Molecular Detection of Hereditary Breast Cancer Susceptibility Genes (BRCA1 / BRCA2) in Palestine

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This thesis was defended successfully on 21/5/2014 and approved by

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Dedication

To my beloved mother…

To my brothers and family …

To my lovely fiancé and his great family…

To my friends…
Acknowledgment

I would like to thank my supervisor Dr. Ashraf Sawafta for his supervision and support.

I appreciate my faculty members at An-Najah University for their help.

I deeply thank my family and friends for their encouragements.
Molecular Detection of Hereditary Breast Cancer Susceptibility Genes (\textit{BRCA1} / \textit{BRCA2}) in Palestine

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List of Abbreviations

µl Microliter
ARMS Amplification refractory mutation system
ASPCR Allele specific polymerase chain reaction
BASC BRCA1-associated surveillance complex
BC Breast cancer
BIC Breast cancer information core
BRCA1 Breast cancer susceptibility gene 1
BRCA2 Breast cancer susceptibility gene 2
CtIP Transcription repressor
DCIS Ductal carcinoma in situ
DNA Deoxyribonucleic acid
DSB Double strand break
E3 Ligase enzyme
EDTA Ethylenediaminetetraacetic acid
EMS1, CCND1 Tumor oncogenes
ER Estrogen receptor
ERBB2 Receptor-tyrosine protein kinase
FGF3 Fibroblast growth factor 2
FNA Fine needle aspiration
HER-2 Human epidermal receptor 2
HR Homologous recombination
IRB Institutional review board
LCIS Lobular carcinoma in situ
LOH Loss of heterozygosity
MOH Ministry of health
MYC Regulator gene
ng Nanogram
NHEJ Non-homologous end joining
p21 Cyclin-dependent kinase inhibitor
p53 Tumor suppressor
PARP Poly (ADP-ribose) polymerase
PHIC Palestinian health information center
PTEN, CHEK2, BRIP1, PALB2, NBS1, RAD50, MSH2, MLH Tumor suppressor genes
RABD1 BRCA-associated ring domain
RAD51 Eukaryote gene
SSB Single strand break
TAE Tris-acetate-EDTA
Molecular Detection of Hereditary Breast Cancer Susceptibility Genes (BRCA1 / BRCA2) in Palestine

By
Rawan Ahmed Hussein Dardouk
Supervisor
Dr. Ashraf Sawafta

Abstract

Background: Breast cancer is the most common cancer among population in Palestine and the most common cancer related death among women. Breast cancer occurs in hereditary and sporadic forms. Hereditary breast cancer accounts for 20-30%, while sporadic breast accounts for 70-80%. Hereditary breast cancer has some distinctive clinical features compared with sporadic breast cancer, hereditary breast cancer appear at an earlier age and also more aggressive than sporadic breast cancer. Breast cancer susceptibility genes (BRCA1/2) account for the majority of hereditary breast and ovarian cancer. The consequences of germ-line mutation of BRCA genes are serious; BRCA1 and/or BRCA2 mutation carriers have a 50%-85% lifetime risk of developing breast cancer. By 50 years of age, 45% of BRCA1 mutation carriers and 20% of BRCA2 mutation carriers will have already developed breast cancer, compared to about 3% of non-carriers.

Objectives: Screening of the most common BRCA1 and BRCA2 mutations (185delAG and 5382insC of BRCA1, and 6174delT of BRCA2) in breast cancer patients and their healthy relatives in Palestine.
Methods: A total of 64 people from West Bank (breast cancer patients and healthy relatives) were included in this study. Genomic DNA was extracted from peripheral blood samples, and then BRCA1 and BRCA2 mutations were screened by Allele-specific polymerase chain reaction (ASPCR) or also known as amplification refractory mutations system (ARMS).

Results: BRCA1 (5382insC) mutation was found with 59.4% incidence rate and no (185delAG) mutation was detected for. While BRCA2 (6174delT) mutation was found with 30% incidence rate. Carriers (heterozygous) of mutations were patients and their healthy relatives.

Conclusion: In West Bank, BRCA1 (5328insC) and BRCA2 (6174delT) mutations are founder mutations.
Chapter One
Introduction
1.1 Overview

Cancer, known medically as a malignant neoplasm, is a broad group of various diseases with many possible factors (Biological, Physical and environmental factors). It is an abnormal cell growth occurred due to multiple changes in genes controlling cell growth and division leading to dysregulation of the normal cellular program for cell division, which results in an imbalance between cell division and cell death, evolving into a population of tumor cells that invade tissues and metastasize throughout the body (1-3). At the molecular level, cancer is triggered by mutations in genes, most of these mutations are acquired in an age-dependant manner and occur in somatic cells, while other mutations are inherited in the germline cells (The gametes and their precursors) (4).

1.2 Epidemiology of Cancer in West Bank

According to the annual report (2012) published by Palestinian Health Information Center (PHIC) (5), 1802 new cancer cases were reported in West Bank, 899 cases were females (49.9%) and 903 were males (50.1%). The cancer incidence rate was 74 per 100,000 of population. The geographical distribution of reported cancer cases (Figure 1.1) shows that Bethlehem governorate reports the highest figures with an incidence rate 114.7 per 100,000 population 232 cases, while Nablus governorate ranked the second place with 399 cases and incidence rate 110.8 per 100,000 population (5).
Figure 1.1 Reported cancer cases by governorate in West Bank in 2012(5).

The above figure illustrates the cancer incidence rate per 100,000 by governorate in West Bank in 2012. Distribution from the highest to the lowest as follows (Bethlehem, Nablus, Jenin, Tulkarm, Qalqiliya, Hebron, Salfit, Ramallah and Al-Bireh, Jericho and Al-Aghwar, Tubas and Jerusalem).

According to the most common cancer cases; breast cancer ranked first with 292 reported cases, 16.2% from all reported cases (Figure 1.2). Breast cancer is the highest among females and focuses in the age group (20-59) years old. The reported figures by Ministry of Health (MOH) shows remarkable increase in cancer mortality in West Bank in 2012 compared with 2007 and 2010, mortality rate increases from (10.3%) in
2007 to (10.8%) in 2010 to (13.6 %) from the total deaths in West Bank in 2012 (5).

![Figure 1.2: Most Common Cancer Cases in West Bank in 2012 (5).](image)

In the above figure, the most reported cancer cases were breast cancer cases (16.2%) followed by colon (10.4%), lung (10.3%), Leukemia (6.1%), brain (5.8%), bladder (5.7%), prostate (4.2%), stomach (4%) and liver (3.9%) cancers respectively, while non-hodgkin lymphoma was the lowest (3.7%).

1.3 Cancer Genes

Cancer is a genetic disease, results from step-wise genetic and epigenetic alterations in major regulatory genes. The human genome contains two major classes of these regulatory genes that are necessary for normal cell growth control, which are:
a) Proto-oncogenes, code for proteins that are important for normal cellular growth regulation such as peptide growth factors, DNA binding proteins, components of the intercellular signaling pathways, cell surface receptors, components of the cell cycle progression pathways and nuclear transcription factors that control cell division and DNA synthesis (4, 6). When proto-oncogenes are activated either by point mutation, amplification, or chromosome translocation, they become oncogenes leads to uncontrollable cell growth and formation of tumors (1). Activation can also occur through environmental factors; environmental factors such as viral infection; gene amplification such that more of protein encoded by the gene is present; point mutation that enhance the function of the protein encoded by the gene (3, 7, 8). HER-2 (Human epithelial receptor 2) is an example for oncogene activation that is responsible for 20% of breast cancer cases (7).

b) Tumor suppressor genes, code for proteins that restrict uncontrollable cell growth, regulation of cellular differentiation, suppression of abnormal proliferation and DNA repair (1). Pathogenic mutations in tumor suppressor genes act by loss of function as opposed to the gain of function mutations in oncogenes (4). The role of tumor suppressor genes in cancer development is explained by the ‘two-hit hypothesis’ proposed by Alfred Knudson in 1971, the first “hit” is a germline mutation and therefore is found in all somatic cells while the second “hit” is a single cell mutation during the mitotic cell cycle (9). Tumor suppressor genes are recessive at the cellular level, requiring complete loss of function in order to reveal a
phenotype. Conversely, germline mutations of tumor suppressor genes function dominantly at the organism level, predisposing the carrier to early onset of disease by supplying one of the required two hits at birth (10).

1.4 Breast cancer (BC)

It is a form of cancer that affects the cells of breast. It’s an extremely complex, heterogeneous and multi-factorial disease caused by interaction of both genetic and possible other epigenetic factors (11). BC is divided into two types based on type of affected tissue:

a) Ductal carcinoma starts in the ducts that move milk from the breast to the nipple. Most of breast cancers are of this type.
b) Lobular carcinoma starts in the lobules that produce milk.

In rare cases, breast cancer start in other areas of breast.

Breast cancer may be invasive or noninvasive (in-situ). Invasive means it has spread to other tissues. Noninvasive means it has not yet spread thus can be classified into:

a) Ductal carcinoma in situ (DCIS), or intraductal carcinoma
b) Lobular Carcinoma in situ (LCIS) (12).

1.4.1 Epidemiology of breast cancer

Worldwide BC is the most common malignancy in women, accounting for 31% of all female cancers (13) and it is the most common cause of cancer related deaths among women, it’s incidence rates have been increased dramatically, about 1.4 million female worldwide are
diagnosed with BC annually, making early detection of a high priority in medical management of the disease (11).

1.4.2 Etiology of breast cancer

Breast cancer is considered to be the final outcome of multiple environmental, physical and genetic factors, such as: A) lesions to DNA, such as genetic mutations (14); B) exposure to carcinogens; C) levels of various hormones in the body (15); D) failure of immune surveillance; E) abnormal growth factor signaling in the interaction between stromal and epithelial cells facilitating malignant cell growth; F) inherited defects in DNA repair genes, such as BRCA1, BRCA2 and p53 (16).

1.4.3 Breast cancer diagnosis and treatment

Early detection of breast cancer using mammograms could reduce the mortality rate of the disease. Mammography as a mass screening tool is convenient, inexpensive and have become the modality choice for an early detection of breast cancers due to its sensitivity in recognizing breast masses. The positive results from the mass screening preceded to biopsy tests such as; the fine needle aspiration (FNA), core needle biopsy and surgical biopsy (17).

In general, BC treatments include, surgery when the tumor is localized, followed by chemotherapy, radiation therapy, hormone therapy for ER-positive tumors and immune therapy. Depending on clinical criteria (age, type of cancer, size, presence or absence of metastasis) patients are
roughly divided to high risk and low risk cases, with each risk category following different rules for therapy. Early accurate diagnosis is important for optimizing the treatment and potential for cure. During the last decades, breast cancer survival has increased considerably due to earlier diagnosis and increasing use of adjuvant and neo-adjuvant therapies but around 30-70% of the patients eventually develop recurrence and die of metastasis (18, 19).

1.4.4 The multi-step progression model of breast cancer

Carcinogenesis of BC can be described as a multi-step process in which each step is thought to correlate with one or more distinct mutations; mutational activation of oncogenes coupled with inactivation of tumor suppressor genes (20). Clinically and histopathologically, various steps can be identified during progression to malignancy (21), (Figure 1.3) illustrate these steps (22). The first sign of pathology is ductal hyperplasia, characterized by proliferation of unevenly distributed epithelial cells with nuclei of varying shapes, cytologically the cells are benign. The transition from hyperplasia to atypical hyperplasia is clinically associated with an increased risk of breast cancer. The next step is development of carcinoma in situ. As cells detach from the basement membrane and invade the stroma, the tumor becomes invasive. Through dissemination via blood and lymph vessels, invasive cells can give rise to metastasis, either to local lymph nodes or to distant organs. The majority of invasive carcinomas are ductal (85–95%), while invasive lobular carcinoma constitutes approximately 10% of all breast cancers (23, 24).
Figure 1.3 Model of the multi-step carcinogenesis in breast cancer (22).

1.4.5 The genetics of breast cancer

BC classified into sporadic and hereditary BC, sporadic BC result from a serial stepwise accumulation of acquired and uncorrected mutations in somatic genes, without any germline mutation playing a role. Mutational activation of oncogenes (MYC, FGF3, EMS1, CCND1 and ERBB2), often
coupled with non-mutational inactivation of tumor suppressor genes, is probably an early event in sporadic tumors, followed by more, independent mutations in at least four or five other genes (25-32). However, hereditary breast cancer is characterized by an inherited susceptibility to breast cancer on basis of an identified germline mutation in one allele of tumor suppressor genes. Inactivation of the second allele of these tumor suppressor genes would be an early event in the oncogenic pathway (Knudson’s “two-hit” model). Hereditary BC comprises approximately 20%-30% of all BC cases (Figure 1.4) (33) and appear at an earlier age and also more aggressive than normal sporadic BC (34). Genes involved in hereditary BC are:

a) Breast cancer susceptibility genes (BRCA1 and BRCA2). Two high penetrance genes, responsible of 20% of hereditary BC cases (35, 33).

b) p53 (TP53), PTEN, and ATM are rarely associated with hereditary BC. Carriers of p53 mutations develop Li-Fraumeni syndrome and are at high risk of developing early-onset breast cancer, but these mutations are very rare (33).

c) Low to moderate risk genes such as CHEK2, BRIPI, PALB2, NBS1, RAD50, and the mismatch repair genes MSH2 and MLH (36).
In the above figure BRCA1 and BRCA2 are two major high penetrance genes associated with hereditary breast and ovarian cancer syndrome. Mutations in CHEK2 contribute to a large proportion of hereditary BC. TP53 mutations are very rare. Mutations in other genes, such as PTEN, ATM and MSH2/MLH1, are also rare causes of inherited breast cancer and about half of the hereditary BC is unexplained.

Thus, whereas the contribution of other genes to early onset and hereditary BC remains to be clarified, genetic testing for BRCA1 and BRCA2 has become standard of care and an important component of personalized BC risk assessment and prevention.

1.4.5.1 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. In 1994, the breast cancer susceptibility gene BRCA1 was identified by
positional cloning, mapped to chromosome 17q21 (37, 38). This gene is expressed in numerous tissues, including breast and ovary. BRCA1 gene is a large gene spread over approximately 100 kb of genome and composed of 24 exons, exon 1 and exon 4 are non-coding and are not analyzed (37, 39), encodes 1863 amino acid nuclear protein which has a RING finger domain at the amino terminus involved in mediating protein-protein or protein-DNA interactions and BRCA-associated ring domain (RABD1) at the carboxy terminus (4).

In 1995, the BRCA2 gene was identified and mapped to chromosome 13q12.3 (36, 37). BRCA2 gene is even larger than BRCA1, consists of 27 exons, exon 1 is non-coding and is not analyzed. It’s encoding a 3418 amino acid protein. BRCA2 is a histone acetyl transferase that may be involved in regulation of transcription (4).

1.4.5.2 Functions of BRCA1 and BRCA2

a) DNA repair

It is believed that DNA double strand breaks (DSB) are the main cause of genomic instability and chromosomal rearrangements that lead to cancer. In eukaryotes, there are two primary mechanisms of DNA DSB repair: homologous recombination (HR) and non-homologous end joining (NHEJ). HR is used in cells during the S and G2 phases of the cell cycle when an intact sister chromatid is available as template. NHEJ is a process of ligating DSB ends together without a homologous template and therefore is considered an error-prone mechanism. The protection of the
genome from HR involves damage recognition, signal mediation and inititation of repair (Figure 1.5) (40), BRCA1 plays a role in signal mediation while BRCA2 and RAD51 in initiation of repair, which suggests that it is the BRCA1-BRCA2-HR pathway that suppresses tumorgenesis (35, 41).

![Figure 1.5 Model for the role of BRCA1 and BRCA2 in double strand break repair (40).](image)

Moynahan et al. (42) and Snouwaert et al. (43) provided direct evidence linking BRCA1 to HR by showing a significant impairment of homologous repair in BRCA1-deficient mouse embryonic stem cells. This impairment can be corrected by re-expression of wild-type BRCA1 (42, 43). Snouwaert et al. also reported an increase in the frequency of NHEJ in BRCA1-deficient cells (43).

b) Cell cycle control

Cell cycle checkpoints are surveillance mechanisms that stop cell cycle progression until DNA is intact to ensure genomic fidelity. BASC (BRCA1-associated surveillance complex) is a complex of BRCA1 along
with other proteins that are involved in DNA repair and cell cycle checkpoint control (42, 44).

c) Transcription and chromatin remodeling

Several DNA damage-responsive gene and regulator of cell cycle checkpoints are transcriptionally regulated by BRCA1. Activation or repression of these genes depends on the interaction between BRCA1 and the transcriptional repressor CtIP (45, 46). BRCA1 up-regulates tumor suppressors such as p53 and p53-regulated genes (p21) and represses cell proliferation genes (45, 47).

d) Ubiquitination

Ubiquitination, a post-translational modification process of covalently attaching ubiquitin groups to lysine residues in proteins, targets those proteins for destruction by the proteosome. BRCA1 play a role in ubiquitination due to its E3 ligase activity that’s localized to the RING finger domain at the nitrogen terminus (37, 38).

1.4.5.3 Mutations in BRCA1 and BRCA2

It is now well accepted that among all populations, an estimated 5% to 10% of breast cancer cases arise in individuals who inherit highly penetrated mutations in BC susceptibility genes (BRCA1/BRCA2) genes (48-50).

Both BRCA1 and BRCA2 are considered tumor suppressor genes because their wild-type alleles protect against breast cancer and their
mutant alleles are recessive (51). People with the mutation (in the first allele) are likely to acquire a second mutation (in the second allele), leading to expression of the cancer (Knudson two-hit theory). A mutated BRCA gene can be inherited from either parent, thus they are classified as hereditary or germline mutations rather than acquired or somatic mutations. Cancer caused by a mutated gene inherited from an individual's parents is a hereditary cancer rather than sporadic cancer (52).

Humans have a diploid genome; each cell has two copies of the gene (one from each biological parent). Inherited BRCA mutation means, only one copy contains a mutation and the affected person is heterozygous for the mutation. However, if the functional copy is harmed, then the cell will use alternate DNA repair mechanisms, which are more error-prone leading to cancerous transformation of the cell. The loss of the functional copy is called loss of heterozygosity (LOH) (53).

The population-based Australian breast cancer family study (Figure 1.8) showed that among women with two or more relatives with breast and/or ovarian cancer, about 1 in 2 have a BRCA1 or BRCA2 mutation and among women with at least one relative, about 1 in 18 have a detectable mutation (54, 55).
Mutations in both genes are spread throughout the entire gene. More than 600 different mutations have been identified in \textit{BRCA1} gene and 450 mutations in \textit{BRCA2}. However, not all mutants are at high risk; some are harmless variations but the majority of mutations, known to be disease-causing, results in a truncated protein due to frame shift, nonsense, or splice site alternations. Nonsense mutations occur when the nucleotide substitution produces a stop codon (TGA, TAA, or TAG) and translation of the protein is terminated at this point. Frame shift mutations occur when one or more nucleotides are either inserted or deleted, resulting in missing or non-functional protein. Splice site mutations cause abnormal inclusion or exclusion of DNA in the coding sequence, resulting in an abnormal protein. Another kind of mutations resulting from a single nucleotide substitution is missense mutations in which the substitution changes a
single amino acid but does not affect the remainder of the protein translation (56, 57).

*BRCA1* and/or *BRCA2* positive women have a 50% to 85% lifetime risk of developing breast cancer and 15% to 65% risk of developing ovarian cancer, beginning at age 25. However, *BRCA1* mutations have a higher risk of developing breast and ovarian cancer than *BRCA2* mutations. Harmful *BRCA1* mutations may also increase a woman’s risk of other cancers (cervical, uterine, pancreatic, and colon cancer) (58, 59). Harmful *BRCA2* mutations may additionally increase a woman’s risk of pancreatic cancer, stomach cancer, gallbladder and bile duct cancer, and melanoma (60). Men with harmful *BRCA1/2* mutations also have an increased risk of breast cancer and, possibly, of pancreatic cancer, testicular and early-onset prostate cancer (59).

*BRCA* mutations are linked to breast, ovarian cancer and other types of cancer but it’s linked to breast and ovarian cancer in a higher percentage than other types of cancer, because the growth of breast and ovary tissues are hormonally driven, and this process produces reactive oxygen species, which cause measurable oxidative DNA damage. The consequence of oxidative DNA damage is the production of a subset of lesions that cause DNA replication stress and result in DSBs that demands the use of the *BRCA1–BRCA2–HR* pathway (61, 62).

Distinct somatic genetic changes have been found to be associated with tumor progression in carriers of *BRCA1* and *BRCA2* germline
mutation (63). These somatic genetic mutations found in hereditary BC seem to be both quantitatively and qualitatively different from those involved in sporadic BC progression. In sporadic BC, non-mutational disregulation or suppression of BRCA1/2 (64-72), such as hypermethylation of the BRCA1 promoter (72) or binding of BRCA2 by EMSY (73).

Different ethnic and geographical regions have different BRCA1 and BRCA2 mutation spectrum and prevalence. Several studies are carried out worldwide for analysis and identification of BRCA1/2 mutations using different molecular tools including, allele-specific oligonucleotide hybridization, allele-specific PCR, PCR-mediated site-directed mutagenesis, single-strand conformation polymorphism, the protein truncation test and DNA sequencing. Among these studies several founder mutations have been identified in BRCA1/2, represented in Table 1.1. The two most common mutations in BRCA1 are 185delAG and 5382insC) (74), which account for approximately 10% of all the mutations seen in BRCA1. These two mutations occur at a 10-fold higher frequency in the Ashkenazi Jewish population (75, 76) than in non-Jewish Caucasians.
Table 1.1 Selected examples of recurrent and founder mutations in \( BRCA1/2 \) genes.

<table>
<thead>
<tr>
<th>Population</th>
<th>( BRCA1 ) mutations</th>
<th>( BRCA2 ) mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palestinian ‘Gaza’ (77)</td>
<td>185delAG</td>
<td>6174delT</td>
</tr>
<tr>
<td></td>
<td>5382insC</td>
<td></td>
</tr>
<tr>
<td>Ashkenazi Jewish (78)</td>
<td>185delAG</td>
<td>6174delT</td>
</tr>
<tr>
<td></td>
<td>5382insC</td>
<td></td>
</tr>
<tr>
<td>Romanian (79)</td>
<td>5382insC</td>
<td></td>
</tr>
<tr>
<td>Tunisian (80)</td>
<td>1294del40</td>
<td></td>
</tr>
<tr>
<td>Jordanian (81)</td>
<td>Sequence variations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in Exons (2, 11)</td>
<td></td>
</tr>
<tr>
<td>Ukrainian (82)</td>
<td>5382insC</td>
<td></td>
</tr>
<tr>
<td>Egyptian (83)</td>
<td>185delAG</td>
<td></td>
</tr>
<tr>
<td>Lebanese (84)</td>
<td>Deleterious mutations</td>
<td>Deleterious mutations</td>
</tr>
</tbody>
</table>

The above table represents founder mutations among selected population. Founder mutation, it is a gene mutation observed with high frequency in a group that is or was geographically or culturally isolated, in which one or more of the ancestors was a carrier of the mutant gene. This phenomenon is often called a founder effect (85).

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. A study was conducted to delineate the genetic component of BC/ OC among the Palestinian population (86). 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 \textit{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 Palestinian population (86).

1.5 \textbf{Risk reduction for \textit{BRCA1/2} mutation carriers}

The option for whom at risk for hereditary breast and/or ovarian cancer is prophylactic surgery (bilateral mastectomy and / or oophorectomy). Bilateral prophylactic salpingo-oophorectomy is widely used for cancer risk reduction in premenopausal women with \textit{BRCA1/2} mutations, reduces breast cancer risk by approximately 50\% and ovarian cancer risk by 80\% to 95\% (87-89).

1.6 \textbf{Targeted therapy for \textit{BRCA1/2} tumor}

The first study verifying targeted cancer therapy for \textit{BRCA}-tumor was published in 2005, after it had been demonstrated that HR-defective \textit{BRCA1}- or \textit{BRCA2}-deficient cell lines display dramatically increased sensitivity to inhibition of the single strand break (SSB) repair enzyme PARP (Poly (ADP-ribose) polymerase ) (90, 91). The inhibition of PARP leads to the accumulation of SSBs which are converted into DSBs upon encountering DNA replication forks during S-phase when HR is most active. Consequently, in cancer cells lacking \textit{BRCA1} or \textit{BRCA2}, which means absence of HR, PARP inhibition results in the accumulation of DSBs and, ultimately, in apoptosis. Importantly, normal cells survive the
treatment owing to functional HR, providing the kind of selectivity that is considered the ultimate goal of cancer therapy (92).

1.7 Objectives

The general objective of this study is to investigate the incidence of \textit{BRCA1} and \textit{BRCA2} mutations (185delAG and 5382insC of \textit{BRCA1}, and 6174delT of \textit{BRCA2}) in West Bank familial cases of breast cancer in an attempt to establish a genetic profile for this population. This information will facilitate \textit{BRCA1} and \textit{BRCA2} mutational screening in the West Bank population and identify individuals at high risk, who will then be able to seek genetic counseling.
Chapter Two
Materials and Methods
2.1 Study population

The study population was 46 samples; composed of 50 BC patients (49 females, 1 male) and 14 healthy female at high risk of hereditary BC. The population was from West Bank.

2.2 Permission and ethical consideration

According to research ethics, permission was obtained from Institutional Review Board (IRB) and MOH. The objective of the study was explained to all participants and their consent was obtained.

2.3 Materials

2.3.1 Chemicals and Reagents

The chemicals and reagents used in this study are listed below:

- Promega DNA extraction kit
- Absolute Ethanol
- Isopropanol Alcohol
- 2X Ready Mix PCR master mix (1.5 mM MgCl2)
- Direct load step ladder, 50bp
- Ultra-pure Agarose
- TAE buffer
- Ethidium bromide
2.3.2 Disposables

The major disposables used in this study are listed below:

- 2.5 EDTA tubes
- Disposable powder gloves
- Micro tubes, 1.5 ml capacity
- PCR micro tubes, 0.2 ml capacity
- Micropipette tips

2.3.3 Equipments

All experiments of this study were done in the research laboratory of An-Najah National University, biology/biotechnology department. The major equipment’s that were used are listed below:

- Microcentrifuge
- Thermocycler
- Refrigerator 20°C
- Vortex mixer
- Safety capnet
- Micropipette
- Microwave
- Electrophoresis
- UV-Transilluminator
2.4 Methods

2.4.1 Case selection

Selection of patients was mainly based on the following criteria: any patient with BC diagnosed under the age of 55 years; any patient having a family history of BC; any patient having a previous personal history of BC.

2.4.2 Blood sample collection

Peripheral blood sample (5-10 ml) was collected in EDTA tubes, by Palestinian hospitals nurse staff.

2.4.3 DNA extraction

Genomic DNA was extracted from peripheral blood using Promega DNA extraction kit, a rapid procedure for isolating DNA that is ready for direct use in polymerase chain reaction (PCR) according to manufacture protocol as follows:

A) Red blood cell and nuclei lysis

1. 900 µl of cell lysis solution were added to 300-µl blood in 1.5 ml centrifuge tube (mixed by incersion) then it was incubated for 10 minutes at room temperature.
2. The tube was centrifuged at 13000 xg for 20 seconds.
3. The resultants supernatant was discarded and pellet re-suspended by vortex.
4. 300 µl of nuclei lysis solution were added and mixed by pipetting.
B) Protein precipitation

1. 100 µl of protein precipitation solution were added then vortex for 20 seconds.
2. The tube was centrifuged at 13000 xg for 3 minutes.

C) DNA precipitation and rehydration

1. The supernatant was transferred to a new tube containing 300 µl isopropanol and mixed.
2. The tube was centrifuged at 13000 xg for 1 minute.
3. Supernatant was discarded, and then 300 µl of 70% ethanol were added.
4. The tube was centrifuged at 13000 xg for 1 minute.
5. The ethanol was aspirated and air-dried the pellet (10-15 minutes).
6. The DNA was rehydrated in 100 µl of DNA rehydration solution for 1 hour at 65°C or over night at 4°C then it was stored at -20°C for further use.

2.4.4 DNA check

Extracted DNA was checked on 0.7% agarose gel. DNA concentration was estimated by spectrophotometer measurements.
2.4.5 Mutation screening

Three mutations were screened in this study, summarized in Table 2.1.

Table 2.1 list of mutations positions and variation type.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gene position</th>
<th>Variation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>185delAG</td>
<td>BRCA1 Exon 2</td>
<td>Truncated protein</td>
</tr>
<tr>
<td>5382insC</td>
<td>BRCA1 Exon 20</td>
<td>C-terminal deletion (truncated protein)</td>
</tr>
<tr>
<td>6174delT</td>
<td>BRCA2 Exon 11</td>
<td>Framshift (truncated protein)</td>
</tr>
</tbody>
</table>

2.4.5.1 Amplification-refractory mutations system (ARMS)

The amplification-refractory mutation system (ARMS), also known as allele-specific polymerase chain reaction (ASPCR) or PCR amplification of specific alleles, is a simple, rapid, and reliable method for detecting any mutation involving single base changes or small deletions. ARMS technique is based on the use of sequence-specific PCR primers that allow amplification of target DNA only when the target allele is contained within the sample and will not amplify the non-target allele. Following an ARMS reaction, the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele. The ARMS technique is based on the observation that oligonucleotides that are complementary to a given DNA sequence except for a mismatched 3’ terminus will not function as PCR primers under appropriate conditions. An example is given in figure 2.1 below (93).
In the above figure, for the mutant-specific primer (M), the 3’ terminal base of the ARMS primer should be complementary to the mutation; for the normal-specific primer (N), the 3’ terminal base should be complementary to the corresponding normal sequence. The base that is altered is indicated in the normal and mutant DNA sequences by a box. The presence of an arrow indicates that primer/target combinations can be extended by Taq DNA polymerase; an “X” indicates extension does not occur. Bases in the ARMS primers that are not complementary to the target are shown displaced from the target sequence. A single mismatch (in this case a C/C) at the 3’ end is not sufficient to prevent extension whereas a primer with two adjacent mismatches, at the terminal and the penultimate base, is not extended.

In this study, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) presented in table 2.2 were designed for each mutation. The mutant and wild-type primers differed by ~20 bp in size, so the size of amplified mutant and wild-type segments differed by ~20 bp.
Table 2.2 Nucleotide sequences of the primers used for screening of three mutations.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
<th>Expected fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA1 185delAG</strong></td>
<td>5’ GGTTGGCAGCAATATGTGAA 3’</td>
<td></td>
</tr>
<tr>
<td>Common forward (p1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type reverse (p2)</td>
<td>5’ GCTGACTTACCAGATGGGACTCTC 3’</td>
<td></td>
</tr>
<tr>
<td>Mutant reverse (P3)</td>
<td>5’CCCAATTAATACACTCTTGTGACTTACCAGATGGGACAGTA 3’</td>
<td>335 bp</td>
</tr>
<tr>
<td>BRCA1 5382insC</td>
<td>5’ GACGGGAATCCAAATTACACAG 3’</td>
<td></td>
</tr>
<tr>
<td>Common reverse(p1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type forward (p2)</td>
<td>5’ AAAGCGAGCAAGAGAATCGCA 3’</td>
<td></td>
</tr>
<tr>
<td>Mutant forward (p3)</td>
<td>5’AATCGAAGAAAAACCACAAAGCTTACGCGAGCAAGAGAATCACC 3’</td>
<td>271 bp</td>
</tr>
<tr>
<td><strong>BRCA2 6174delT</strong></td>
<td>5’ AGCTGGTCTGAATGTTCTACT3’</td>
<td></td>
</tr>
<tr>
<td>Common reverse (p1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type forward (p2)</td>
<td>5’ GTGGGATTTTTAGCACAGCTAGT 3’</td>
<td></td>
</tr>
<tr>
<td>Mutant forward (p3)</td>
<td>5’CAGTCTCATCTGCAAATACTTCAGGGATTTTAGCACACAGCATGG 3’</td>
<td>151 bp</td>
</tr>
</tbody>
</table>
The figures below illustrate the positions of primers for each corresponding mutations.

Common Forward (p1)
5’--------------
93601 tctgtagctt tctctttctt ggagaagga aaagacccaa gggttggca gcaatatgtg
93611 aaataattca gaatttatgt tgtctaatta caaaaagcaa cttctagaat ctttaaaat
93721 aagagccgttt tcattagtct tttggttgg tattattctta aaccttcca aattcttaaat
93811 ttacctttatt ttaaatgtat aaatgaagt tgcataatcat taaaacctttt aaaaagatat
93841 atatatagtgt ttcttcatat tgttaaaggt cattggaaca gaagaaatg gatttatcctg
Del.
93901 cctcttcggtgt tgaagaatga caaaatgtca ttaatgtcat gcagaaaattt ttagAGtgc
93961 ccatttggta agtcagcaaa agagtgttact tttttgggat tcctatgatt atctcctatg

Wild-type Reverse (p2)
5’------------------------------
Mutant Reverse (p3)

Figure 2.2 Positions of respective primers on BRCA1 exon 2.

In the above figure, FASTA sequence of BRCA1 exon 2 from Gene Bank. Mutation (185delAG) position is shown; deleted A and G nucleotides are in bold and capitalized letter. Primers sequences in bold letters, mutant-reverse primer was designed without A and G.
Mutant forward (p3)
5’----------------------------------
Wild type forward (p2)
--------------------
160861 gtcagaggag atgtggtc aa tggagaagg caccaaggtc caaagcgagc aagagagac
    Ins. C
160921 Caggacagaa aggttaagct ccctccctca agttgacaaa aatctcacc caccactcctg
160981 tattccactc cccctttgcaq agatgggcgg cttcattttg taagacttat tacatacata
161041 cacagtgcta gatactttca cacaggttct tttttcactc tttccatcaca accacataaa
161101 taagtattgt ctctacttta tgaatgataa aactaagaga tttagagagg cgtgtaatt
    3’←
161161 tggattcccg tc cgggttc agatcttagc tgataagtgg aagagctggg actttaagca
    ----------= 5’
Common reverse (p1)

**Figure 2.3** Positions of respective primers on *BRCA1* exon 20.

In the above figure, FASTA sequence of *BRCA1* exon 20 from Gene Bank. Mutation (5382insC) position is shown; insertion of C nucleotide after the capitalized C nucleotide. Primers sequences in bold letters, mutant-forward primer was designed with inserted C.

Mutant forward (p3)
5’----------------------------------
Wild type forward (p2)
5’--------------------
29761 tagggaagct tcataagt ca gtctcatctg caaataccttg tggatatattt agcagacaca
    3’
    Del.
298221 gTgggaatc tgctccaggt aattacaaaa cgcaagacaa gttttttcttg tcagatgctt
29881 aaataagaag tagtaccaag caaagtctttt ccaaaggtatt gtttaaaggt aacgaacatt
    3’←
29941 cacagcagct caaagagaa gaaaatacctg ctatacgatc tccgaacat ttaatatccc
    ----------= 5’
Common reverse (p1)

**Figure 2.4** Positions of respective primers on *BRCA2* exon 11.
In the above figure, FASTA sequence of *BRCA2* exon 11 from Gene Bank. Mutation (6174delT) position is shown; the deleted T nucleotide is in bold and capitalized. Primers sequences in bold letters, mutant-forward primer was designed without T.

ASPCR was performed in a 25 µl volume using 1X Ready mix PCR master mix (0.625 u ThermoPrime Taq DNA polymerase, 75 mM Tris-HCl (pH 8.8 at 25 °C), 20mM (NH4)SO4, 1.5mM MgCl2, 0.01% (v/v) Tween 20, 0.2 mM each of dATP, dCTP, dGTP and dTTP, precipiant and red dye for electrophoresis. The concentrations of primers used were 0.2 µM of each primer.

The amplifying program was as follows; each ASPCR reaction consisted of an initial denaturation of 10 min at 94 °C, followed by 35 cycles of 30 sec of denaturation at 94 °C, 30 sec of annealing at 57 °C, and 45 sec of extension at 72 °C, and a final extension step of 10 min at 72 °C.

### 2.4.5.2 Genotyping

PCR products were separated by electrophoresis on 2% agarose gel (70V, 90 minutes) and stained with ethidium bromide.
Chapter Three

Results and Discussion
3.1 DNA check

Extracted DNA was checked by Gel electrophoresis (figure 3.1) and by spectrophotometer (Table 3.1). The range of DNA concentration for all samples was (15-223 µg/µl).

![Figure 3.1](image)

Figure 3.1 Representative samples for the extracted genomic DNA.

The above figure shows the separation of extracted genomic DNA for ten samples by gel electrophoresis (0.7% agarose gel, 70V, 120 min).

Table 3.1 Spectrophotometer measurements for representative samples.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>A(λ260)</th>
<th>A(λ260/280)</th>
<th>DNA conc. (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.223</td>
<td>0.996</td>
<td>223</td>
</tr>
<tr>
<td>2</td>
<td>0.136</td>
<td>1.051</td>
<td>136</td>
</tr>
<tr>
<td>3</td>
<td>0.049</td>
<td>1.061</td>
<td>49</td>
</tr>
<tr>
<td>18</td>
<td>0.107</td>
<td>1.961</td>
<td>107</td>
</tr>
<tr>
<td>19</td>
<td>0.055</td>
<td>2.683</td>
<td>55</td>
</tr>
<tr>
<td>20</td>
<td>0.033</td>
<td>2.439</td>
<td>33</td>
</tr>
<tr>
<td>29</td>
<td>0.069</td>
<td>2.327</td>
<td>69</td>
</tr>
<tr>
<td>32</td>
<td>0.065</td>
<td>2.19</td>
<td>65</td>
</tr>
<tr>
<td>57</td>
<td>0.147</td>
<td>1.963</td>
<td>147</td>
</tr>
<tr>
<td>61</td>
<td>0.104</td>
<td>1.367</td>
<td>104</td>
</tr>
</tbody>
</table>
In the above table, quantitative (Absorbance on λ260) and qualitative (Ratio of absorbance λ 260/ λ 280) results were obtained for extracted DNA. While quantity of DNA samples was good, unexpectedly, the purity (quality) was not, since it is a genomic DNA extracted from whole blood.

DNA concentration was estimated following the equation:

\[
\text{OD}_{260} \times 50 \text{ng/µl} \times \text{dilution factor.}
\]

### 3.2 BRCA1 mutations

**A. 185delAG**

From 64 DNA samples, the number of samples with normal homozygous (Both alleles are normal) was 56 and no mutation was found in any sample (Table 3.2).

**Table 3.2 Percentage of wild-type alleles and mutant alleles for 185delAG mutation.**

<table>
<thead>
<tr>
<th>185delAG</th>
<th>Number</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (w)</td>
<td>56</td>
<td>87.5%</td>
</tr>
<tr>
<td>Mutated (m)</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>12.5%</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>100%</td>
</tr>
</tbody>
</table>

Amplification with wild-type specific primers (p1, p2) gave 335 bp bands (Figure 3.1, all lanes); according to bands size, it represents the normal exon sequence size without mutation (deletion of AG nucleotides).
Figure 3.2 Representative samples for amplification of *BRCA1* wild-type allele by wild-type specific primers.

In the above figure, ASPCR products were checked by gel electrophoresis (2% agarose gel, 70 V, 90 min.). M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the figure. In all lanes, 335 bp amplified PCR products were detected; normal gene sequence. C refers to no-template control (proving the lack of contamination).

Mutation screening was carried by ASPCR using mutant specific primers (p1, P3); negative results were detected for all samples, i.e., absence of this type of sequence alterations on exon 2; normal gene sequence.
Figure 3.3 Representative samples for amplification of *BRCA1* 185delAG mutation by mutant specific primers.

In the above figure, ASPCR products were checked by gel electrophoresis (2% of agarose gel, 70 V, 90 min.). M refers to 50bp DNA ladder. C refers to no-template control (proving the lack of contamination). In all lanes, negative amplification results are shown; no mutations.

8 samples were negative for ASPCR reactions with wild-type specific primers and mutant-specific primers, indicating the DNA was not amplifiable for this type of primers, even after dilution to reduce PCR inhibitors.
B. 5382insC

Among 64 DNA sample, the number of samples with 5328insC mutation was 38; all 38 samples were heterozygous, which means, mutation present in one allele while the second allele is normal, those who are heterozygous for mutation are carriers of mutation. 26 of samples have wild-type alleles (both alleles are normal) (Table 3.3).

**Table 3.3 Percentage of BRCA1 5382insC mutation.**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>26</td>
<td>40.6%</td>
</tr>
<tr>
<td>Heterozygous (5382insC)</td>
<td>38</td>
<td>59.4%</td>
</tr>
<tr>
<td>Homozygous (5382insC)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>100%</td>
</tr>
</tbody>
</table>

In the above table; the frequency of 5382insC mutation was 59.4%. Mutation was found in one allele only (heterozygous). However, 40.6% of the study population was normal homozygous (Both alleles are normal).

Amplification using wild-type specific primers (P1, P2), result in 271 bp fragment, i.e, normal exon sequence without alterations (Figure 3.3). While amplification by mutant-specific primers was carried out to
screen for mutation, 295 bp fragments were observed, i.e., presence of mutation (Figures 3.4 and 3.5).

Figure 3.4 Representative samples for heterozygous mutation results.

In the above figure; gel electrophoresis (2% of agarose gel, 70 V, 90 min.) for heterozygous samples. M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the figure. Two lanes represent the sample; lane 21W (wild-type specific amplification, 271bp fragment) and 21M (mutant-specific amplification 295bp fragment). Wild-type fragments are in lanes (21w, 22w, 24w, 28w, 29w and 30w). Mutant fragments are in lanes (21m, 22m, 24m, 28m, 29m, and 30m). Cw and Cm refers to no-template controls for both amplifications (proving the lack of contamination).
Among the 38 heterozygous samples (Table 3.4); the number of patients were 30 female and 1 male, 7 samples were healthy female relatives, which mean it is an inherited mutation.

![Figure 3.5 Representative samples for mplification of BRCA1 by wild-type specific primers.](image)

In the above figure, ASPCR products were checked by gel electrophoresis (2% of agarose gel, 70 V, 90 min.). M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the figure. C refers to no-template control (proving the lack of contamination). In all lanes, 271 bp ASPCR products were detected (normal gene).
Figure 3.6 Representative samples for amplification of *BRCA1* 3582insC mutation by mutant specific primers.

In the above figure, ASPCR products were checked by gel electrophoresis (2% of agarose gel, 70 V, 90 min.). M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the panel. C refers to no-template control (proving the lack of contamination). In lanes 60, 61, 62 and 63, 295bp fragments (presence of mutation in these samples).
Table 3.4 Distribution of heterozygous mutation results in study population.

<table>
<thead>
<tr>
<th></th>
<th>5382insC (heterozygous)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>30 women, 1 man</td>
<td>81.6%</td>
</tr>
<tr>
<td>Healthy relatives</td>
<td>7 female</td>
<td>18.4%</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>100%</td>
</tr>
</tbody>
</table>

The above table shows the distribution of the resulted heterozygous mutation among study population; 81.6% were patients, while 18.4% were healthy relatives. It is an inherited germ line mutation, since it was detected in patients and their healthy relatives.

3.3 BRCA2 mutation (6174delT)

Ten samples were screened for 6174delT mutation in BRCA2 (exon 11). From these 10 samples; 7 samples were normal (both alleles are normal) and 3 were heterozygous for mutation (One allele is mutated and the second is not), the results illustrated in table 3.5, figure 3.7 and figure 3.8.
Table 3.5 Percentage of *BRCA2* 6174delT mutation

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wil-type</td>
<td>7</td>
<td>70%</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td><em>(6174delT)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><em>(6174delT)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Figure 3.7* Amplification of *BRCA2* by wild-type specific primers.
In the above figure, ASPCR products were checked by gel electrophoresis (2% of agarose gel, 70 V, 90 min.). M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the panel. C refers to no-template control (proving the lack of contamination). In lanes 3, 17, 19, 20, 21, 29, 31, 32, 34 and 35, 151bp fragments (normal gene sequence without any mutation). However, in lane 3, faint product was detected.

![Image](image.png)

**Figure 3.8** Amplification of *BRCA2* exon 11 by mutant specific primers and by wild-type specific primers (heterozygous results).

In the above figure; gel electrophoresis (2% of agarose gel, 70 V, 90 min.) for heterozygous results (one allele is mutated while the other is normal for same the sample). M refers to 50bp DNA ladder; the molecular sizes (bp) of the DNA marker are shown on the left side of the figure. Two lanes represent the sample; lane 19w (wild-type specific amplification,
151bp fragment) and 19m (mutant-specific amplification 171bp fragment). Wild-type fragments are in lanes (19w, 29w and 34w). Mutant fragments are in lanes (19m, 29m and 34m). C refers to no-template controls for both amplifications (proving the lack of contamination).

Among the 3 heterozygous samples; 2 samples were for patients (female) and 1 sample was for healthy female relative, which mean it an inherited mutation. And also, the 2 heterozygous samples for BRCA2 6174delT mutation are heterozygous for 5382insC BRCA1 mutation too.

3.4 Discussion

Efforts are underway to reduce the high incidence and mortality associated with BC, which can be achieved by the early detection of women at high risk. Since genetic predisposition is the strongest risk factor, molecular testing can be considered as the only way for early detection of BC. DNA testing for BC susceptibility became an option after the identification of the BRCA1 and BRCA2 genes. Germline mutations in either of the two predisposing genes, BRCA1 and BRCA2, account for a significant proportion of hereditary BC. Generally, it has not been possible for clinician to determine which individual in a high risk families are carriers of BRCA mutations. So the availability of the BRCA analysis has beneficial impact on the care and counseling of women at risk.

Like other PCR techniques, allele specific PCR or ARMS technique was required careful optimization of each reaction condition, including
magnesium concentration, cosolvents (dimethyl sulfoxide or glycerol), length and temperature of cycling stages. More importantly, the concentrations (relative and absolute) of individual primers were determined empirically to give relatively optimum amplification.

**BRCA** mutational spectrum has not been entirely characterized. Over one thousand small sequence variations have been reported in the Breast Cancer Information Core (BIC) database (94). More than half of these mutations (over 300 in **BRCA1** and 200 in **BRCA2**) cause the lost of function by premature protein synthesis termination (95). Different ethnic and geographical regions have different **BRCA1** and **BRCA2** mutation spectrum and prevalence.

The **BRCA1** (185delAG, 5328insC) mutations and **BRCA2** 6174delT mutation are perfect candidate for molecular screening; these mutations predispose to the majority of hereditary breast and ovarian cancer (HBOC). These mutations were previously described as Ashkenazi Jewish founder mutations (58), also found frequently in other populations, include, Palestine-Gaza (77), Egypt (83), Poland (96), Belarus (97), Hungary (98) and Russia (99). 185delAG is the most frequent mutation after 5382insC. At a lesser extent, the mutations were detected in Greece (100) and Turkey (101).

In our results; 5382insC mutation in **BRCA1** was detected in a high percentage (59.4%) among study population, with no 185delAG mutation in **BRCA1**. However, in **BRCA2** (6174delT) mutation was detected in a
moderate percentage (30%). As expected, \textit{BRCA1} (185delAG) mutation was detected in male breast cancer patient. The \textit{BRCA1} 185delAG mutation is a perfect candidate for molecular screening, as recurrent in many populations, but unexpectedly we observed that neither the patients nor the healthy relatives had any of this type of mutation (185delAG). That’s because, types and frequencies of \textit{BRCA} mutations vary among different ethnic and geographical regions.

The prevalence of 5328insC and 6174delT mutations in both patients (hereditary breast cancer cases) and their healthy relatives, confirms that it is an inherited germ-line mutations.

\textbf{3.5 Conclusion and Recommendation}

ARMS assay eliminates the need for radioisotopes, endonuclease digestion and high resolution electrophoresis. We conclude that this method is simple, reliable and can be considered for routine use.

In conclusion, \textit{BRCA1} mutation (5382insC) and \textit{BRCA2} (6174delT) are a founder mutations among West Bank population. The frequency of \textit{BRCA1} (5382insC) mutation was 59.4%, 81.6% of mutation was found in patients and 81.4% in healthy relatives. However, the frequency of \textit{BRCA2} (6174delT) mutation was 30%. Our observations of the three mutations could suggest the clinician to provide a preventive genetic test of the \textit{BRCA1/2} defect in the healthy and affected family members as early as possible.
Due to the few reports on the founder mutations in \textit{BRCA1} and \textit{BRCA2} from West Bank, the result of this study has provided the preliminary information to understand the level of involvement of \textit{BRCA1} and \textit{BRCA2} mutations in the breast cancer occurrence of Palestinian population.

We recommend further studies or mutational screening for the whole \textit{BRCA1} and \textit{BRCA2} genes using gene sequencing technique, to determine whether the founder mutations of these genes are unique to Palestinian population and if there is other type of mutation.
References


85) Available at: 


94) Breast Information Core (BIC) database (http://research.nhgri.nih.gov/bic/).


تشخيص جزيئي لجينات سرطان الثدي الوراثي (BRCA1/BRCA2) في فلسطين

إعداد
روان أحمد حسين دردوك

إشراف
د. أشرف صوافطة

قدمت هذه الأطروحة استكمالاً لمنطبيات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين. 2014
التشخيص الجزيئي لجينات سرطان الثدي الوراثي (BRCA1/BRCA2) في فلسطين

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الملخص

نبذة: سرطان الثدي هو أكثر أنواع السرطانات شيوعاً في فلسطين، و يعد من بين أبرز الأمراض المسببة للوفاة بين الإناث، ويمثل سرطان الثدي الوراثي ما نسبته 20% إلى 30% من مجمل حالات سرطان الثدي الذي نتيجة وجود طفرة جينية متواترة في العائلة. حيث أن هناك العديد من الدراسات والأبحاث للكشف عن هذه الجينات ومعرفة الخلل الجيني فيها، ومن أشهر الجينات المسؤولة عن هذا الورم جين سرطان الثدي 1 (BRCA1)، و جين سرطان الثدي 2 (BRCA2). حيث انه يوجد ما يقارب 600 طفرة تحدث لجين سرطان الثدي 1 و حوالي 450 طفرة تحدث لجين سرطان الثدي 2، حيث أن وراثة الطفرة المسرونة تزيد احتمالية الإصابة بسرطان الثدي بنسبة 80%.

الهدف: تحديد الطفرات الشائعة في جيني سرطان الثدي 1 و 2 (BRCA1/BRCA2) في فلسطين، بهدف التقليل من احتمالية الإصابة بسرطان الثدي عن طريق الكشف المبكر لدى العائلات التي تحمل الطفرة الوراثية، والوقاية من الإصابة بالمرض بواسطة الجراحة الوقائية أو الوقاية الكيماوية وبالتالي التقليل من نسبة الوفيات بسبب هذا المرض.

منهجية الدراسة: تم إجراء الدراسة على 64 عينة دم من مرضى سرطان الثدي و أقربائهم الأصحاء، عن طريق عزل الحمض النووي (DNA) من عينات الدم، ثم عمل فحص جيني (ASPCR) بواسطة تقنية تفاعل البوليميراز المتسلسل (ASPCR) للطفرات الشائعة في الجينين BRCA1/2. تم فحص طفرتين في BRCA1: حذف نيكلوتيد G و A في موقع 185.
ج

(5382insC) في موقع 5382 (185delAG) و إدراج نيكليوتيد C في موقع 2000 و حذف نيكليوتيد T في موقع 6174 (6174delT).

النتائج: أثبتت الدراسة انتشار الطفرة الجينية (5382insC) في جين سرطان الثدي 1 بنسبة 59.4% في آليل واحد بينما الآليل الثاني يحتوي على الجين السليم، وتم اكتشاف الطفرة الجينية (6174delT) في جين سرطان الثدي 2 بنسبة 30% في آليل واحد بينما يحتوي الأليل الثاني على الجين السليم. وجدت الطفرات عند المرضى وأيضاء عند الأقارب الأصحاء ما يدل على أن هذه الطفرات هي طفرات متوارثة.

الاستنتاجات: نستنتج انتشار الطفرة الوراثية (5382insC) في جين سرطان الثدي 1 و الطفرة الوراثية (6174delT) في جين سرطان الثدي في فلسطين.