRCA1* and in four of 44 breast and ovarian tumors [78, 79]. To date, at least 100 unique mutations have been identified by an international collaboration [80]. Sixty-three mutations, 38 of which were distinct, have been identified through a complete screen of the *BRCA1* gene. About 85% of all alterations are frame shift or nonsense mutations and lead to a truncated protein product. They are spread throughout all of the coding sequence, leading to considerable heterogeneity in the size of the putative truncated mutant product. The biochemical consequence of these truncations is not known. The remaining15%of the mutations are either missense alterations which affect the cysteine residues within the RING domain or inferred regulatory mutations that lead to the absence of a stable transcript from the mutant allele [81, 82]. Although there is no clustering of mutations, identical mutations have been found in several unrelated individuals. Three of them appear very common: 5382insC in codon 1756, 185delAG in codon 23, and 4184del4 in codon 1355 [82].Not all *BRCA1* mutations are alterations in the coding sequence, since there are a number of families strongly linked to *BRCA1* for which no mutations in coding sequences have been detected [81]. *BRCA1* or *BRCA2* germline mutations increase the risk of developing breast cancer. Tumors cells from germline mutation carriers have frequently lost the wild-type allele. This is predicted to result in genomic instability where cell survival depends upon dysfunctional checkpoint mechanisms. Tumorigenic potential could then be acquired through further genomic alterations. Surprisingly, somatic *BRCA* mutations are not found in sporadic breast tumors. *BRCA1* methylation has been shown to occur in sporadic breast tumors and to be associated with reduced gene expression [82]. The mutational spectrum of the *BRCA1* gene has not yet been completely characterized and frequency of the mutations may have different geographic and ethnic distributions. Overall, women of Eastern

European decent have slightly raised relative risk of breast cancer. In contrast, the cumulative incidence of breast cancer in Japan is low. This phenomenon has been mainly explained by environmental factors. However, it has also been suggested that genetic factors, which may vary between races, might be involved in this difference. Within hereditary breast cancer families, affected and unaffected individuals will harbour similar perspectives; consequently, disagreement with regard to the most suitable application of genetic testing information may not present an additional source of stress and confusion in the health-related decision making of individual family members. However, the large standard deviations for preference scores indicate that this may not hold for a substantial number of families in the population from which this sample was selected. Findings from this study did not support the hypothesis that women indicating they would like to undergo *BRCA1/BRCA2* testing in the near future would display greater preferences for the genetic testing-related health states than women who either did not want the test or who were uncertain. The question thus arises as to the applicability of utility assessment for use in clinical decision making. This non significant finding suggests, for example, that women who view the notion of prophylactic mastectomy and oophorectomy as extremely undesirable and perhaps not even a viable option are no less likely to want to know their *BRCA* status than women who would be more prepared to undertake such preventive measures [12].

Deciding among treatment options requires patients and their physicians to evaluate numerous disease- and patient-related factors, not least of which are the anticipated ways in which certain therapeutic outcomes may affect the physical, psychological, and social functioning of the patient. The decision-making process has been complicated further by the cloning of *BRCA1* and BRCA2, specific genes associated with hereditary breast cancer [16, 18, 23, 83]. With the implementation of *BRCA* genetic testing, women are now faced with several new possibilities and outcomes to consider in the management of their current and future health, including prophylactic mastectomy and/or oophorectomy and participation in preventive drug trials. Specifically, older participants assigned lower utilities to dialysis and kidney transplantation but higher utilities to health states requiring hospitalization. In the context of breast cancer, a decision analysis of prophylactic mastectomy and oophorectomy in *BRCA*-positive women revealed that participants' preferences differed by age, sex, occupation, and self-perceived health status [84].

Several anthropometric measures have been found to be associated with the risk of breast cancer. Current weight, body mass index, and adult weight gain appear to be predictors of postmenopausal breast cancer.

These factors have been associated with a reduced risk of premenopausal breast cancer. They asked whether there is an association between changes in body weight and the risk of breast cancer in women who carry a mutation in either breast cancer susceptibility gene, *BRCA1* or *BRCA2*. A matched case–control study was conducted in 1,073 pairs of women carrying a deleterious mutation in either *BRCA1* (n = 797 pairs) or *BRCA2* (n = 276 pairs). Women diagnosed with breast cancer were matched to control subjects by year of birth, mutation, country of residence, and history of ovarian cancer. Information about weight was derived from a questionnaire routinely administered to women who were carriers of a mutation in either gene. Conditional logistic regression was used to estimate the association between weight gain or loss and the risk of breast cancer, stratified by age at diagnosis or menopausal status. A loss of at least 10 pounds in the period from age 18 to 30 years was associated with a decreased risk of breast cancer between age 30 and 49 (odds ratio (OR) = 0.47; 95% confidence interval (CI) 0.28–0.79); weight gain during the same interval did not influence the overall risk. Among the subgroup of *BRCA1* mutation carriers who had at least two children, weight gain of more than 10 pounds between age 18 and 30 was associated with an increased risk of breast cancer diagnosed between age 30 and 40 (OR = 1.44, 95% CI 1.01– 2.04). Change in body weight later in life (at age 30 to 40) did not influence the risk of either premenopausal or postmenopausal breast cancer. The results suggested that weight loss in early adult life (age 18 to 30) protects against early-onset *BRCA*-associated breast cancers. Weight gain should also be avoided, particularly among *BRCA1* mutation carriers who elect to have at least two pregnancies [85].

The relationship between health state utilities and age, sex and social class has been examined; only age emerged as a significant predictor of patient preferences [86].

2.7 Risk factors of breast cancer

2.7.1 Age-dependent penetrance mutations in the BRCA1 gene

Age is associated with increasing breast cancer risk. However, remarkably, most risk increase occurs during the reproductive years as breast cancer incidence is very low before age 25, and increases up to 100 fold by age 45 [86].

Various genotype–phenotype correlation attempts have yielded important data pertaining to the consequences of *BRCA1* mutations. However, little is known about the effects of recurrent *BRCA* mutations on expressivity and the age of onset of cancer in a population. It was addressed whether different exon mutations have variable expressivity especially in relation to the age of

onset of breast cancer. It was shown that different *BRCA1* gene mutations have distinct effects that influence the age of onset of breast or ovarian cancer. Mutations in exon 2 of the BRCA1 gene had significantly lower penetrance compared with mutations of exons 11, 13 and 20. The median age of affliction with breast cancer was 55 years for 185delAG in exon 2 (95% confidence interval (CI) 46.7 to 59.5), 47 years for the 4184delTCAA mutation in exon 11 (95% CI 39 to 55.4), and 41 years for exon 13 duplication (95% CI 32.9 to 49.7) of the *BRCA1* gene. Moreover, 14 novel mutations in *BRCA1* and BRCA2 genes in the Yorkshire/Humberside population were identified. The conclusion of the study was that 185delAG mutation of the *BRCA1* gene is a low penetrance mutation that is age dependent especially when compared with the exon 13 duplication mutations [87].

They found age and education level to be negatively correlated with and independently predictive of, patients' utilities for colonoscopy with perforation [88].

They examined the relationship between health state utilities and age, sex and social class; only age emerged as a significant predictor of patient preferences [89].

Median age at presentation was 45 years. A total of 288 (33.2%) patients were aged ≤ 40 years. Hormone receptors were positive in 69% of patients 40 and 78.2% of patients above 40 (p= 0.00). Younger patients had a greater probability of recurrence at all time periods (p= 0.03). Adjuvant chemotherapy was administered to 87.9% of younger and 65.6% of older patients (p< 0.00). No significant difference in radiation therapy between the two groups. The young age (≤ 40) is an independent risk factor for relapse in operable Saudi breast cancer patients [90].

2.7.2 Pregnancies and breast feeding

Multiparity, young age at first childbirth and breast-feeding are associated with a reduced risk of breast cancer in the general population. The breast cancer predisposition gene, *BRCA1*, regulates normal cell differentiation.. There was no statistically significant difference in the risk of breast cancer between porous and nulliporous women. Among porous women, an increasing number of full-term pregnancies was associated with a statistically significant decrease in the risk of breast cancer (P trend = 0.008); risk was reduced by 14% (95% confidence interval [CI] = 6% to 22%) for each additional birth. This association was the same for carriers of mutations in either *BRCA1* or *BRCA2* and was restricted to women older than 40 years. In *BRCA2* mutation carriers, first childbirth at later ages was associated with an increased risk of breast cancer compared with first childbirth before age 20 years (20 – 24 years), hazard ratio [HR] = 2.33 [95% CI = 0.93 to 5.83]; 25 – 29 years, HR = 2.68 [95% CI = 1.02 to 7.07]; ≥ 30 years, HR = 1.97 [95% CI = 0.67 to 5.81]), whereas in *BRCA1* mutation carriers, first childbirth at age 30 years or later was

associated with a reduced risk of breast cancer compared with first childbirth before age 20 years (HR = 0.58 [95% CI = 0.36 to 0.94]). Neither history of interrupted pregnancies (induced abortions or miscarriage) nor was history of breast-feeding statistically significantly associated with the risk of breast cancer. So *BRCA1* and *BRCA2* mutation carriers older than 40 years show a similar reduction in breast cancer risk with increasing parity as non-carriers [91]. A negative association between risk and duration of breast feeding was observed only in the mutation carriers. These associations were not statistically significant, but the effects of the two variables differed significantly according to mutation status (P=0.007 and P=0.045 for interaction with number of births and with duration of breast feeding, respectively). That was maintained when limiting the analysis to women diagnosed older than the age of 40 years. They concluded that the association between breast cancer and the number of pregnancies and between breast cancer and the duration of breast feeding was not the same for carriers and non carriers of a detrimental *BRCA2* mutation. In the context of other epidemiological and laboratory studies, this may indicate that the product of the *BRCA2* gene has a function relating to the differentiation of epithelial tissue in the breast. An increasing age at menarche, a low age at first birth, an increasing parity and breast feeding are associated with a reduced risk of breast cancer in the general population [92 – 95].

2.7.3 Hormonal and oral contraceptives influence breast cancer

The author and the colleagues (Hulka BS and Moorman PG. 2001) suggested that the involvement of reproductive hormones in breast cancer etiology. Other known risk factors involved life style factors, environmental factors, a history of benign proliferated breast lesions, and genetic factors. Reproductive factors such as nulliparity, early menarche and older age at first have pregnancy been associated with an increased breast cancer risk [86, 96].

In addition, exogenous hormonal influences like using oral contraceptives may increase breast cancer risk. However, the opposite was demonstrated in many epidemiologic studies in which no association between the use of oral contraceptive and the risk of breast cancer was shown. Recently, however, a large meta-analysis calculated a small but significant increase in relative risk of breast cancer (RR = 1.24) in current oral contraceptive users [97].

The use of hormone replacement therapy (HRT) by postmenopausal women was also shown to be associated with enhanced breast cancer risks, predominantly affecting the chance of development of a hormone receptor-positive breast cancer [98].

Other factors associated with breast cancer included dietary factors such as high fat intake, low vegetables/fruit and low fiber intake may also increase risk [99]. Furthermore, alcohol consumption was significantly associated with a higher risk of breast cancer [100, 101]. Some studies related socio-economic status to breast cancer risk, but these findings can probably be explained by differential life styles such as alcohol, diet and reproductive patterns [102].

2.7.4 Familial history and hereditary breast cancer

BRCA1 or *BRCA2* have been estimated to include approximately 45% breast cancer susceptibility syndromes that are transmitted as a dominant autonomic trait, accounting for about 40% cases of families with both early onset breast cancers [103].

BRCA1 and *BRCA2* account for most cases of hereditary breast cancer in the United States and Europe. These are suppressor genes that are inherited in an autonomic dominant fashion. Several studies showed that the histological and molecular phenotype of *BRCA*-associated tumors is different from that of nonhereditary tumors. There is a difference in steroid receptor status between *BRCA1* and 2 tumors regard to chemoprevention of breast cancer with anti estrogens. 93-100% of *BRCA2* associated breast cancers are ER/PR+. Breast cancers associated with *BRCA1* mutations are frequently of a higher grade and are hormone receptor-negative in one third of them. A higher proportion of cancers related to a *BRCA1* mutation have atypical or typical modularly histological features. The lifetime cumulative risk of invasive breast cancer for individuals with *BRCA1* or *BRCA2* mutations ranges from 50% to 87%. Ongoing clinical trials will determine who the optimal subjects are for screening, how screening and counseling should be conducted and what type of societal involvement is needed so that genetic screening can be used without exposing the subject to unexpected risks and consequences [104].

Only 5–10% of newly diagnosed BC patients and about 5% of OC cases are attributable to high penetrate breast cancer predisposing genes. *BRCA1* and *BRCA2* can be identified in about half of the breast cancer patients with positive family history [105].

There has been a significant increase in breast cancer incidence in Palestine. Young age onset and cancer family history are suggestive of genetic predisposition. Molecular screening for *BRCA1* and *BRCA2* mutation is an established component of risk evaluation and management of familial breast cancer. Carriers of germ line mutations in these two genes are known to be at high risk of breast cancer. They had studied the family history of 300 Palestinian women affected with breast cancer. They had identified those families with cancer history and utilized direct sequencing to screen for mutation in *BRCA1* and *BRCA2*.

A novel *BRCA2* mutation, E2229X in exon 11 was found in 3 unrelated patients. No carriers for the mutation were detected among 296 probands with breast cancer. The mutation did not appear among 1000 healthy Palestinian controls. The full *BRCA1/2* screening should be offered to patients with characteristic family history [106].

Both the maternal and paternal family history should be detailed even if the history of cancer or familial gene mutation is known to be on one side of the family. A thorough family history may uncover other cancer syndromes or hereditary conditions [107].

The inheritance of genetic disorders in the PA population. The Arab population is genetically heterogeneous; therefore the distribution of genetic disorders in this population is not uniform. Most of the PA population in Israel and in the Palestinian Authority lives in villages/ tribes that were founded by few individuals less than 10 generations ago and often includes less than 10,000 inhabitants. Consanguineous marriages are frequent in this population, therefore, each of the villages may be considered as a small isolated community [108]. Diseases that are relatively frequent among Muslim Arabs are more homogenously prevalent in the PA population in Israel as well [109].

2.7.5 Body weight, height and the risk of breast cancer

Several anthropometric measures have been found to be associated with the risk of breast cancer. Current weight, body mass index, and adult weight gain appear to be predictors of postmenopausal breast cancer. These factors have been associated with a reduced risk of premenopausal breast cancer. They asked whether there is an association between changes in body weight and the risk of breast cancer in women who carry a mutation in either breast cancer susceptibility gene, *BRCA1* or *BRCA2*. A matched case–control study was conducted in 1,073 pairs of women carrying a deleterious mutation in either *BRCA1* (n = 797 pairs) or *BRCA2* (n = 276 pairs). Women diagnosed with breast cancer were matched to control subjects by year of birth, mutation, country of residence, and history of ovarian cancer. Information about weight was derived from a questionnaire routinely administered to women who were carriers of a mutation in either gene. Conditional logistic regression was used to estimate the association between weight gain or loss and the risk of breast cancer, stratified by age at diagnosis or menopausal status. A loss of at least 10 pounds in the period from age 18 to 30 years was associated with a decreased risk of breast cancer between age 30 and 49 (odds ratio (OR) = 0.47; 95% confidence interval (CI) 0.28–0.79); weight gain during the same interval did not influence the overall risk. Among the subgroup of *BRCA1* mutation carriers who had at least two children,

weight gain of more than 10 pounds between age 18 and 30 was associated with an increased risk of breast cancer diagnosed between age 30 and 40 (OR = 1.44, 95% CI 1.01– 2.04). Change in body weight later in life (at age 30 to 40) did not influence the risk of either premenopausal or postmenopausal breast cancer. The results suggested that weight loss in early adult life (age 18 to 30) protects against early-onset *BRCA*-associated breast cancers. Weight gain should also be avoided, particularly among *BRCA1* mutation carriers who elect to have at least two pregnancies [85].

2.8 Genetic testing

Genetic testing enables women at risk for hereditary breast and/or ovarian cancer to find out whether they have inherited the gene mutation (*BRCA1/BRCA2*), and if so, to option for frequent surveillance and/or prophylactic surgery (bilateral mastectomy and / or oophorectomy). Here, a follow-up is described for 63 healthy women at 50% risk of being a *BRCA1/BRCA2* mutation carrier who underwent genetic testing. The course of distress and problems regarding body image and sexuality up to 1 year after disclosure of the test-outcome were described separately for mutation carriers undergoing mastectomy (n=14), for mutation carriers opting for surveillance (n=12) and for non-mutation carriers (n=37). Women opting for prophylactic mastectomy had significantly higher distress levels than mutation carriers who opted for surveillance, and the non-mutation carriers. This difference in levels of distress was highest at pre- and post-test and had almost disappeared at 1-year follow-up. Besides, mutation carriers opting for prophylactic mastectomy were more often in their thirties, more often had young children and had a longer awareness of the genetic nature of cancer in the family than those opting for regular surveillance. Adverse effects were observed in women who underwent prophylactic mastectomy (mostly in combination with immediate breast reconstruction) regarding the perception of how their breast region looked like and felt, the intimate relationship and physical wellbeing whereas women opting for prophylactic mastectomy reported more distress than the other women in the study, their distress levels had significantly decreased 6 months or longer after surgery, possibly due to the significant risk reduction of developing breast cancer. This might explain why most women who underwent prophylactic mastectomy were satisfied with this decision, despite a perceived negative impact on body image, the intimate relationship and physical wellbeing [110].

One of the major benefits of genetic testing is the possibility of a more individually tailored application of risk management strategies in women from at-risk families. Options of

intensive surveillance or radical prophylactic surgical interventions (bilateral mastectomy and/or oophorectomy) may be discussed with *BRCA1/BRCA2* gene mutation carriers, whereas surveillance can be discontinued in most non-mutation carriers (those who are older than 50 years and/or have relatives with breast cancer on the other side of the family may continue some form of breast surveillance). Reported proportions of *BRCA1/BRCA2* gene mutation carriers expressing interest in prophylactic mastectomy or oophorectomy are highly variable [82, 111].

The process of genetic testing is often deemed a family affair. Several studies have indicated that individuals undergo *BRCA1/2* testing not only to learn about their own cancer risks and options for screening and prevention, but also to gather information for potentially at-risk relatives. However, many individuals are not prepared for the medical and emotional implications that accompany the genetic testing process. While genetic providers have an obligation to inform individuals of the implications of *BRCA1/2* test results for at-risk relatives, they must also strive to respect and maintain autonomy and confidentiality. In addition, methods of post-test support and follow-up to facilitate the disclosure process for patients and their family members as well as foster positive communication will be discussed [112].

2.8.1 Genetic counseling

Genetic counseling is the process of helping people understand and adapt to the medical and familial implications of genetic contributions to disease. This process integrates the interpretation of family and medical histories to assess the chance of disease occurrence and recurrence, education about inheritance, testing, management, prevention, resources and research, and counseling to promote informed choices and adaptation to the risk or condition. Information is provided in a non-directive manner, allowing patients to make decisions regarding genetic testing compatible with their personal beliefs, experiences, religious conviction and financial status. However, even in the setting of non-directive counseling, heightened cancer screening and prevention measures may sometimes be presented as recommendations [113].

During the genetic counseling process, women considering genetic testing must be thoroughly informed about the potential drawbacks in the event of a positive test result. For instance, the options available to them to prevent the onset of cancer are quite limited at this time, and those that are available bring no guarantees of future cancer avoidance. A clear understanding of these potential shortcomings of *BRCA* testing will ensure that the complete and accurate 'cost benefit analysis' required for informed consent is achieved. As advances in treatment for cancer continue to extend survival of patients, the quality of life following treatment

has become a topic of considerable focus. It is now well accepted that in establishing decision models for medical interventions, it is not sufficient to look only at improvements in life expectancy; there must also be an incorporation of the health-related quality of life afforded to patients by a particular treatment [12].

2.8.2 Mutation testing during adjuvant radiotherapy

The study assessed psychological distress during the first year after diagnosis in breast cancer patients approached for genetic counseling at the start of adjuvant radiotherapy and identified those vulnerable to long-term high distress. There were no differences between the subgroups of approached patients. Predictors for long-term high distress or an increase in distress over time were pre-existing high distress and a low quality of life, having children, and having no family members with breast cancer. It was concluded that breast cancer patients can be systematically screened and approached for genetic counseling during adjuvant radiotherapy without imposing extra psychological burden. Patients vulnerable to long term high distress already displayed high distress shortly after diagnosis with no influence of their medical treatment on their level of distress at long-term [114].

2.9 Prevalence of BRCA1 and BRCA2 Mutations

A complete list of the seven founder mutations can be found in Table 2.1. These mutations are so common that often individuals of French Canadian ancestry initiate testing of the *BRCA* genes with a panel of the founder mutations and if negative then consider reflex to complete evaluation of the genes [115].

Table 2.1 Selected examples of recurrent and founder mutations in the *BRCA* gene [115].

POPULATION	*BRCA1*	*BRCA2*
Ashkenazi Jewish	185delAG 5382 ins C	6174delT
Icelandic		999del15
British	6-kb dup exon 13 4184 del4	
Dutch	2804delAA del exon 13 del exon 22	
Chinese	1081delG	
African American	943ins10 1832del5 5296del4	
Hispanic	185delAG del exon 9–12	
French Canadian	4446C>T 2953del3 + C 3768insA	8765delAG 2816insA 6085G>T 6503delTT

About 60% of Jews are Ashkenazi (Eastern European) and others are Sephardi (Middle Eastern/ African and Iraqi) or of mixed ancestry. Other major populations include Muslim Arabs, Christian Arabs, Druze, and Bedouin. Three Ashkenazi Jewish founder mutations in *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) genes have combined frequency of 2.5% in general Ashkenazi population and appear in f10% [116, 117] of breast cancer cases in Ashkenazi Jewish women. In Ashkenazi Jewish breast cancer patients with family history of breast and ovary cancer, 45% carry a mutation in one of these genes [117]. The mutations in other breast cancer susceptibility genes (PTEN, ATM, and p53) are not frequent enough in Israel to explain the rest of *BRCA*-negative familial breast cancers [117, 118].

Sixty breast cancer Egyptian patients, derived from 60 families, were selected for molecular genetic testing of *BRCA1* and *BRCA2* genes. The study also included 120 healthy first degree female relatives of the patients, either sisters and/or daughters, for early detection of presymptomatic breast cancer mutation carriers. the studied exons. Mutations in both *BRCA1* and *BRCA2* genes were detected in 86.7% of the families. The study indicated that 60% of these families were attributable to *BRCA1* mutations, while 26.7% of them were attributable to *BRCA2* mutations. Four mutations were detected in the *BRCA1* gene, while one mutation was detected in the *BRCA2* gene. Asymptomatic relatives, 80(67%) out of total 120, were mutation carriers. The results showed that *BRCA1* and *BRCA2* genes mutations are responsible for a significant proportion of breast cancer. *BRCA* mutations were found in individuals with and without family history [119]. The frequency of *BRCA1* (185delAG) mutation in Egyptian female patients with breast cancer was estimated. Forty selected female patients with breast cancer, 80 of their female relatives and 10 healthy females as a control group were included. The age of onset of breast cancer was below 40 years in 25 (62.5%) patients and above 40 years in 15 (37.5%) patients. There were significant differences among the patients regarding the age at menarche before 13 years ($p=0.011$), onset of breast cancer ($p<0.001$), parity ($p<0.001$), first delivery before 30 years of age ($p=0.04$), breast feeding ($p=0.002$), and positive family history ($p<0.001$). The frequency of *BRCA1* (185delAG) mutation was 10% in the patients. Eight percent of patients with early onset below 40 years and 13.5% of patients with onset after 40 years were heterozygotes for the mutation [120]. The incidence of breast cancer in Korea has been increasing in recent years, such that it is now the most common female cancer. Breast cancer in Korea is characterized by an earlier age of onset than in Western countries, suggesting that it would be related with genetic background. They assayed germline mutations in the *BRCA* genes to evaluate their genetic pathology in Korean breast cancer patients. The study subjects consisted of 173 patients at clinically higher risk and 109 unselected patients. Germline mutations in the entire coding sequences of the *BRCA1* and *BRCA2* genes were analyzed by Conformation-Sensitive Gel Electrophoresis (CSGE), and any aberrantly-sized band was sequenced. *BRCA* mutations were present in 12.7% of the high risk patients, compared with 2.8% of the unselected patients. Among high risk patients, mutations were most prevalent in patients with a family history of breast or first-degree ovarian cancer (22.1%), followed by those with male breast cancer (20%), bilateral breast cancer (20%), multiple organ cancer including breast (13%) and younger breast cancer patients (aged <35 yr)(8.1%). Moreover, *BRCA* mutations were

detected in 34.8% of patients having two high risk factors. These findings suggest that *BRCA* gene mutation analysis should be performed on Korean patients with high-risk factors for breast cancer [121]. Breast cancer rates and median age of onset differ between Western Europe and North Africa. In Western populations, 5 to 10 % of breast cancer cases can be attributed to major genetic factors such as *BRCA1* and *BRCA2*, while this attribution is not yet well defined among Africans. To help determine the contribution of *BRCA1* mutations to breast cancer in a North African population, they analyzed genomic DNA from breast cancer cases ascertained in Algiers. Both familial cases (at least three breast cancers in the same familial branch, or two with one bilateral or diagnosed before age 40) and sporadic cases less than 38 years of age were studied. Complete sequencing plus quantitative analysis of the *BRCA1* gene was performed. Early-onset sporadic was 9.8 % (5/51) and 36.4 % (4/11) of familial cases were found to be associated with *BRCA1* mutations. One mutation, c.798_799delTT, was observed in two Algerian families and in two families from Tunisia, suggesting a North African founder allele. Algerian non-*BRCA1* tumors were of significantly higher grade than French non-*BRCA* tumors, and the age at diagnosis for Algerian familial cases was much younger than that for French non-*BRCA* familial cases. In conclusion, the observation was that the frequency of *BRCA1* mutations among young breast cancer patients was higher than observed in Europe, suggesting biological differences and that the inclusion criteria for analysis in Western Europe may not be applicable for the Northern African population. Studies of breast cancer in the Maghreb (including Morocco, Algeria, Tunisia, Libya and Mauritania) have shown striking differences in breast cancer patterns. Age-standardized incidence per 100,000 for breast cancer in 2002 was 23.5 in Algeria versus 91.9 in France. The size and grade of breast tumors in the Maghreb was increased, while the median age of onset (48) was more than ten years younger than the European/North American median of 61. About 11 % of breast cancer cases in Algeria occur in women < 35 years old, and 55 % of cases at < 50 years. The differences may be due to differences in exposure to female hormones, diet, physical activity, or other factors. The germline mutations of breast cancer susceptibility genes (*BRCA1*) and breast cancer susceptibility genes (*BRCA2*) have been associated with a significant increase in breast cancer risk and certain other cancers. Among the most known mutations in these tumor suppressor genes are 5382insC and 185delAG in *BRCA1* and 6174delT in *BRCA2* [122].

It was indicated that the aforementioned founder mutations were not detected in the groups studied. The results indicate that 5382insC and 185delAG mutations in *BRCA1* and 6174delT in *BRCA2* have much less frequency in Iranian breast cancer patient [123].

Thirty patients with early onset breast cancer or familial breast cancer from Malaysia were analyzed for germline mutation in the early onset breast cancer gene (*BRCA1*). Direct sequencing of the entire coding region of *BRCA1* identified a frame shift mutation, c.5447-5448insC (insC5447) (codon 1776 of exon 21) in a patient aged 32 of the Malay ethnic origin, who had no family history of breast and/or ovarian cancer. Eight polymorphisms (2201C>T, 2430T>C, P871L, E1038G, K1183R, 4427T>C, S1613G and IVS8- 57delT) were identified in the samples tested [125].

Early onset breast cancer susceptibility gene (*BRCA1*) has been linked to 52% of families with breast cancer [125, 126]. Germline *BRCA1* mutations have been identified in young-onset breast cancer patients without a strong family history of breast cancer. Greenman et al., reported *BRCA1* mutations in 18% patients (five out of 27) with families with one to three relatives with breast or ovarian cancer [127]. Another study identified 15 mutations from 208 breast cancer patients below the age of 45 who had first degree breast cancer family history (affected mother and/or sister with breast cancer [128].

Germline mutations in the *BRCA1* or *BRCA2* genes predispose their carriers to breast or/and ovary cancers during their lifetime. The most frequent mutations: 5382insC, 185delAG, C61G and 4153delA in *BRCA1*, and 6174delT and 9631delC in *BRCA2* were studied in a group of 148 probands admitted for genetic counseling, using allele-specific amplification (ASA) PCR test. Fifteen carriers of three different mutations: 5382insC, 185delAG and C61G in *BRCA1* were found. Two families carried the 185delAG mutation and additional two C61G in *BRCA1*. Nobody carried the mutation 4153delA in neither *BRCA1* nor 6174delT or 9631delC in *BRCA2*. Most of the carriers of a germline mutation were observed among the patients who developed bilateral breast cancer (17%). The lowest frequency of the germline mutations was found in the healthy persons who had two or more relatives affected with breast or ovarian cancer. A few recurrent mutations which accounted for more than 80% of all germ-line mutations described in the Polish population. The most important finding in both papers was a very high frequency of single *BRCA1* 5382insC mutation. Mutations 185delAG and C61G were less frequent. A recurrent mutation was earlier described also in the *BRCA2* gene–9631delC [129]. It was likely that this particular mutation is limited to the Southern part of Poland because all families carrying

this mutation lived in Silesia and came from Silesia or a neighboring district. In this group of patients we did not find any family with this mutation. It confirmed the distribution of recurrent mutations in the Polish population and it is not different from the rest of Poland [130].

A large number of distinct mutations in the *BRCA1* and *BRCA2* genes have been reported worldwide, but little was known regarding the role of these inherited susceptibility genes in breast cancer risk among Indian women. Indian breast cancer patients were selected with regard to early onset disease (≤40 years) and family history of breast and ovarian cancer. Two hundred four breast cancer cases along with 140 age-matched controls were analyzed for mutations. In total, 18 genetic alterations were identified. Three deleterious frame-shift mutations (185delAG in exon 2; 4184del4 and 3596del4 in exon 11) were identified in *BRCA1*, along with one missense mutation (K1667R), one 5'UTR alteration (22C>G), three intronic variants (IVS10-12delG, IVS13+2T>C, IVS7+38T>C) and one silent substitution (5154C>T). Similarly three pathogenic protein-truncating mutations (6376insAA in exon 11, 8576insC in exon19, and 9999delA in exon 27) along with one missense mutation (A2951T), four intronic alterations (IVS2+90T>A, IVS7+ 75A>T, IVS8+56C>T, IVS25+58insG) and one silent substitution (1593A>G) were identified in BRCA2. Four previously reported polymorphisms (K1183R, S1613G, and M1652I in *BRCA1*, and 7470A>G in *BRCA2*) were detected in both controls and breast cancer patients. Rare *BRCA1/2* sequence alterations were observed in 15 out of 105 (14.2%) early-onset cases without family history and 11.7% (4/34) breast cancer cases with family history. The *BRCA1* and *BRCA2* mutations appear to account for a lower proportion of breast cancer patients at increased risk of harboring such mutations in Northern India (6/204, 2.9%) than has been reported in other populations [131].

Despite substantial differences in age-standardized incidence rates between developed and developing countries (age standardized rates per 100,000 women (ASR) ranging from 99.4 to 16.5 in North America and Central Africa, respectively). In all, breast cancer accounts for 14.1% of female cancer deaths. Most alarmingly, incidence rates have continued to increase worldwide, with an overall annual increase of approximately 0.5% since 1990. However, changes in incidence rates are greatest in developing countries, attaining annual increases of 3–4%. Should these trends continue, it is estimated that 1.5 million new cases of breast cancer will be diagnosed in 2010 [132].

In India, an average of 80,000 women was diagnosed with carcinoma of the breast, and 40,000 women die of the disease every year [133].

To date 1536 distinct mutations, polymorphisms and variants in *BRCA1* and 1885 in BRCA2 have been reported [BIC database, [134], which are distributed throughout the entire coding regions of both genes. Together, mutations in both the genes account for the great majority of families with hereditary susceptibility to breast and ovarian cancer [126]. Epidemiological studies indicate that *BRCA1* mutation carriers have a lifetime risk of breast cancer that is on the order of 60–80%. The lifetime breast cancer risk for *BRCA2* mutation carriers approaches that of *BRCA1* carriers: however, disease onset has been documented to be at a later age [126, 135].

In other words, women with an altered *BRCA1* or *BRCA2* gene are 3 to 7 times more likely to develop breast cancer than women without alterations in those genes [136], with very high relative risks for early disease onset (before age 40) of about 30-fold. Carriers of *BRCA1* and *BRCA2* mutation(s) are also at increased risk for other cancers – in particular, both genes increase the risk of ovarian cancer, while *BRCA2* confers greatly increased risks of male breast cancer. Additional, but more modest risks are found for uterine, cervical, early onset prostate and pancreatic cancers for *BRCA1* [137], and prostatic, pancreatic, gallbladder, bile duct, stomach cancers and melanoma for *BRCA2* [138].

However, the contribution of mutations in these two genes to breast cancer patients in the Indian population remains relatively unexplored apart from a few small studies [139, 140]. The *BRCA1* 185delAG mutation was identified in an early onset index case [age 35] without any family history. This mutation is common in Ashkenazi Jews, having attained a 1% carrier frequency within the population [141]. Population studies have shown that the 185delAG mutation predates the separation of Sephardi and Ashkenazi Jewish populations and is probably 2000 years old [142].

In follow-up studies of other families, they have now identified this mutation in seven unrelated Finnish families. The other recurrent mutations, the exon 18 nonsense mutation (8555TG) and the Of the 10 *BRCA1* mutations identified here, six are so far novel Finnish mutations while four have been described previously in other European populations or in the USA. [143].

Of these, 2803delAA (also called 2804delAA) is a prominent founder mutation in the Netherlands and Belgium, but has not been found previously in other populations [144]. Another mutation, 3604delA, has been described in Dutch and German families [144, 145]. The 5385insC (also named as 5382insC) mutation found in one Finnish family has been described as a founder mutation in Russia [146], and in Hungary [147], in families of Jewish and non-Jewish ancestry. Finally, the 4446CT mutation in exon 13 has been found multiple times on different haplotypes

and may represent a mutational hotspot [148]. Of the six different *BRCA2* mutations, five were novel mutations so far unique to Finland, while one recurrent, and a proposed ancient founder mutation (999del5), has been previously described as a strong founder in Iceland [149-153].

2.10 Breast cancer in Gaza Strip

Breast cancer is a common malignancy affecting women around the world, including Gaza Strip. No studies on breast cancer has been published. This is the first study that documents collected data about cancer patients.

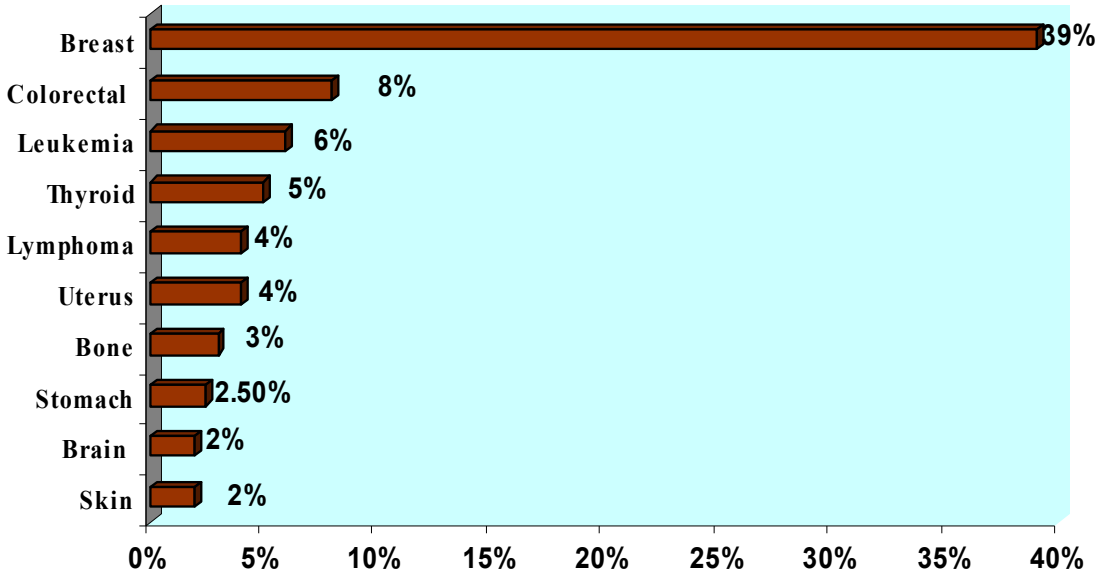

Figure 2.1 Most common types in female malignancies. Palestinian National Authority-MOH-Gaza Cancer Registry Center-Reported Cases 2003 [154].

- The incidence of cancer cases in the whole population is about 40/100.000.
- The median age of cancer in female was 52 years and most common types Figure 2.1 **were:**

Breast 39%, Colorectal 8%, leukemia 6% Thyroid 5%, Lymphoma 4%, Uterus 4%, Bone 3%, Stomach 2.5%, Brain 2%. [154]

Chapter Three

Materials and methods

3.1 Study design

This study is a cross sectional study.

3.2 Study population

The study population comprised 122 breast cancer patients in El- Shifa Hospital and The European Hospital of Gaza Strip in the period between March 2010 to July 2010 and control sample consist of 55 married healthy females. Only 94 samples of breast cancer patients were processed and analyzed in the genetic laboratory at the Islamic University of Gaza.

3.3 Questionnaire

The questionnaire (see Annex 1) has been filled by all patients and agreement to carry out mutational genetic analysis has been signed on the questionnaire papers. It concluded the risk factors associated with breast cancer risk such as the age of patients, the height and length to calculate the BMI, the age at menarche, the age at first birth, number of pregnancies, Time of breast feeding of the population of the study, using of contraceptives, the age of onset of the disease of the patients, and the family history of the study population**.**

3.4 Permission and ethical considerations

According to research ethics, permission was obtained from Helsinki committee (Annex 2) and MOH (Annex 3) for specimen collection and performing the study. The objective of the study was explained to all participants and their consent was taken. The age of the women of the study population was under 50 years old.

3.5 Materials

3.5.1 Chemicals and Reagents

The chemicals and reagents used in this study are summarized in Table 3.1. All chemicals were analytical and molecular biology grade.

Table 3.1. Chemicals and reagents used in the present study

Reagent	supplier
Wizard Genomic DNA purification Kit	PROMEGA, Madison, WI, USA
Absolute Ethanol	Sigma, USA
Isopropanol Alcohol	Sigma, USA
Agaros	PROMEGA, USA
Taq DNA polymerases	PROMEGA Cat. # M-1661.
PRONTO® Kits	Pronto diagnostic. Isreal

3.5.2 Disposables

The major disposables used in this study are listed in Table 3.2.

Table 3.2. List of disposables

Item	Supplier
2.5 K3-EDTA Tube	Canelli, Italy
Disposables powder gloves	Weihai Sun Genius – China
Micro Tubes, 1.5 ml capacity	Labcon, USA
Micro Tubes, 0.2 ml capacity	Labcon, USA
Micropipettes tips	Labcon, USA

3.5.3 Equipments

All experiments of this study were done at the Islamic University of Gaza- Genetic Laboratory. The major equipment that were used are listed in Table 3.3.

Table 3.3 A list of major equipment used in the present study.

Instrument	Manufacture
Thermocycler	Eppendrof, Germany
Microcentrifuge	Centurrion Scientific LTD, UK
Refregerator -20ºC	LG, Koriaad, USA
Vortix Mixer	Turbo Mixer, Georgia
Micropipette	Lab Mate, Poland
Microwave	Nanodrop, USA
Safety Cabinet	Heraeus, Germany
UV-Transilluminator	Scie- pals LTD, UK
Electrophoresis	BioRad, USA

3.6 Methods

3.6.1 Case selection

Selection of patients mainly based on the following criteria: any patient with breast cancer diagnosed under the age of 50 years; any patient having a family history of breast, and any patient had a previous personal history of cancer.

3.6.2 Blood sample collection

Peripheral blood samples (5 -.10 ml) was collected in EDTA Tube. The buffy coat was separated and frozen at - 70°C for further use.

3.6.3 DNA extraction

Genomic DNA was extracted from peripheral blood. Promega' DNA Extraction kit features a rapid, procedure for isolating DNA from whole blood that is ready for direct use in DNA amplification reactions according to manufacture protocol as follows:

3.6.3.1 Red blood cell lysis

1. Nine hundred microliter of cell lysis solution was combined with three hundred microliter blood in a tube (mixed by inversion) then it was incubated for ten minutes at room temperature.
2. The tube was centrifuged 13000xg; twenty seconds.
3. Supernatant was discarded, then pellet was vortex.

3.6.3.2 Nucleic lysis and protein precipitation

1. Three hundred microliter of nucleic lysis solution were added and mixed by inversion.
2. One hundred microliter of protein precipitation solution were added then vortex for twenty seconds.
3. The tube was centrifuged 13000 x g; three minutes.

3.6.3.3 DNA precipitation and rehydration

1. The supernatant was transferred to a new tube containing three hundred microliter isopropanol then mixed.
2. The tube was centrifuged 13000 x g; one minutes.
3. Supernatant was discarded, then three hundred micrometer ethanol (70%) were added.
4. The tube was centrifuged 13000 x g; one minutes.
5. The ethanol was aspirated and air- dried the pellet (10- 15 minutes).
6. The DNA was rehydrated in one hundred microliter of DNA rehydration solution for one hour at 65˚C or over night at 4˚C then it was refrigerated for further use.

3.6.4 Mutation detection

Design of PRONTO technology

The Pronto system allows detection of disease causing mutations and Single Nucleotid Polymorphisms (SNPs) in DNA sequences. The PRONTO technology is based on a single nucleotide primer extension assay that is visualized by ELISA in a 96-well format. Its accuracy comes mostly from the high specificity in which DNA polymerase incorporates nucleotides into the elongating strand. In the detection process, a 5´ labeled primer binds to the tested DNA (post PCR) next to the suspected mutation site. DNA polymerase extends the 5´ labeled primer with a single biotinylated nucleotide. The mutation site is tested in two separate reactions on a thermostable 96 well microplate (see Annex 4).

Each reaction utilizes a different complementary bio-dNTP for the normal and the mutant alleles. Only the extended primers are labeled with biotin (see Annex 5).Mutation detection was carried out using Pronto diagnostic commercial kit which include the following steps:

3.6.4.1 DNA amplification:

Amplification of target DNA to visible levels (10-100 ng/ µl) on an EtBr-stained gel is recommended. It include the following steps;

1- Tow microliter template DNA (from an initial concentration of about 150 ng/µL) were dispensed to a thermoplate well or tube.

2- A master mix were prepared in a sterile vial, according to the volumes indicated in the table below, plus one spare reaction volume. The Taq DNA polymerase was added to the amplification mix shortly before dispensing the mix. And gently mixed by pipetting in and out several times.

3.a PCR master mix

Volume for one sample	Solution
17.5 µL	Amplification mix BRCA
(5 u /µL) 0.5 µL	Taq DNA Polymerase

Taq DNA polymerases were validated for use with this procedure (lacking $3' \rightarrow 5'$ exonuclease activity): PROMEGA Cat. # M-1661.

3- Eighteen microliter master mix was dispensed to each well or tube.

4- One drop of ColoRed-Oil was added to each well.

5- The thermo plate well or tube was placed in a thermocycler previously programmed with the following protocol:

3.b Cycling protocol

Start,	1. 94º C	2 minutes
35 cycles,	2. 94º C	30 seconds
	3. 59º C	30 seconds
	4. 72º C	60 seconds
End,	5. 72º C	5 minutes

To verify amplification, five microliter of the amplified product were subjected to electrophoresis in a 2% Agarose gel.

3.c Sizes of amplified fragments

Fragment size	Mutation	Gene Position
258 bp.	185 delAG	BRCA1 Exon 2
400 bp.	5382 insC	BRCA1 Exon 20
550 bp.	6174 delT	BRCA2 Exon 11

3.6.4.2 Post - amplification treatment

This reaction inactivated free nucleotides, which was remain after the amplification steps:

1- A post-amplification treatment mix was prepared shortly before use. Combined in a single test tube the volumes appearing in the following table, (multiplied by the number of tested samples, plus one spare volume) .

*** Solution volumes for one sample**

1. PRONTO Buffer 3 45.0 µL
2. Solution 2.0 µL
3. Solution D 1.5 µL

2- Gently by pipetting this solution was mixed in and out five times.

3- Forty microliter of the post-amplification mix were added into each well or tube containing fifteen microliter of each amplified DNA sample.

4- One drop of ColoRed oil was added to each tube.

5- The tubes were incubated for thirty minutes at 37°C, then for ten minutes at 95°C in a thermocycler (see Annex 6).

3.6.4.3 Primer extension reaction

This post-amplification mutation detection system utilizes a single nucleotide primer extension assay. It benefits from the high specificity with which DNA polymerase incorporates nucleotides into the elongating strand. In the complete genotyping format of the PRONTO system each mutation site is tested in two wells of a 96-well microtiter plate. Every sample is separately and internally controlled for each mutation tested. DNA polymerase extended a 5´FITC-labeled primer with a single biotinylated nucleotide, which was complement the nucleotide at the tested site. Every reaction utilized a different complementary bio-dNTP for the normal and

the mutant alleles. Each mutation is tested in two wells: one for the mutant allele and one for the wild-type allele. The amplified DNA is dispensed into a 96-well thermowell plate using a multichannel pipette (see Annex 7). The plate is placed in a thermocycler, where a short primer extension reaction takes place as shown in Figure 3.1.

Results are clearly determined visually.

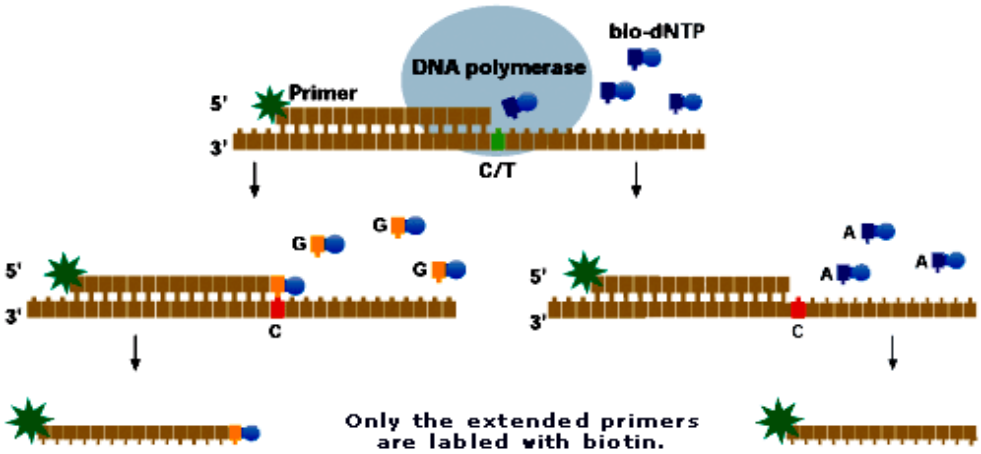

Figure 3.1 primer extension reaction.

1- The thermocycler was programmed as follows:

Cycle	**Temperature**	**Time**
Start:	94ºC	15 sec.
20 cycles:	94ºC	30 sec.
	57ºC	10 sec.
End:	18-25ºC - Cool down to room temperature	

2- A PRONTO plate was taken out of its pouch.

3- Eight microliters of post-amplification treated DNA were dispensed into the first six wells in The first row. Continued with the remaining samples.

4- The plate was titled and then added one drop of ColoRed oil to each well.

5- The thermocycler was turned on and started the cycling protocol.

6- When the thermal cycling is complete, you can proceed to the ELISA assay, or store the reaction products in the refrigerator and carry out the visualization steps within 24 hours.

3.6.4.4 Elisa assay- color development

3.A. Detection by ELISA

Reaction products were diluted and transferred to a streptavidin coated 96-well ELISA plate. Following a short incubation period, unbound primers were washed away and an HRP anti-FITC conjugate is added. The peroxidase reaction, that takes place in the presence of the HRP substrate, resulted in the appearance of a deep blue color in every positive well. Each well of a PRONTO Plate contains 5′-labeled primers that hybridize to the tested DNA next to the suspected mutation sites, and biotinylated nucleotides (bio-dNTPs), which are complementary to the nucleotide bases at the tested sites.

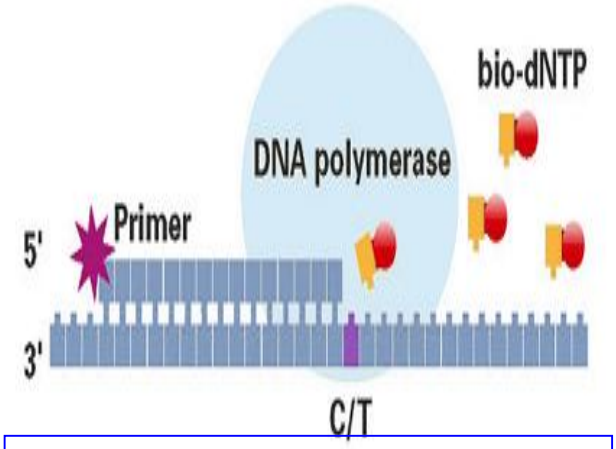

Figure 3.2 labeled primers with biotin.

Depending on the tested individual's genotype, the relevant primers are extended and thus become labeled with biotin as in Figure 3.2.

The ELISA assay consists of the following steps:

- **Binding** the biotinylated primer to the Streptavidin-coated plate Figure 3.3.
- **Washing** away the unbound primer.
- **Incubating** with the HRP conjugate.
- **Washing** away the unbound conjugate.
- **Incubating** with the TMB substrate (color development).

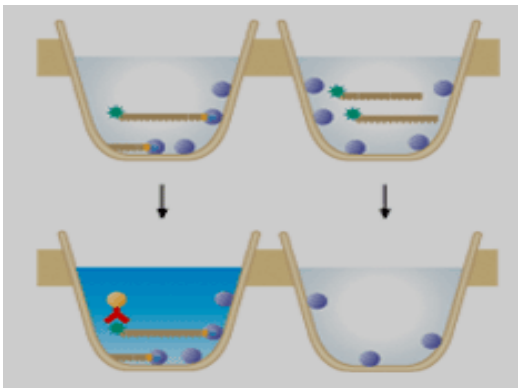

Figure 3.3 ELISA assay of biotinylated primer

The results of this assay were determined **Visually**, by monitoring the development of the blue color.

3.B. Preparation

1- The 20x wash solution were diluted to 1x with deionized water.

2- The plastic cover of the detection plate were peeled off . The side of the plate was marked with the kit name and test number.

3- The PRONTO plate was placed and the detection plate side by side, oriented in the same direction.

3.C. Transfer to the detection plate

1- A reagent reservoir /trough was filled with the green colored assay solution.
About eleven microliter will be required for a 96-well plate.

2- One hundred microliters of Assay Solution were added to the bottom of each well in column 1 of the PRONTO plate with a multichannel pipette. We mixed the assay solution with the solution in the wells.

3- One hundred microliters from each well in this column without changing tips, were transferred to the first column in the detection plate .

4- This procedure was repeated, using a new set of tips for each column. It is essential to maintain the order of the samples.

5- The PRONTO plate was incubated for ten minutes at room temperature (18-25ºC).

3.D. Visual genotype assignment

For every mutation site tested, at least one of the wells should develop a deep blue color.

Otherwise, results are invalid Figure 3.4.

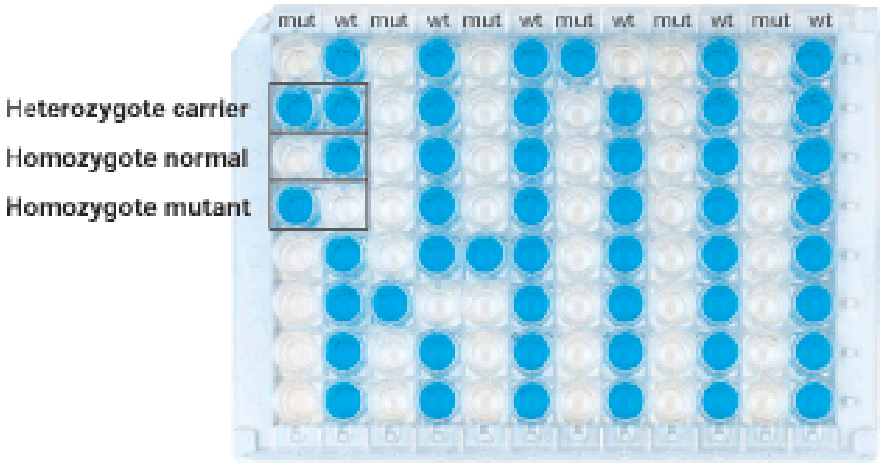

Figure 3.4 Visual genotype assignment**.**

3.6.4.5 Genotype assignment according to visual inspection of test results

Genotype assignment according to visual inspection of test results as in figure 3.5.

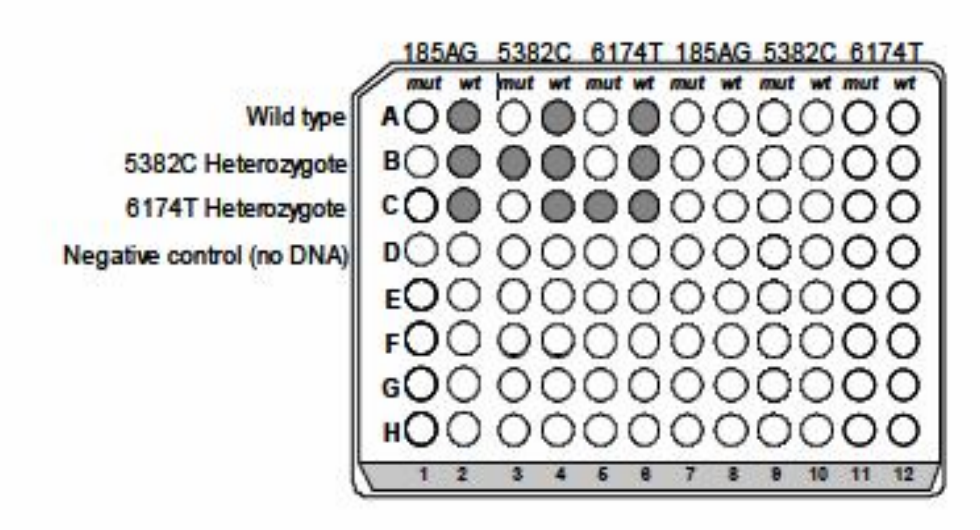

Figure 3.5 Visual inspection of test results.

3.7 Statistical analysis

Both qualitative and quantitative data analysis methods were used. The data analysis were made utilizing SPSS version 16.

Statistical analysis test for analysis of binominal variables outcome (dependent variable) the study used the following tests:

- Count, percentage and descriptive statistics.
- Odds ratio which used to show the difference in risk between independent variables (groups)
- Chi–square test to build 2x2 tables for categorical data and cross tabulation and examine if there is statistically significance proportion differences.
- Fisher's exact test for small data sets when variable groups less than 5.

Chapter Four

Results

4.1 Risk of breast cancer associated with reproductive health determinants for sporadic and hereditary patients.

4.1.1 Patients' age groups

The study population has been divided into two groups, a control group of 55 individual and breast cancer patients group which consist of 122 patients. Both groups have been divided according to age into two groups as shown below in Table 4.1.

Table 4.1. Age groups of the control individual and breast cancer patients

		Normal	BC patients	OR**	(95 % CI)**	P- value
Age	< 40	39	31	0.14	(0.07-0.29)	0.00*
		55.7%	44.3%			
	≥ 40	16	91			
		15.0%	85.0%			

It was found that more than 41.2 % of the patients were < 40 years. The mean age of the study population is 40.3 ± 7.9 as shown below in Table 4.2.

Table 4.2. Statistical descriptive of the population under study

	Minimum	Maximum	Mean	Std.
Age (years)	22	50	40.3	7.9
BMI (kg/ m^2)	17.9	53.1	29.2	5.9
Age at menarche (years)	11	18	13.7	1.4
Age at marriage (years)	Na*	42	20.3	6.6
Age at first birth (years)	Na*	43	20.7	8.5
Have been pregnancy	1	2	1.1	0.3
Number of pregnancy	Na*	16	3.5	3.7
Breast feeding	1	2	1.2	0.4
Time of feeding (years)	Na*	4	1.9	1.2
Using of contraceptives	1	2	1.8	0.4
Age of onset of disease (years)	25	50	39.6	5.9
Parents consanguinity	1	2	1.5	0.5
If there is relatives with cancer	1	2	1.5	0.5
Valid N =177				

Na*mean non of them.

Table 4.1 revealed that there was statistical significant relationship between the control individual and breast cancers patients related to women age group (P= 0.00) and OR= 0.14 with confidence interval 0.07-0.29. This means that breast cancer incidence increase with age of 40 years.

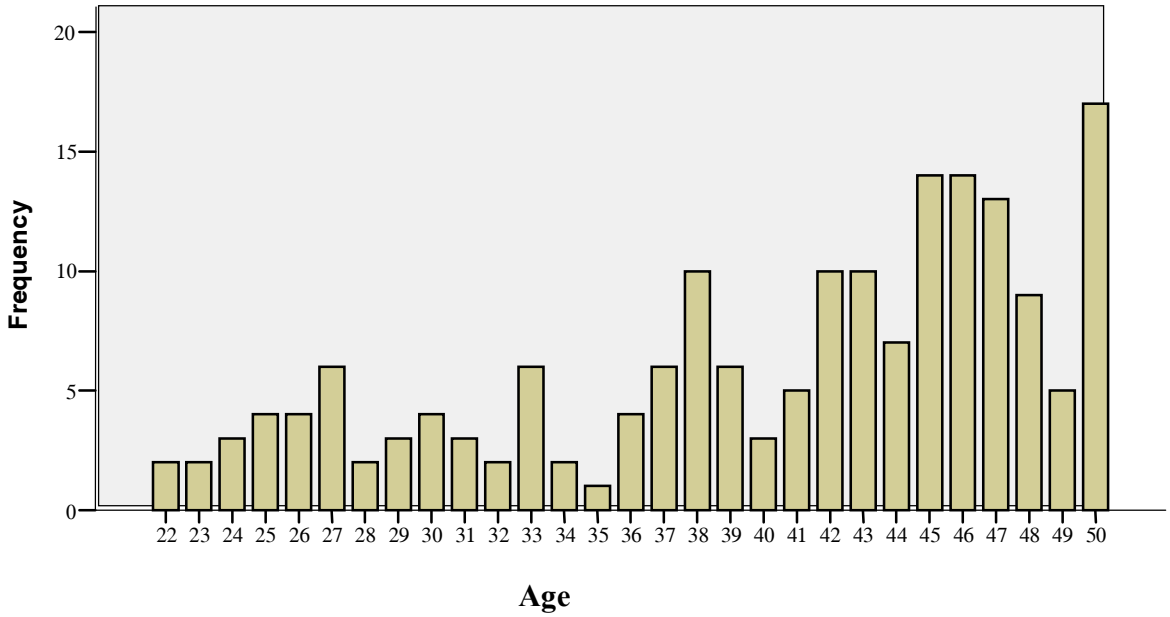

Figure 4.1 The frequency of age of the population under study.

Also when we compare the patients of mutated and non- mutated in *BRCA1/2* genes as shown below in Table 4.3. The results revealed a statistical significant relationship between breast patients related to women age group (P= 0.05) and OR ratio = 2.6 with confidence interval 1.04 - 6.50 at 95%. This means that women with age >40 years has 2.6 times hazard risk having BC compared with women <40 years which indicating the incidence of mutations tend to increase with the age of patients ≥ 40.

Table 4.3. Risk of breast cancer associated with age of patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Age	< 40	18	10	2.6	(1.04 - 6.50)	0.05*
		64.3%	35.7%			
	≥ 40	27	39			
		40.9%	59.1%			

4.1.2 Patients' body mass index (BMI)

The patients' weight ranged from 41 kg to 136 kg. Patients have been grouped based on BMI into underweight, normal, obese, and overweight. The minimum BMI was 17.9, the maximum BMI was 53.1, with a mean of 29.2± 5.9 as shown in Table 4.2.

Both patients and controls BMI has been classified into two groups BMI under 28 has been considered normal within the normal range and BMI above 28 has been considered within abnormal weight range. There is statistical significant difference between the two groups in relation to BMI (P= 0.04) with confidence interval 0.28-1.00 as shown below in Table 4.4. This mean that breast cancer incidence increase with the increase of the BMI of the individual.

Table 4.4. Risk of breast cancer associated with BMI of the population of the study

		Normal	BC patients	OR**	(95 % CI)**	P- value
BMI	< 28	31	49	0.53	(0.28-1.00)	0.04*
		38.8%	61.2%			
	≥ 28	24	73			
		25.0%	75.3%			

*OR= odds ratio, CI= confidence interval

4.1.3 Age at menarche

The minimum age of menarche was found to be 11 years and the maximum age of menarche is 18 years, with a mean age of 13.7±1.4 as shown in Table 4.2. When they grouped it was found that 83 (46.9%) of the patients their ages at menarche were at ≤13 years old while 94 (53.1%)of the patients their ages at menarche were at >13 years old and there were no statistical significant relationship between control cases and the patients related to the age at menarche (P= 0.46) as shown in Table 4.5.

Table 4.5. Risk of breast cancer associated with the age of menarche of population under study

		Normal	BC patients	OR**	(95 % CI)**	P- value
Age at menarche	≤ 13	25	58	1.09	(0.57 -2.06)	0.46
		30.1%	69.9%			
	> 13	30	64			
		31.9%	78.0%			

Also there was no statistical significant relationship between of mutated and not mutated breast cancer patients related to women age at menarche (P= 0.68) as shown in Table 4.6.

Table 4.6. Risk of breast cancer associated with age at menarche of mutated and not mutated patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Age at menarche	≤ 13	23	22	1.28	(0.57 - 2.89)	0.68
		51.1%	48.9%			
	> 13	22	27			
		44.9%	55.1%			

*OR= odds ratio, CI= confidence interval.

4.1.4 Age at marriage

Only 9 of the patients were single, 113 patients were married as shown in Table 4.7, the minimum age of married was 13 years and the maximum was 42 years of age. The mean age of marriage was 20.3± 6.6 as shown in Table 4.2.

Table 4.7. Social status of the population under study

	Number	Percentage %	Cumulative Percent
Married	168	94.9	92.6
Single	9	5.08	100.0
Total	177	100.0	

4.1.5 Age at first birth

It was found that 19 of patients have never been pregnant and the rest were pregnant. The minimum age at first birth was 16 years and the maximum age at first birth was 43 years old as shown in Figure 4.2. The mean age of first birth was 20.7± 8.5 as shown in Table 4.2.

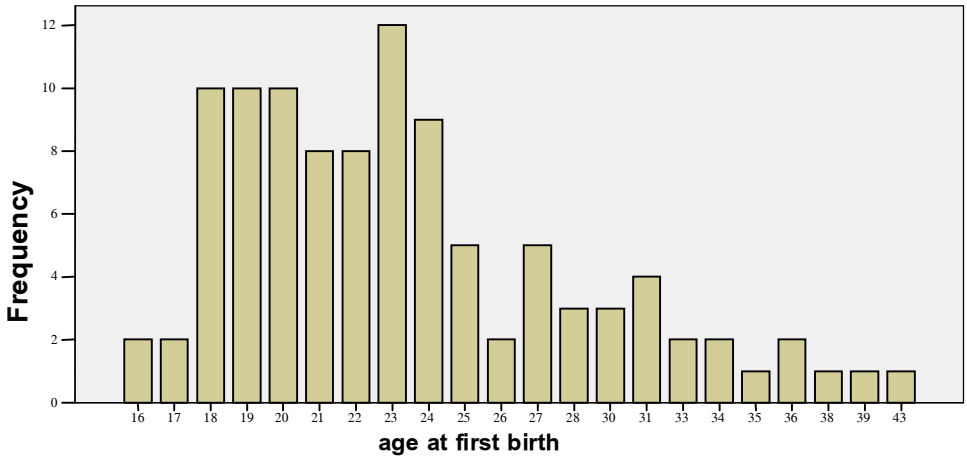

Figure 4.2. The frequency of age at first birth.

There were no statistical significant relationship between control individual and breast cancer patients related to the age at first birth (P= 0.38) as shown in Table 4.8.

Table 4.8. Risk of breast cancer associated age at first birth controls breast cancer patients

		Normal	BC patients	OR**	(95 % CI)**	P- value
Age at first birth	≤ 20	16	34	1.20	(0.59- 2.45)	0.38
		32.0%	68.0%			
	> 20	39	69			
		36.1%	63.9%			

But there were statistical significant relationship between breast cancers patients related to women age at first birth (P= 0.04) which in the other hand the result indicated the OR ratio was 2.6 with confidence interval 1.12 – 5.96 at 95% this means that women with age more than 20 years of age at first birth has 2.6 times hazard risk having breast cancer compared with women their age at first birth was less than or equal 20 years as shown in Table 4.9.

Table 4.9. Risk of breast cancer associated with age at first birth of mutated and not mutated patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Age at first birth	≤ 20	25	16	2.6	(1.12 - 5.96)	0.04*
		61.0%	39.0%			
	> 20	20	33			
		37.7%	62.3%			

*OR= odds ratio, CI= confidence interval.

4.1.6 Number of pregnancies

There were 19 patients had not been pregnant at any time during their life as shown in Table 4.10. The number of pregnancies of patients had ranged from Na*-16 times which showed a mean of 3.5±3.7 as shown in Table 4.2.

Table 4.10. Patients and pregnancy

		Number	Percentage %	Cumulative Percent
Number of pregnancies	Yes	158	89.26	89.26
	No	19	10.73	100.0
	Total	177	100.0	

The result revealed that there was statistical significant relationship between breast cancer patients and control individual related to the number of pregnancy (P= 0.00) within confidence interval 0.12-0.55 as shown in Table 4.11.

Table 4.11. Risk of breast cancer associated with pregnancy of the study population

		Normal	BC patients	OR**	(95 % CI)**	P- value
No. of pregnancies	≤ 3	23	16	0.26	(0.12- 0.55)	0.00*
		59.0%	41.0%			
	> 3	32	87			
		26.9%	73.1%			

When we compared the patients who are mutated and non- mutated in BRCA1/2 genes, there were no statistical significant relationship (P=1.0) as shown in Table 4.12.

Table 4.12. Risk of breast cancer associated the number of pregnancies of patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Number of pregnancies	≤ 3	12	14	0.91	(0.37- 2.25)	1.0
		46.2%	53.8%			
	> 3	33	35			
		48.5%	51.5%			

*OR= odds ratio, CI= confidence interval.

4.1.7 Breast feeding

From 158 individual were pregnant, there were 157 patients had fed their children, there are just one patients had not feed their children. The total number of patients who did not feed their child was 20 patients (15.8%) as shown Table 4.13. The mean of the study population 1.2± 0.4 as shown in Table 4.2.

Table 4.13. Feeding or not of the study population

		Number	Percentage %	Cumulative Percent
Feeding or not	Yes	157	88.7	88.7
	No	20	11.3	100.0
	Total	177	100.0	

The study population has been divided into two groups, there were statistical significant relationship between breast cancer patients and control cases (P= 0.00) as shown in Table 4.13.

Table 4.14. Time of breast feeding of the study population

		Normal	BC patients	OR**	(95 % CI)**	P- value
Time of breast feeding	Not feeding	0	20	0.43	(0.34- 0.54)	0.00*
		0%	100.0%			
	<1yr	35	40			
		40.7%	53.3%			
	>1 yr	20	62			
		24.4%	75.6%			

There was no statistical significant relationship between breast cancer patients related to duration of breast feeding (P= 0.65) as shown in Table 4.15.

Table 4.15. Risk of breast cancer associated with duration of breast feeding of mutated and not mutated patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Time of breast feeding	<1yr	13	17	0.79	(0.32 - 1.99)	0.65
		43.3%	56.7%			
	> 1 yr	24	25			
		49.0%	51.0%			

4.1.8 Use of contraceptives

As 168 had married and 103 were pregnant, so 132 had not use contraceptives and 45 patients had contraceptives. The mean of the study population 1.8± 0.4 as shown in Table 4.2. When compared between the control individual and breast cancer patients related to the use of contraceptives, there was statistical significance (P= 0.01) as shown in Table 4.16.

Table 4.16. Risk of breast cancer associated with using of contraceptives of the study population

		Normal	BC patients	OR**	(95 % CI)**	P- value
Using contraceptives	Yes	27	36	0.43	(0.23- 0.84)	0.01*
		42.9%	57.1%			
	No	28	86			
		24.6%	75.4%			

*OR= odds ratio, CI= confidence interval.

However, there was no statistical significant relationship between breast cancer patients related to use of contraceptives (P= 0.26) of mutated and not mutated patients as shown in Table 4.17.

Table 4.17. Risk of breast cancer associated with using of contraceptives of mutated and not mutated patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Using of contraceptives	Yes	15	11	1.73	(0.69 - 4.31)	0.26
		57.7%	42.3%			
	No	30	38			
		44.1%	55.9%			

*OR= odds ratio, CI= confidence interval.

4.1.9 Age of onset of disease

The minimum age of onset of cancer was 25 years, the maximum age of onset was 50 years, and the mean of age of onset was found to be 39.6± 5.9.

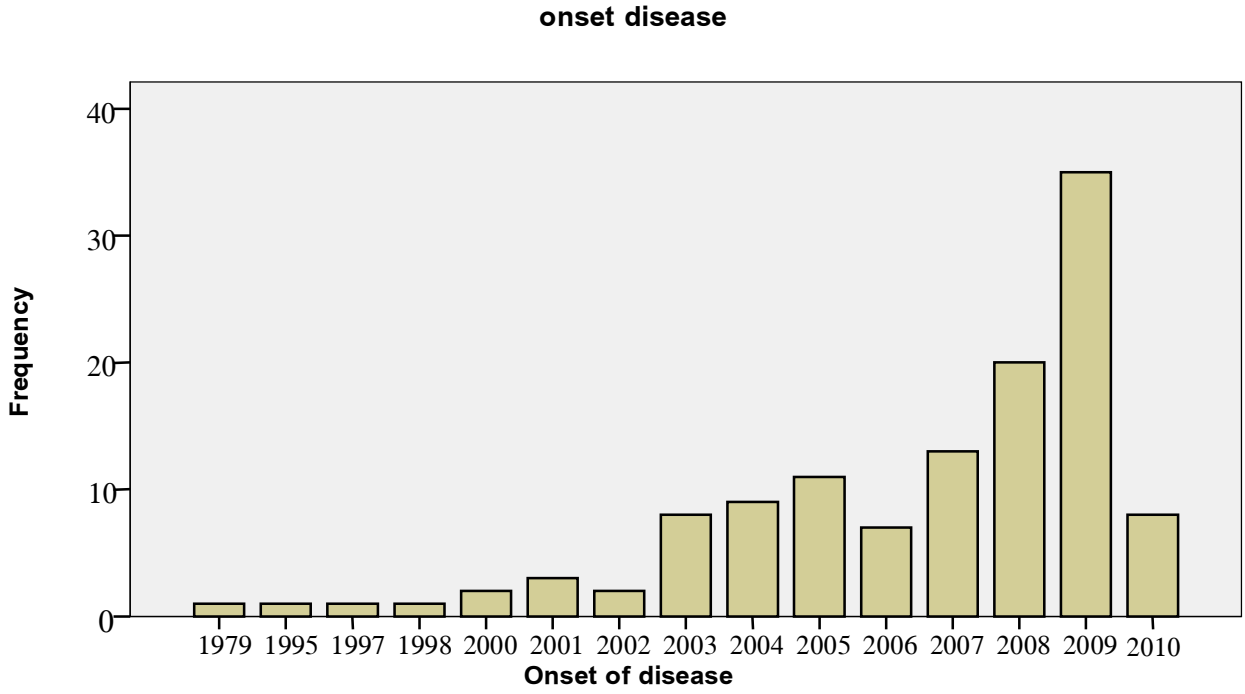

Figure 4.3. Frequency of onset of the disease.

As shown in Figure 4.3 the most frequency of onset of the disease among the breast cancer patients of the population under study was in the year of 2009.

The number of patients their ages of onset < 40 were 52 (42.6%), and the number of patients their ages of onset ≥ 40 were 70 (57.4%) as shown in Table 4.18.

Table 4.18. Age of onset the disease pf patients

		Number	Percentage %	Cumulative percent
Age of onset	< 40	52	42.6	42.6
	≥ 40	70	57.4	100.0
	Total	122	100.0	

There was statistical significant relationship between breast cancers patients related to women age of onset of disease (P= 0.00) which in other hand the result indicated the OR ratio was 4.1 with confidence interval 1.74 - 9.77 at 95% this means that women with age more than 40 years of onset of disease has 4 times hazard risk having BC compared with women less than 40 years as shown in Table 4.19.

Table 4.19. Risk of breast cancer associated age of onset of disease of patients

		Not mutated	Mutated	OR**	(95 % CI)**	P- value
Age of onset	< 40	28	14	4.1	(1.74 - 9.77)	0.00*
		66.7%	33.3%			
	≥ 40	17	35			
		32.7%	67.3%			

*OR= odds ratio, CI= confidence interval

4.2 Familial history

4.2.1 Parents' consanguinity

It was found that 86 (48.6%) of the patients their parents were relatives, and the rest 89 patients (51.4%) their parents were not related to each other. The mean of the study population 1.5± 0.5 as shown in Table 4.2. There were no statistical significant relationship between the mean groups relates to the parents consanguinity as shown in Table 4.20.

Table 4.20. Risk of breast cancer associated with Parents' consanguinity of the study population

		Normal	BC patients	OR**	(95 % CI)**	P- value
Parents' consanguinity	Yes	28	58	0.87	(0.46- 1.65)	0.40
		32.6%	67.4%			
	No	27	64			
		29.7%	70.3%			

*OR= odds ratio, CI= confidence interval

4.2.2 Familial cases

Out of the 177 individual, there were 76 (42.9%) of the individuals had relatives with cancer while 101 (57%) did not have any relatives with cancer, as shown in Table 4.21. There was statistical significant relationship between breast cancers patients and control individual related to women age of onset of disease (P= 0.00) which in other hand the result indicated the OR ratio was 5.1 with confidence interval 2.45 – 11.48 at 95%.

Table 4.21. Risk of breast cancer associated with relatives with cancer of the study population

		Normal	BC patients	OR**	(95 % CI)**	P- value
Relatives with cancer	Yes	10	66	5.3	(2.45- 11.48)	0.00*
		13.2%	86.8%			
	No	45	56			
		44.6%	55.4%			

*OR= odds ratio, CI= confidence interval

There were 2 patients had relatives from all kinds of relatives, 12 patients had relatives from first and second degree relatives, one patient had relatives from first and third degree relatives, and one patient had relatives from second and third degree relatives. The number of patients with

only first degree relatives were 13 (39.3%), while patients with only second degree relatives with cancer were 13 (44.3%), and patients with only third degree relatives who have cancer history were 9 patients (16.4%) as shown in Table 4.22.

Table 4.22. Number of relatives with cancer of the breast cancer patients

		Number	Percentage %
Relatives with cancer	First degree relatives	48	39.3
	Second degree relatives	54	44.3
	Third degree relatives	20	16.4
	Total	122	100.0

There was no statistical significant relationship between breast cancer patients related to relatives with cancer (P= 1.0) and first degree relatives with cancer (P= 0.83) as shown in Table 4.23.

Table 4.23. Risk of breast cancer associated with relatives with cancer specially first degree relatives of the patients

	Answer	Not mutated	Mutated	OR**	(95 % CI)**	P- value
Relatives with cancer	Yes	27	29	1.03	(0.45 - 2.36)	1.0
		48.2%	51.8%			
	No	18	20			
		47.4%	52.6%			
First degree relatives with cancer	Yes	15	15	1.13	(0.48- 2.70)	0.83
		50.0%	50.0%			
	No	30	34			
		46.9%	53.1%			

*OR= odds ratio, CI= confidence interval.

4.2.3 BRCA1 and BRCA2 mutations

We managed genetic analysis for 94 patients from the population under study (122 patients). The number of the patients with mutations were 49 patients (52.1%) and the rest 45 patients (47.9%) were normal (wt) as shown in Table 4.24.

Table 4.24. Percentage of normal and mutated of patients

	Number	Percentage %
Wilde type (wt)	45	47.9
Mutated (mt)	49	52.1
Total	94	100.0

There were 24 patients had BRCA1 (49%), 13 patients had BRCA2 (26.5%) and 12 patients had both mutations (24.5%) as shown in Table 4.25.

Table 4.25. Percentage of the mutations of the patients

		Number	Percentage %
Mutations of patients	BRCA1	24	49.0
	BRCA2	13	26.5
	Both	12	24.5
	Total	49	100.0

4.2.3.1 BRCA1 mutations

A. 158AG mutation

There were 2 patients had the three mutations, 17 patients had two mutations, and the rest (30 patients) had one mutation. We found 25 patients had BRCA2 mutation (51%) and 24 patients had BRCA1 mutations (49%). The number of patients with 158AG mutation were 60, 37 patients were normal homozygous, 10 were heterozygous and 13 patients were homozygous as shown in Table 4.26.

Table 4.26. Percentage of the 158AG mutation of the patients

		Number	Percentage %
158AG mutation	Normal	37	61.7
	Heterozygous	10	16.7
	Homozygous	13	21.7
	Overall	60	100.0

B. 5382C mutation

The number of patients with 5382C mutation were 57, 35 patients were normal homozygous, 17 patients were heterozygous and 5 of them were homozygous mutant as shown in Table 4.27.

Table 4.27. Percentage of the 5382C mutation of the patients

		Number	Percentage %
5382C mutation	Normal	35	61.4
	Heterozygous	17	29.8
	Homozygous	5	8.8
	Overall	57	100.0

4.2.3.2 BRCA2 mutation

A. 6174T mutation

The number of patients with 6174T mutation were 48 normal homozygous, 12 were found to be heterozygous while the rest 13 patients were found homozygous as shown in Table 4.28.

Table 4.28. Percentage of the 6174T mutation of the patients

		Number	Percentage %
6174T mutation	Normal	23	47.9
	Heterozygous	12	25.0
	Homozygous	13	27.1
	Overall	48	100.0

Chapter Five

Discussion

5.1 Age of patients and breast cancer

Breast cancer is the first occurring type of cancer in women in Gaza Strip. As known earlier studies have discussed the risk factors which lead to high rate of incidence, we aim in this study to discuss the main risk factors of breast cancer.

Worldwide studies have shown that breast cancer increases with age specially after 40 years of age. In our study it seems that our population is not different from other populations and that the incidence of breast cancer increase with age as compared between patients < 40 and patients ≥ 40.

Also the incidence of the mutation causing breast cancer in patients has been shown to increase with age. This contradicts earlier study on Arab population in Israel which showed that mutation incidence increases before the age of 50. This indicate that mutation tend to be somatic rather than inherited. This could be due to the different environmental disturbances that the Gaza population are exposed to that come from continuous explosives and attacks by Israel.

The mean age of the study population was 40.3±7.9. The incidence of patients their ages were < 40 (25.5%) and 74.5% of patients with ages ≥ 40. There was a statistical significant relation related to women age between the control and the patients (P= 0.00), hazard ratio OR= 0.14 [95%CI=0.07-0.29], and between mutated and not mutated of the breast cancer patients (p= 0.05), hazard ratio (OR)= 2.6 [95%CI=1.04-6.49]. It was reported that breast cancer incidence is very low before age 25, and increases up to 100 fold by age 45 [86].

5.2 BMI and breast cancer

In order to study the relation between BMI and breast cancer incidence we divided patients in two groups according to their BMI. ≤ 20 group which include normal and under weight patients and > 20 group which include overweight and obese patients. Statistical analysis showed an association between rate of BMI and breast cancer incidence. Another study suggested that weight loss in early adult life (age 18 to 30) protects against early-onset *BRCA*-associated breast cancers. Weight gain should also be avoided, particularly among *BRCA1* mutation carriers who elect to have at least two pregnancies [85].

5.3 Age at menarche and breast cancer

Age at menarche was suggested to be a strong and consistent prediction of breast cancer risk in the general populations. However our results showed that in cancer patients there was no association between age at menarche and incidence in breast cancer in this population. That study was consistent with other population in which this relation has been observed. Although a study has shown that early age at menarche is determinant of breast cancer among women with *BRCA1* mutations [92- 95].

Our study showed that age at menarche have no risk effect on breast cancer incidence among women with *BRCA1* and *BRCA2* mutations.

5.4 Age at first birth and breast cancer

The younger the women is when she begins childbearing, the lower the risk of breast cancer. The relative risk of developing breast cancer is estimated to increase by 3 % for each year of delay.

There is evidence that reduction in risk of breast cancer with childbirth, and higher risk with later age at first full time birth, may be limited to estrogen reception positive tumors. This has been confirmed in mutated and not mutated patients, as the odd ratio for the risk of breast cancer in mutated related to age at first birth was found to be OR= 1.2 [95% CI= 0.59-.2.45] when we compared patients under and over 20 years of age at first childbirth. There was no association between breast cancer incidence and age at first birth between the control and the patients (P= 0.38).

However, this association has been observed in other population. In *BRCA2* mutation carriers, first childbirth at later ages was associated with an increased risk of breast cancer compared with first childbirth before age 20 years (20 – 24 years, hazard ratio [HR] = 2.33 [95% CI = 0.93 to 5.83]; 25 – 29 years, HR = 2.68 [95% CI = 1.02 to 7.07]; ≥ 30 years, HR = 1.97 [95% CI = 0.67 to 5.81]), whereas in *BRCA1* mutations carriers, first childbirth at age 30 years or later was associated with a reduced risk of breast cancer compared with first childbirth before age 20 years (HR = 0.58 [95% CI = 0.36 to 0.94]. [91]

5.5 Breast feeding and breast cancer

Earlier studies has shown that Women who breastfeed reduce their risk compared with women who do not breastfeed. The longer a woman breastfeeds, the greater the protection: risk is reduced by 4% for every 12 months of breastfeeding. However we found that the numbers of breast cancer patients who fed for more than one year higher than the number in normal

population. There is no statistical significant relationship between breast cancer patients related to duration of breast feeding (P= 0.65).

This contradiction could be due to several reasons i.e. the duration of breast feeding as we did not calculate the total duration of breast feeding or because other studies use the cut time of duration of breast feeding from three months [155].

5.6 Oral contraceptives and breast cancer

In cancer patients' population the findings that more women use oral contraceptives than normal population. This correlation does not seem to agree with the findings of our study as there was no statistical significant relationship between breast cancer patients related to use of contraceptives (P= 0.26).

To obtain more realistic correlation the duration of time of use should be considered rather than only used or not as the relation seem to be affected by the duration of use contraceptives [156-158].

In addition, using oral contraceptives may increase breast cancer risk. However, the opposite was demonstrated in many epidemiologic studies in which no association between the use of oral contraceptive and the risk of breast cancer was shown. Recently, however, a large meta-analysis calculated a small but significant increase in relative risk of breast cancer (RR = 1.24) in current oral contraceptive users [97].

5.7 Breast cancer correlation with number of pregnancies

In our study there was no association between breast cancer incidence and number of pregnancies. Mutated and non- mutated in *BRCA1/2* genes, showed no statistical significant relationship (P=1.0) However this association has been observed in other population. An increased number of births were associated with a decreased risk of breast cancer in *BRCA2*-negative cases but not in *BRCA2*-positive cases. An increasing age at menarche, a low age at first birth, an increasing parity and breast feeding are associated with a reduced risk of breast cancer in the general population [92 – 95].

5.8 Breast cancer correlation with onset of disease

In our study there was statistical significant relationship of breast cancer patients related to women age of onset of disease (P= 0.00), hazard ratio (OR) = 4.1 [95%CI= 1.73-9.77]. The study revealed that the most frequency of onset of the disease was in the year of 2009.

5.9 Breast cancer correlation with common genetic mutations

Among 122 women with sporadic and hereditary breast cancer, there were 24 patients had *BRCA1* (49%), 13 patients had *BRCA2* (26.5%) and 12 patients had both mutations (24.5%). The number of the patients with mutations were 49 patients (52.1%) and the rest 45 patients (47.9%) were normal (wt). *BRCA1 a*nd *BRCA2* mutation is an established component of risk evaluation and management of familial breast cancer. Carriers of germ line mutations in these two genes are known to be at high risk of breast cancer [106].

Chapter Six

Conclusions and recommendations

6.1 Conclusions

- The risk of breast cancer increased with the age, age at first birth, age of onset of disease.
- There was no influence risk of breast cancer with the factors; age at menarche, breast feeding, no. of pregnancies, using oral contraceptives, and the presence of relatives with breast cancer.
- The incidence of *BRCA1* mutations (185AG and 5382C mutations) was more than *BRCA2* mutation (6174T mutation).

6.2 Recommendations

- This study has highlighted the need of a breast cancer awareness campaign, which should also stress the importance of early detection and reporting of breast cancer.
- Extensive mutation screening of high-risk breast cancer primarily targeting early-onset cases should be undertaken in Gaza Strip with proper genetic counseling, since female carriers of mutations in these genes are also at a high risk for developing a second malignancy either in the breast or ovary.
- Personal risk information may help in taking preventive measures and also motivate a high-risk woman to adopt breast screening that may promote early detection and improve chances of surviving breast cancer.
- Women found to carry a pathogenic variant can be advised to undergo regular screening by mammography or magnetic resonance imaging and to consider risk-reducing surgery. In addition, their relatives who do not carry the pathogenic variant can be able to seek genetic counseling of breast self-examination (BSE) and clinical breast examination (CBE) recommendations.

Chapter Seven

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Annex (1)

Questionnaire

أختكم الباحثة : ميسرة سليم صالح.
طالبة ماجستير في العلوم الحياتية في الجامعة الإسلامية وأقوم بالبحث النهائي من رسالة الماجستير والتي تدرس الطفرات الوراثية المسببة لمرض سرطان الثدي في قطاع غزة .
وهو فحص جيني غير متوفر في قطاع غزة لذا أرجو من سيادتكن المساعدة في تعبئة الاستبيان و الإجابة على الأسئلة بصدق ليعطي الغاية المرجوة منه.
علماً بأن المعلومات الواردة في الاستبيان سرية جداً و خاصة للبحث العلمي فقط.
كما وأرجو الموافقة على سحب عينة دم لعمل الفحص الجيني عن الطفرات المسببة للمرض.

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Questionnaire

- Name: ---------- ● Birth date: / /
- Age: ---------- Years.
- Length:---------- cm ● Weight: ---------- kgs ● Obesity: □ Yes □ No
- Address:----------

- The age at the first ministration (menarche): ----------
- The age at marriage: ----------
- Parents consanguinity: ----------
- The age at first birth: ----------
- No. of pregnancies: ----------
- No. of siblings: ----------
- The time of breast feeding:----------
- Using contraceptives: ----------
- Duration of use: ----------
- Onset of the disease: ----------
- Any treatment or hormones medicine:----------
- Family history of the disease: ----------

استبيان

١- الاسم :.....

٢- تاريخ الميلاد : / / م العمر : .....

٣- الطول : .....

٤- الوزن : .....

٥- العنوان: .....

.....

٦- العمر عند أول دورة شهرية : .....

٧- العمر عند الزواج : .....

٨- العمر عند أول ولادة : .....

٩- عدد مرات الحمل : .....

١٠- فترة الرضاعة في كل حمل : ١-.....٢- .....٣-.....

٤-.....٥-.....٦-.....٧-.....٨-.....

١١- هل تناولت حبوب منع الحمل: □ نعم □ لا

١٢- فترة الاستخدام : □ ٦شهور□ سنة □ سنة ونصف □ سنتان □ أكثر □ أقل من ذلك .

١٣- تاريخ بداية (اكتشاف) المرض : .....

١٤- هل تناولت أدوية لعلاج الهرمون : □ نعم □ لا

١٥- هل الأب والأم أقارب : □ نعم □ لا

١٦- هل يوجد أفراد في عائلتك مصابين بالمرض: □ نعم □ لا

١٧- الأفراد المصابين بالمرض من العائلة : .....

بارك الله فيكم وجزاكم الله خيراً و عافاكم .

Annex (2)

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

التاريخ6/12/2010

Name:

الاسم: ميسرة صالح

I would like to inform you that the committee has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

Commom Mutations of BRCA1 and BRCA2 Genes in early onset breat Cancer Patient In Gaza Strip

In its meeting on December 2010 and decided the Following:-

و ذلك في جلستها المنعقدة لشهر ديسمبر 2010 و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member
عضو

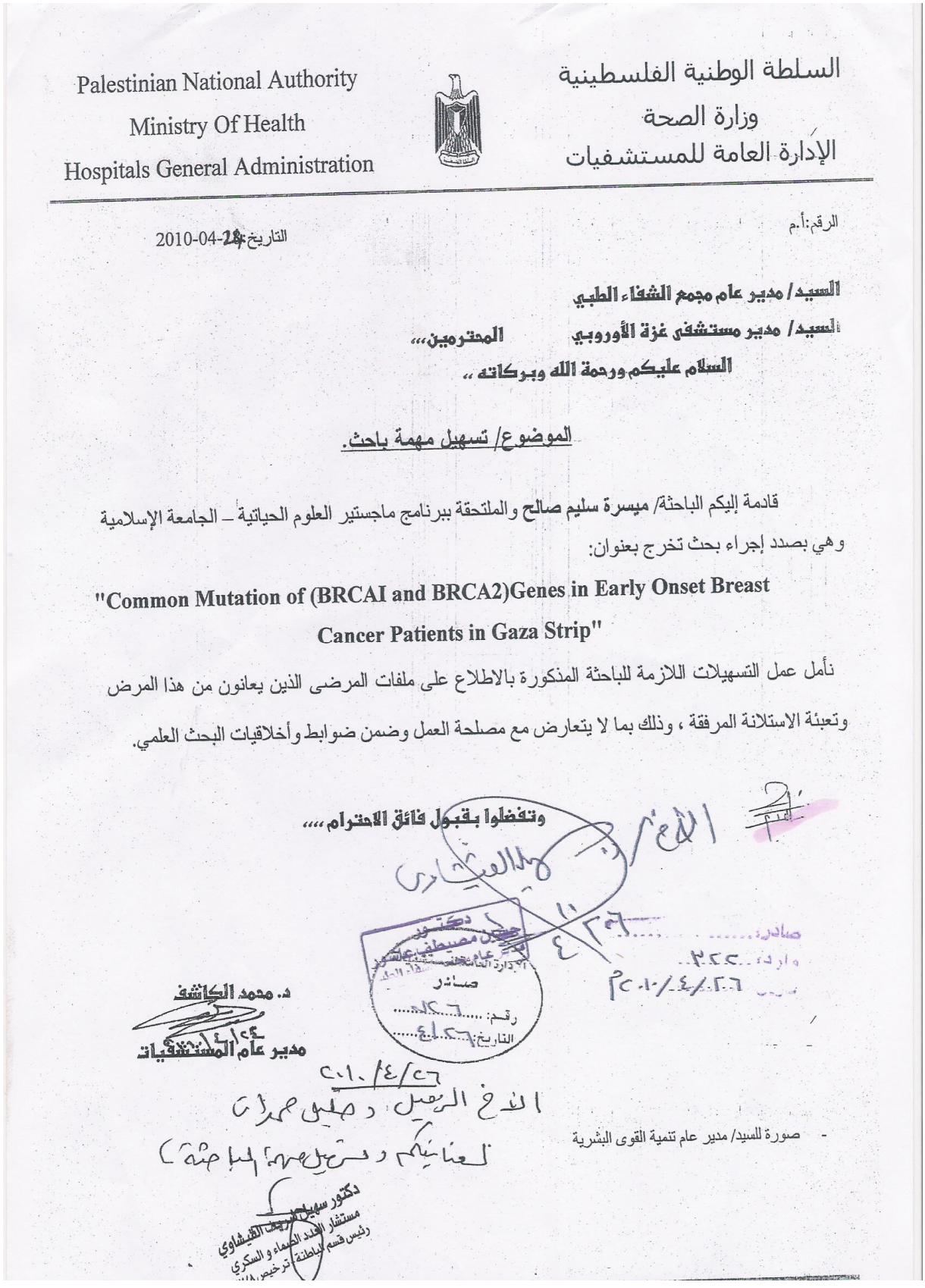
Member
عضو

Chairperson

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

Annex (3)



السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة للمستشفيات

Palestinian National Authority
Ministry Of Health
Hospitals General Administration

الرقم:أ.م

التاريخ: 28-04-2010

السيد/ مدير عام مجمع الشفاء الطبي
السيد/ مدير مستشفى غزة الأوروبي المحترمين،،،
السلام عليكم ورحمة الله وبركاته ،،

الموضوع/ تسهيل مهمة باحث.

قادمة إليكم الباحثة/ ميسرة سليم صالح والملتحقة ببرنامج ماجستير العلوم الحياتية – الجامعة الإسلامية وهي بصدد إجراء بحث تخرج بعنوان:

"Common Mutation of (BRCAI and BRCA2)Genes in Early Onset Breast Cancer Patients in Gaza Strip"

نأمل عمل التسهيلات اللازمة للباحثة المذكورة بالاطلاع على ملفات المرضى الذين يعانون من هذا المرض وتعبئة الاستلانة المرفقة ، وذلك بما لا يتعارض مع مصلحة العمل وضمن ضوابط وأخلاقيات البحث العلمي.

وتفضلوا بقبول فائق الاحترام،،،

د. محمد الكاشف
مدير عام المستشفيات

- صورة للسيد/ مدير عام تنمية القوى البشرية

Annex (4)

BRCA - PROCEDURE SUMMARY

DNA EXTRACTION: from human whole blood, using a validated method.

DNA AMPLIFICATION:

Volumes per reaction: 2 µL Template DNA + 17.5 µL Amplification Mix + 0.5 µL Taq Polymerase.

Cycling protocol:

94°C 2 min→35 cycles of {94°C 30 sec. /59°C 30 sec./ 72°C 60 sec.} →72°C 5 min.

POST-AMPLIFICATION PROCEDURE:

- Volumes per reaction:

PRONTO® Buffer 3	45.0 µL
Solution C	2.0 µL
Solution D	1.5 µL

- Pipette in and out to mix.
- **Add** 48 µL into each well containing 15 µL amplified product, mix well.
- **Add** one drop of ColoRed® oil.
- **Incubate** 30 minutes at 37°C, then 10 minutes at 95°C.

PRIMER EXTENSION REACTION:

- **Dispense** 8 µL of each Post-Amplification treated DNA into six wells of the PRONTO® Plate.
- **Add** one drop of ColoRed™ oil.
- **Start** the cycling protocol:
- 94°C 15 sec→20 cycles of {94°C 30 sec. / 57°C 10 sec.} → Cool.

Insert the PRONTO® Plate in the thermocycler when the temperature is 90°C

DETECTION:

- Add 100 µL Assay Solution to each well in the PRONTO® Plate and mix.
- Transfer 100 µL from each well of the PRONTO® Plate to the identical position in the Detection Plate. Incubate 10 minutes at RT.
- Empty the wells and wash four times with 350 µL of 1x Wash Solution.

	Visual Detection	Colorimetric Detection
Add 100 µL of Conjugated HRP to every well and incubate for 10 minutes at RT.	Dilution 1:100	Dilution 1:300
Empty the wells and wash four times with 350 µL of 1x Wash Solution.	✓	✓
Add 100 µL of TMB Substrate to each well and incubate at RT for:	30 minutes	15 minutes
Add Stop Solution	—	100 µL per well
Read O.D. at:	620 nm	450 nm

Annex (5)

Figures of the experimental technology of the materials and methods of the study

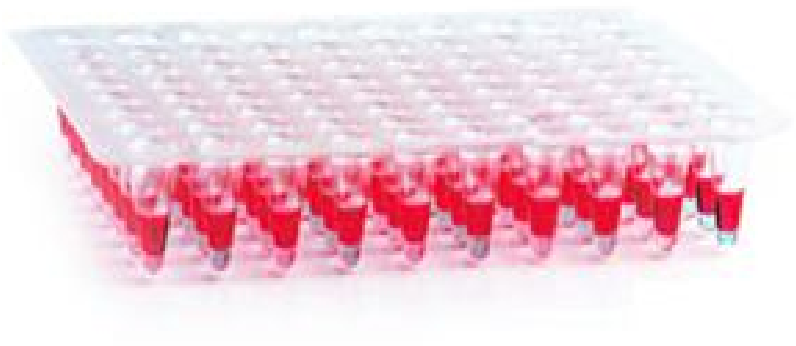


Figure 5.1; The streptavidin coated 96-well ELISA plate.

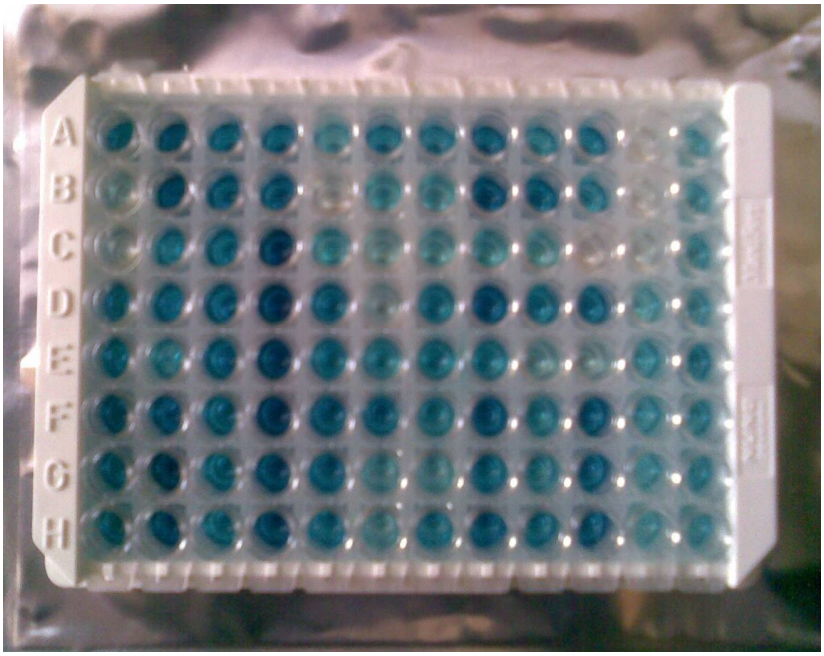


Figure 5.2; The visual genotype results of detection plate of BC patients.

Annex (6)

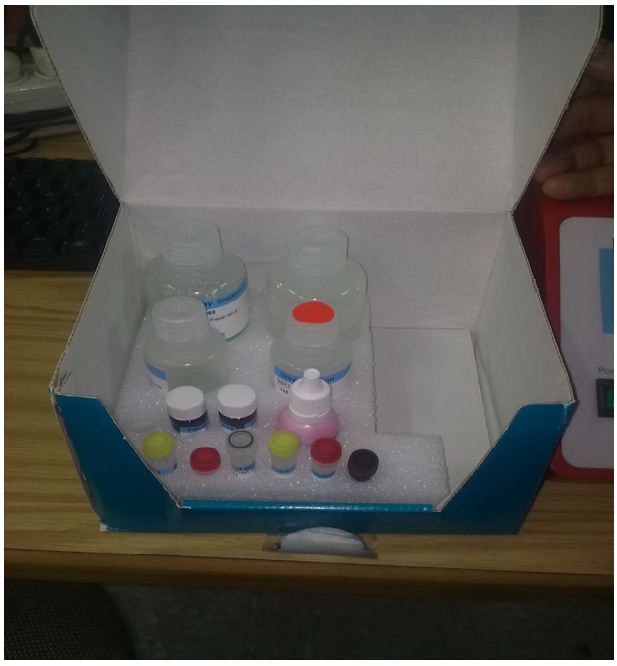


Figure 5.3; The pronto kit.

Figure 5.4;The thermocycler (PCR).

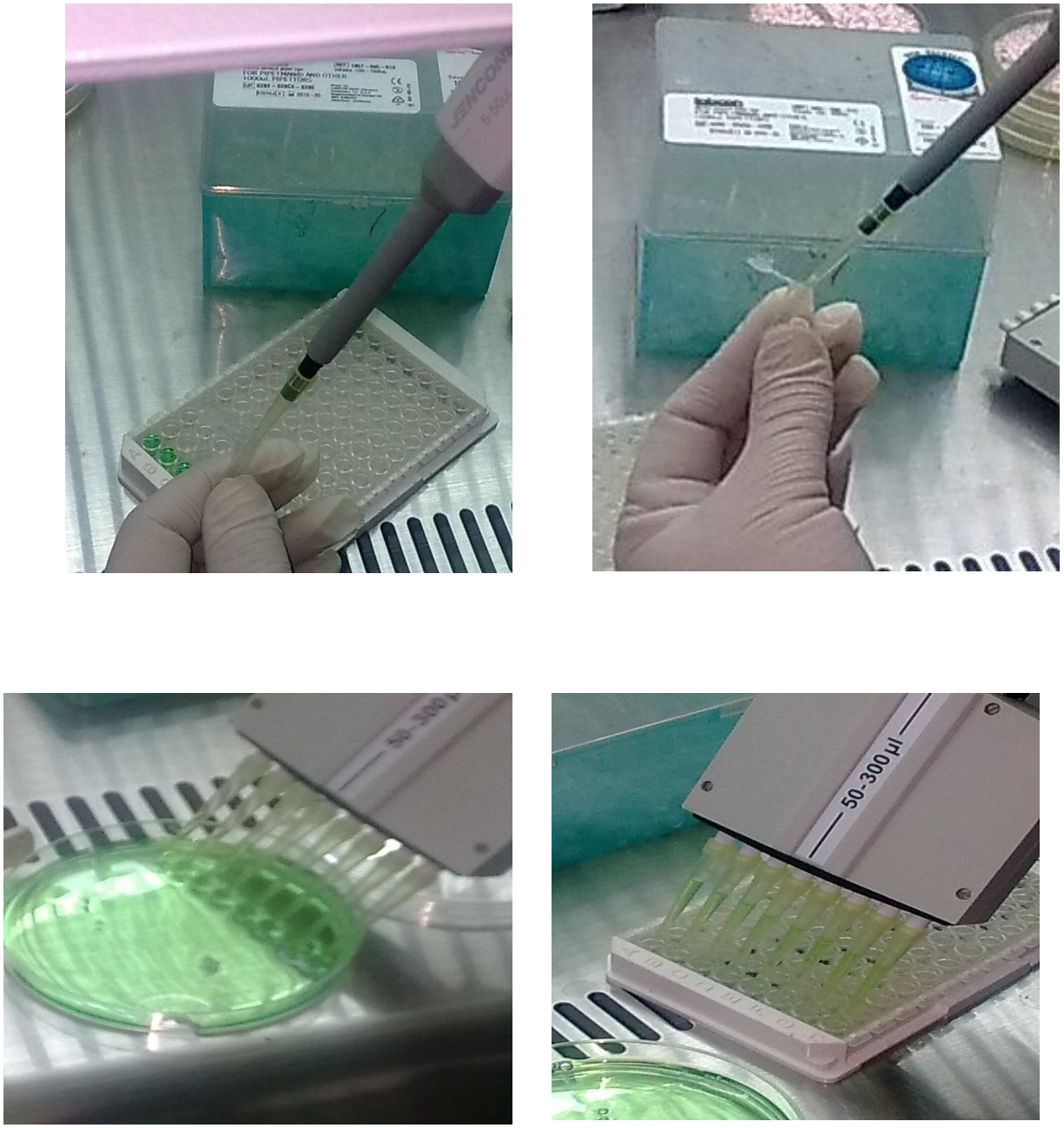


Figure 5.5; Primer extension reaction using a multichannel pipette.