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**PREVALENCE OF BRCA1 AND BRCA2 MUTATIONS AMONG
BREAST AND OVARIAN CANCER PATIENTS IN NORTHERN
EMIRATES**

Zahra Ahmed Mohammed Saeed

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PREVALENCE OF *BRCA1* AND *BRCA2* MUTATIONS AMONG
BREAST AND OVARIAN CANCER PATIENTS IN NORTHERN
EMIRATES

Zahra Ahmed Mohammed Saeed

This thesis is submitted in partial fulfilment of the requirements for the degree of
Master of Science in Molecular Biology and Biotechnology

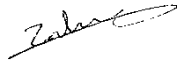
Under the Supervision of Dr. Yusra Saif Al Dhaheri

November 2020

Declaration of Original Work

I, Zahra Ahmed Mohammed Saeed, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Prevalence of BRCA1 and BRCA2 Mutations among Breast and Ovarian Cancer Patients in Northern Emirates*”, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Yusra Saif Al Dhaheeri, in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Breast Cancer (BC) is the most common cancer and the second most cause of death among women. Mutations in *BRCA1* and *BRCA2* genes confer high susceptibility to both breast and ovarian cancer. However, data on the prevalence of the *BRCA1/2* mutations among breast and ovarian cancer patients is limited. The genetic component of breast cancer in UAE is largely unknown and no study has evaluated the *BRCA* mutations status in breast and ovarian cancer patients in UAE population. This retrospective study aimed to establish mutation frequencies of the *BRCA* genes in breast and ovarian cancer patients from Northern Emirates and sought to examine potential association of *BRCA* carriers and Triple- Negative Breast Cancer (TNBC). The study population included patients who underwent *BRCA* genetic testing at Sheikh Khalifa Specialty Hospital (SKSH) to determine hereditary breast/ovarian cancer. Mutations in *BRCA1* and *BRCA2* were analyzed by Sanger sequencing or next generation sequencing (NGS) along with Multiple Ligation Probe Amplification (MLPA).

Among 262 patients, 224 (85.5%) had no mutation. *BRCA* mutations were identified in 38 patients (14.5%). *BRCA1* and *BRCA2* mutations were detected in 6.9% and 7.6% of the patients, respectively. Variant of unknown significance in *BRCA1* was found in 0.4% of patients (one patient). TNBC accounted for 22% of all patients with Breast Cancer (BC) who underwent immunohistochemistry (28/127). Importantly, one novel *BRCA1* mutation: c. (80+1_81-1) _ (441+1_442-1) dup in exons 3,5,6,7 was observed in one patient with ovarian cancer who showed positive family history and age ≤ 45 . Moreover, two novel deletion mutations were identified in the *BRCA2* gene. One deletion in exon 5 in ovarian cancer patient who showed positive family history and age ≤ 45 . The second was a deletion in exons 10-13 observed in male patient with breast cancer with age ≤ 45 . The current study results will help to establish the spectra of *BRCA* mutations and risks associated with breast and ovarian cancer in UAE patients.

Keywords: UAE, Hereditary, Breast cancer, Ovarian cancer, *BRCA1*, *BRCA2*, MLPA, NGS, TNBC.

Title and Abstract (in Arabic)

نسبة انتشار الطفرات الجينية *BRCA1* و *BRCA2* بين مرضى سرطان الثدي والمبيض في الإمارات الشمالية

الملخص

يعتبر سرطان الثدي أكثر أنواع السرطان شيوعاً و ثاني أحد أسباب الوفاة بين النساء. تمنح الطفرات في جينات *BRCA1* و *BRCA2* قابلية عالية للإصابة بسرطان الثدي والمبيض. و مع ذلك، فإن البيانات المتعلقة بنسبة إنتشار طفرات *BRCA1/2* في مرضى سرطان الثدي و سرطان المبيض محدودة. يعتبر التكوين الجيني لسرطان الثدي الى حد كبير غير معروف و لم تقم أي دراسة بتقييم حالة طفرات سرطان الثدي *BRCA* لدى مرضى سرطان الثدي و المبيض في دولة الامارات العربية المتحدة. تهدف هذه الدراسة إلى تحديد معدل إنتشار الطفرات الوراثية في جينات *BRCA* لدى مرضى سرطان الثدي والمبيض من الامارات الشمالية، كما سعت إلى فحص العلاقة المحتملة بين حاملين سرطان الثدي *BRCA* و سرطان الثلاثي السلبي (TNBC). شملت الدراسة على المرضى الذين خضعوا للاختبار الجيني *BRCA* في مستشفى الشيخ خليفة التخصصي (SKSH) لتحديد الاصابة بسرطان الثدي / المبيض الوراثي. تم تحليل الطفرات الوراثية في جينات *BRCA1* و *BRCA2* عن طريق إجراء فحص معرفة التسلسل الجيني Sanger أو تقنية الجيل الجديد لتحديد التسلسل الجيني (NGS) بالإضافة إلى فحص التضخيم مسبار الربط المتعدد (MLPA).

من إجمالي عدد 262 مريض، لم يكن لدى 224 مريض طفرة جينية في *BRCA* (85.5%)، بينما تم تحديد طفرات *BRCA* لدى 38 مريضاً (14.5%). حيث تم العثور على طفرات *BRCA1* و *BRCA2* بنسبة 6.9% و 7.6% من المرضى على التوالي. كما تم العثور على طفرات مجهولة الاهمية في *BRCA1* بنسبة 0.4% من المرضى (مريض واحد). يمثل سرطان الثلاثي السلبي (TNBC) نسبة 22% من إجمالي عدد المرضى (127/28) الذين يعانون من سرطان الثدي و الذين خضعوا لفحوصات الكيمياء النسيجية المناعية (Immunohistochemistry).

الجدير بالذكر هو العثور على طفرة جديدة في جين *BRCA1* (80+1_81-1) c. وهو حدوث تكرار في اكسون 3,5,6,7 exons في حالة واحدة ((441+1_442-1) dup)،

لمريضة مصابة بسرطان المبيض، لم تتجاوز عمر 45 سنة ولديها تاريخ عائلي. علاوة على ذلك، تم اكتشاف طفرتين جديدتين للحذف في جين *BRCA2*، احدهما حذف اكسون 5 exon في حالة لمريضة لم تتجاوز ال 45 سنة مصابة بسرطان المبيض و لديها تاريخ عائلي. و الحالة الثانية هو حذف في اكسونات 10-13 exons لوحظت في مريض ذكر مصاب بسرطان الثدي، لم يتجاوز ال 45 سنة. هذه النتائج ستساعد في تأسيس محتوى واضح للطفرات الجينية *BRCA* و المخاطر المرتبطة بسرطان الثدي والمبيض لدى مرضى شمال دولة الامارات العربية المتحدة.

مفاهيم البحث الرئيسية: دولة الامارات العربية المتحدة، سرطان الثدي والمبيض الوراثي، سرطان الثدي ثلاثي السلبية، فحص التسلسل الجيني، فحص التسلسل الجيني التالي، فحص تضخيم الكشف ذو الربط المستقل المتعدد.

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Hadeel, who was always ready to drive me till Al Ain and back again to home and sorry for annoying more than usual.

Finally, to all women who sacrificed their time, believe what Napoleon hill said “Great achievement is usually born of great sacrifice, and is never the result of selfishness”.

Dedication

To my beloved parents and family

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List of Abbreviations

BC	Breast Cancer
BRCA1	Breast Cancer 1
BRCA2	Breast Cancer 2
ER	Estrogen Receptor
GLOBOCAN	Global Cancer Observatory
HAAD	Health Authority of Abu Dhabi
HBOC	Hereditary Breast and/or Ovarian Cancer
HER2	Human Epidermal Growth Factor 2 Receptors
MLPA	Multiplex Ligation-dependent Probe Amplification Analysis
NGS	Next-Generation Sequencing
NCD	Non-Communicable Disease
OC	Ovarian Cancer
SKSH	Sheikh Khalifa Specialty Hospital
PR	Progesterone Receptor
TNBC	Triple Negative Breast Cancer

Chapter 1: Introduction

1.1 Cancer

Cancer is uncontrolled cell division that can occur in any place of the body. In normal cell division, an accurate genome duplication will occur to ensure that the two daughter cells will have the same genetic material as their parent cell. Any failure to achieve this purpose will develop a genomic instability, which generates random mutations including chromosomal rearrangements and different forms of genome alterations. The accumulation of these genetic alterations may varies between different subgroups of cells and leads to form the heterogeneity of cancer [1-2].

Cancer cells are distinct from their normal counterparts through six essential biological features. In 2002, these biological features were first introduced and defined as a cancer hallmarks which includes sustaining proliferative signaling, evading growth suppressors (antigrowth), resisting cell death (apoptosis), enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis (Figure 1). Four additional hallmarks of cancer were introduced in 2011, including deregulating cellular energetics, avoiding immune destruction, genomic instability and mutation and tumor promoting inflammation [3].

The pathogenesis of cancer is multifactorial, as for many other malignancies, involving interactions between different life style and genetic characteristics. Cancer remains one of the most health burden disease worldwide. The incidence of cancer has been rapidly increased resulting in over 8.7 million deaths [4]. Based on 2018 GLOBOCAN statistics, the total number of cancer new cases was around 18 million while the death cases was 9 million in 2018 (Figure 1) [5].

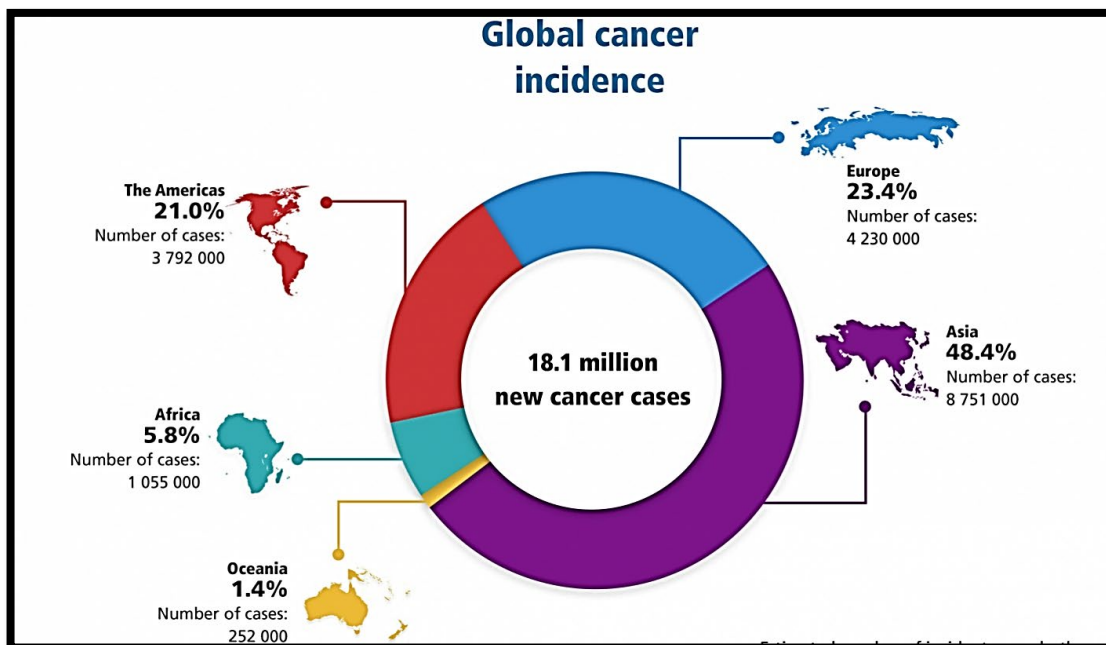


Figure 1: Global cancer statistics 2018 (GLOBOCAN) [5].

In the United Arab Emirates (UAE), the burden of cancer in 2010 was high and ranked the third leading cause of non-communicable diseases (NCD)-related mortality after cardiovascular and injury [6]. The GLOBOCAN data from 2018 shows that the cancer incidence rate reached 135.5 per 100,000 people and the mortality rate was 54.7 per 100,000 people in UAE [5].

1.1.1 Breast Cancer

Breast cancer is the most prevalent type of cancer among women worldwide with 25.4% of total cancer cases in 2018 [7]. It is considered a serious threat to global development with estimated 1,384,155 new cases and approximately 459,000 deaths cases. It has been estimated that one every eight women will develop breast cancer during her life [8].

Breast cancer is not a single disease but a complex multifactorial disease resulting from interactions between a number of different environmental, lifestyle,

hormonal, and genetic factors. There are three most common diagnostics markers in clinical management of BC patients which are human epidermal growth factor 2 (HER-2), estrogen (ER), and progesterone (PR). BC cell expression of these receptors has an impact in disease progression and also in the therapeutic management. The majority of BCs are sporadic, which means it develops from damage to a person's genes that occurs by chance after they are born. There is no risk of the person passing this gene on to their children. However, the familial susceptibility to BC accounts for more than 25% of all BCs [9].

BC is the most dominant cancer among women in Arab countries [10]. Women in Arab world are diagnosed with breast cancer at more advanced stages, and the incidence rate has increased over the past three decades [11]. According to American Cancer Society, prediction of new cases of breast cancer in women will reach to about 3.2 million in 2050 [12]. These statistics demonstrate the vastness of breast cancer incidence and its effect on societies worldwide.

The Health Authority of Abu Dhabi (HAAD) reported that the prevalence of breast cancer among all cancers diagnosed in Abu Dhabi reached 20.3% in 2014, with an associated mortality rate of 12.2%, making breast cancer the most incident in that Emirate [13]. Of all cancer cases, breast cancer in women from the GCC countries is the most common cancer type. It was reported by the Gulf Center for Cancer Registration that annually, for every 100,000 female there are 53.4 cases of breast cancer in Bahrain, 22.8 cases in the United Arab Emirates, 17.5 cases in Saudi Arabia, 48.2 cases in Qatar, and 46.6 cases in Kuwait [14]. However, the effect of geographical and ethnical differences in breast cancer incidence signs the impact of environmental conditions and the way of life (Figure 2) [15].

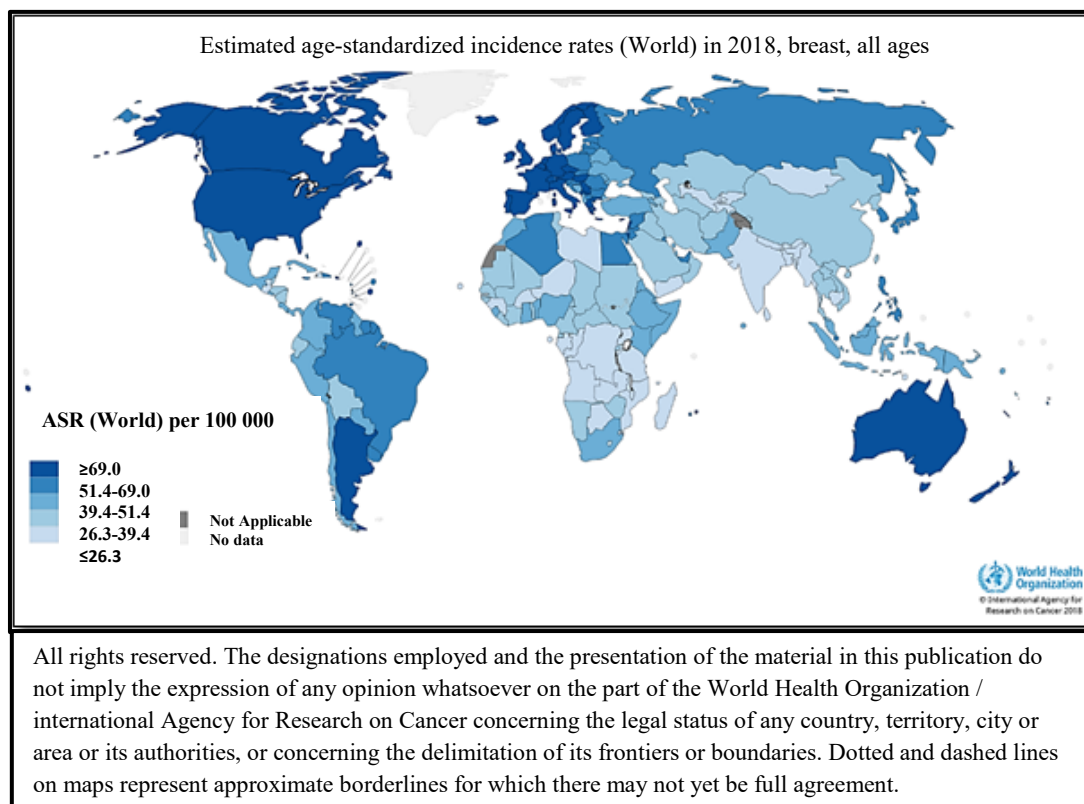


Figure 2: Breast cancer prevalence around the world [5].

1.1.2 Ovarian Cancer

Worldwide, ovarian cancer (OC) is the seventh most common cancer and the eighth leading cause of cancer-related mortality in females, contributing nearly 4% of the total number of new cases diagnosed in 2018 [7]. A number of clinical features are considered to affect progression-free-survival and overall survival rates in ovarian cancer, which includes disease grade, stage and surgical outcome. There are five main histologically defined subtypes of OC which are high grade serous, endometrioid, clear cell, low grade serous and mucinous OC serous and mucinous OC. As the majority of OC is high grade serous, the studies focused on this subgroup more than others [16]. Ovarian cancer, like other types of cancer, may occur spontaneously, and mostly developed due to genetic predisposition involving dysfunctional of dominant genes [17]. However, more than 23% of all ovarian cancer cases are thought to be hereditary [18]. Also, it was reported that in ovarian cancer relatives, the risk of breast cancer is

increased and vice versa [19]. In Saudi Arabia, the OC accounted for 2.9% of all cancers, whereas in western population it accounted for 3.4%. The median age of OC Saudi population (55 years) and Middle East Epithelial OC population (50 years) were lower than western population (65 years), which could influence the genetic predisposition factors in their ethnicity. Therefore, determination such genetic predisposition will make efficient early diagnosis and cancer prevention process [20].

1.2 Genetic Mutation and Hereditary Breast and/or Ovarian Cancer (HBOC)

In human, genes are located on 23 pairs of chromosomes. During the process of cancer development, malignant transformation is known to involve genetic and epigenetic changes that lead to uncontrolled cellular proliferation and/or abnormal programmed cell death (apoptosis). These cellular abnormalities resulted through accumulation of mutations that are frequently associated with molecular abnormalities in certain types of genes, such as proto-oncogenes and tumor-suppressor genes [8, 10]. These mutations can result from genetic predisposition and/or exposure to several risk factors such as physical inactivity and sedentary lifestyles, the overconsumption of high-caloric and poorly-nutritive meals, an increase in obesity rates, an increase in smoking prevalence, elevated air pollutant levels, different forms of ionizing radiations, and synthetic chemicals. Mutations are either inherited from a parent (germline) or acquired affecting only the cells that grow from the mutated cell (somatic). Somatic mutation may determine the phenotype of a particular cancer and maybe of clinical value in determining cancer prognosis. However, only germline mutations can predetermine an individual's risk of developing cancer. Among all cancer types, only about 5-10% are thought to be related to an inherited gene change [21].

Hereditary Breast and/or Ovarian Cancer (HBOC) syndrome is an inherited genetic disorder with high level of risk compared to the normal breast and ovarian cancer in related family members [17]. Approximately 5-10% of breast cancer cases [18] and about 18% of ovarian cancer cases [22] are due to genetic mutation which is thought to be hereditary.

1.2.1 *BRCA1/2* Genes

Minority of breast cancers can show dominant gene mutations. The most common gene mutations are those affecting the Breast CAncer 1 (*BRCA1*) and BREast CAncer 2 (*BRCA2*) genes [8]. *BRCA* genes, important tumor suppressor genes, are involved in DNA damage repair and recombination, cell-cycle checkpoint control, apoptosis and transcriptional regulation [23]. *BRCA 1* and 2 genes were reported and identified as breast cancer susceptibility genes for the first time in 1994 and 1995 respectively. Since the time of their discovery, the number of studies reporting the frequency of mutations in these genes in high-risk breast and breast-ovarian cancer families have been increased largely. The breast cancer susceptibility gene *BRCA1* is expressed in several tissues including breast and ovary. *BRCA1* gene (Figure 3) is a large gene extend to about 100 kb of genomic DNA. It consists of 24 exons, the first and fourth exons are non-coding [24]. Eleventh exon is the largest exon providing more than 60% of the whole coding sequence of *BRCA1* gene [25]. The final product of the protein composed of 1863 amino acids, produce about 220 kd nuclear protein. This *BRCA1* gene product contains a protein motif, a Ring Finger domain near the amino acid terminus and a conserved acidic carboxyl terminus that functions in transcriptional co-activation. It was proven that *BRCA1* protein plays a crucial part in the DNA repair process. *RAD51* gene, encodes a protein called *RAD51*, which is

important in the stability of the genome and essential for the repair of damaged DNA. *BRCA1* protein reacts with RAD51 protein during homologous recombination and double strand break repair [23]. The *BRCA2* gene (Figure 3) is larger compared to *BRCA1*, composed of 27 exons, where one is non-coding. It codes for a protein of 3418 amino acids that produce a nuclear protein of 380 kd. *BRCA2* gene has a different structure than *BRCA1* and the protein lacks a well-defined functional domain. Also, the *BRCA2* protein reacts with RAD51 protein and during this cross interaction with RAD51, both *BRCA1* and *BRCA2* interrelate at sites of DNA synthesis after the induction of DNA damage. Thus, the proteins made by *BRCA1* and *BRCA2* genes are both essential for repairing damaged DNA and any changes in these genes may lead to hereditary predisposition to breast and ovarian cancer [24].

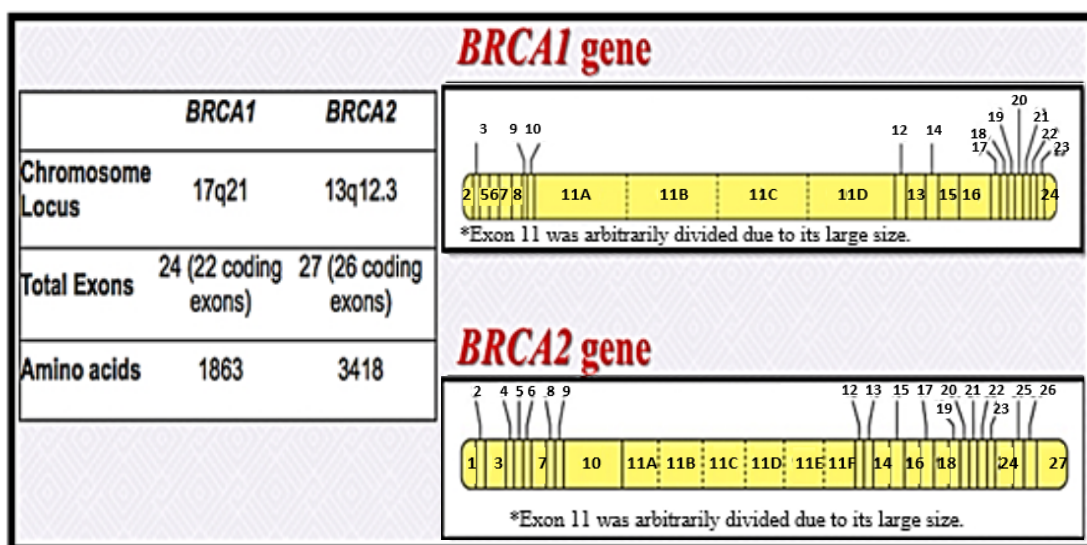


Figure 3: *BRCA1* and *BRCA2* genes [26].

1.2.2 *BRCA1/2* Genes Mutations

Mutations in *BRCA* genes induce defective DNA repair mechanisms, which are associated with the risk of developing breast and/or ovarian cancers. Changes in *BRCA* genes are spread all over the entire gene. Different mutations have been reported

that exceeds 600 and 400 in *BRCA1* and *BRCA2*, respectively. Most types of gene mutations considered to be risky and disease-causing that produce a defective and non-functional protein. There are several forms of mutations reported in *BRCA* genes such as frame shift mutation when either deletion or insertion (indels) of one or more nucleotides resulting in missing or non-functional product, nonsense mutation when change in one nucleotides produce a stop codon that terminate the protein, or splice site alternations that affect the DNA identity and results in abnormal protein [24]. About 12% of women in the general population will develop breast cancer sometime during their lives and about 1.4% of women in the general population will develop ovarian cancer sometime during their lives. One in 400 to 1,000 persons in the overall population are expected to have a germline mutation in *BRCA1* and *BRCA2* genes that passed down through their families known as autosomal dominant predisposing genes [27]. Some studies showed that a mutation that found towards the 5'-end of *BRCA1* gene tends to develop ovarian cancer whereas mutation towards the 3'-end seems to develop breast cancer which means that there is a possible relation between the mutation site and the corresponding phenotype [25]. Inherited deleterious mutations in the *BRCA1* or the *BRCA2* genes are associated with an increased risk of developing breast and/or ovarian cancer in both woman and men. Women with an inherited *BRCA1* mutation have a lifetime risk of 65-80% of developing breast cancer and 37-62% of developing ovarian cancer, while *BRCA2* carriers have a lifetime risk of 45-85% for breast cancer and 11-23% for ovarian cancer. There is an increased risk of developing several other cancers in addition to breast and ovarian cancer among *BRCA* carriers. *BRCA1* mutations may increase a woman's risk of developing fallopian tube cancer and peritoneal cancer. Men with pathogenic *BRCA1* or *BRCA2* mutations have a higher risk of prostate cancer (5-25%), and breast cancer among men with *BRCA2*

mutations (6%). Men and woman with *BRCA1* and *BRCA2* mutations maybe at increased risk of pancreatic cancer, stomach, and head and neck. Together, *BRCA1* and *BRCA2* mutations cause about 20-25% of hereditary breast cancers. In addition, mutations in *BRCA1* and *BRCA2* account for around 15% of ovarian cancers overall. Studies on *BRCA1* mutation occurrence suggested that nearly half of the families at high risk for breast cancer carried *BRCA1* mutation. However, other analysis suggests that the actual incidence of *BRCA1* in high risk families (>3 cases of breast and/or ovarian cancer) might be as low as 12.8% to 16%. It has been observed that large variation in the prevalence of *BRCA1* mutations in high risk families of different countries more common than the prevalence of *BRCA2* mutations [19, 25, 28, 29].

1.3 Triple Negative Breast Cancer (TNBC)

Triple-negative breast cancer is an aggressive subtype of breast cancer with a higher risk of both local and distant recurrence and poor overall prognosis and it accounts for about 10-20% of all cases of breast cancer. TNBC is characterized by a lack of the expression of estrogen (ER), progesterone (PR) and human epidermal growth factor 2 receptors (HER2/neu), thus, no validated molecular targets for treatment are available .TNBC shows an estimated range between 10% and 20% of all breast cancers and having a family history of TNBC and/or *BRCA* mutations may increase the possibility of having TNBC [30]. It has been unclear how much *BRCA1/2* mutation frequency may influence the expression of ER, PR, and HER2/neu [31]. With the development of targeted therapies for breast cancer patients, designation of treatment regimens has become more specific, and breast cancer patients with *BRCA* mutations should be treated differently from the patients without *BRCA* mutations. The exact relationship between *BRCA* status and TNBC is still under investigation.

1.4 Prevalence of *BRCA1/2* Gene Mutations

The overall prevalence of pathogenic *BRCA1/2* gene mutations is low in the general population (~0.2-0.3%). This prevalence is increased to approximately 3% in women with breast cancer, 6% in women with breast cancer onset at young age (before the age of 40), and higher prevalence is associated with a positive family history of breast or ovarian cancer (20%) [32]. Although it has been demonstrated that the overall prevalence of *BRCA1* or *BRCA2* oncogenic mutations may differ widely according to the study populations, other studies conclude that mutation prevalence is similar across diverse races and ethnicities [33-34].

1.4.1 Prevalence of *BRCA1/2* Mutation in the World

Recent studies indicate that there is a significantly variation in the prevalence of *BRCA1* or *BRCA2* mutations among ethnic groups and geographical areas. Mutations in *BRCA1* and *BRCA2* are more common in certain racial/ethnic populations. There are common mutations in specific population that have been described among Ashkenazi Jews population [35] as well as patients of Spanish ancestry and that due to common ancient ancestor [36]. Founder *BRCA1* and *BRCA2* mutations have also been found in several European populations in Austria, Slovenia, Italy, France, Spain, Portugal, Belgium, the Netherlands (Holland), Germany, Czech Republic, Slovakia, Hungary, Greece, Cyprus, Denmark, Sweden, Norway, Finland, Iceland, the United Kingdom, Ireland, Poland, Latvia, Lithuania, Estonia, Belarus, and Russia [36]. Prevalence of *BRCA1* and *BRCA2* mutations varied widely between key clinical and demographic subgroups and across countries. Data from Table 1 were adopted from Armstrong et al. [37], which summarizes the prevalence of both genes mutation in several studies reported on *BRCA* mutation prevalence in patients with

different stage of breast cancer and different hormone receptor status. The highest germline mutation of *BRCA* was reported in the United States [37].

Table 1: Prevalence of Germline *BRCA* Mutation Internationally (Unselected for Family History, Age, Sex, or Ethnicity) [37].

Country	Study Reference	Hormone Receptor status	Breast Cancer Stage	N at risk of mutation	% <i>BRCA</i> 1	% <i>BRCA</i> 2	% <i>BRC</i> A 1/2
United States	Sharma et al. [60]	TNBC	Mixed	207	11.1	4.3	15.4
	Couch et al. [56]		NR/unclear	1824	8.5	2.7	11.2
	Tung et al. [61]	HR+/HER2-	Stages I-III	301	1.7	3.3	5
	Bayraktar et al. [62]	Mixed	Stages I-III	488	3.7	2.5	6.1
			Metastatic BC	195	15	6	21
Vidula et al. [63]	NR/unclear		178	NA	NA	15.2	
Spain	Gonzalez-Rivera et al. [64]	TNBC	Stages II-III	105	12.4	1.9	14.3
Australia	Wong-Brown et al. [65]		Mixed	439	5.9	3.4	9.3
Sardinia/ Italy	Palomba et al. [66]		NR/unclear	726	1	1.9	2.9
Germany	Fasching et al. [67]	Mixed	Metastatic	1462	1.4	2.9	4.3
France	Meynard et al. [68]		Metastatic	407	0.7	2	2.7
South Korea	Kim et al. [69]	NR/unclear	NR/unclear	471	1.5	1.5	3
Israel	Dagan et al. [70]	Female, Ashkenazi, Early onset	Locally advanced BC	13	23.1	30.8	53.8

Abbreviations: BC, breast cancer; *BRCA*, BC susceptibility gene; HER2-, human epidermal growth factor receptor 2-negative; HR+, hormone receptor-positive; NR, not reported; TNBC, triple-negative BC.

1.4.2 Prevalence of *BRCA1/2* Mutations in the Arab Countries

The incidence and mortality of cancer in Arab countries is increasing rapidly and this will obstruct human development and well-being. The most common cancer among women in Arab countries was Breast cancer with 49.9% and Ovarian cancer with 5% in 2012. WHO estimated that by 2030, there will be an increase of 1.8 fold in cancer incidence in the Gulf States and the Eastern Mediterranean region [38]. The number of cancer researches and publications from Arab region is considered low compared to worldwide publications and this due to less number of researchers in this field, the high cost of research and low research output [10]. This explains the rarity of high quality data on *BRCA* mutations in Arab populations. Breast cancer is the first leading cause of all cancer deaths among Arab females with approximately rate of 14 to 42% of all female cancers in Arab region [18]. According to Abdurashid et al. [18] it has been shown that HBOC patients in Arab countries have *BRCA* mutations and the prevalence of *BRCA2* mutations were more common (17%) than *BRCA1* mutations (11%) among the breast cancer patients in the Arab region [18].

1.4.3 Prevalence of *BRCA1/2* Mutations in the Gulf Countries

There are six member countries of the Arabian Gulf Cooperation Council that are: Saudi Arabia, Bahrain, Kuwait, Oman, Qatar and United Arab Emirates. Due to the migration from Africa, the first Eurasian populations formed and found to be genetically related to the original Arabs. In the GCC, populations are divided to genetic subgroups, about 48.1% of GCC populations was expats because of recent migration of migrant workers [14]. The fact of being descended from the same ancestors has led to an increase in genetic diseases among GCC populations and these factors contribute to a unique genetic makeup in their populations. According to Abulkhair et al. [27]

prevalence of *BRCA1* gene mutation (82.5% of mutations) in Saudi patients is more than *BRCA2* gene mutations [27]. In Oman, Kuwait and Bahrain, the knowledge about the frequency of *BRCA1/2* gene mutations among breast cancer patients is not yet available. Although it was reported that Qatari breast cancer patients with *BRCA1* mutations carriers were triple negative breast cancer [14]. Although patients with hereditary breast and/or ovarian cancer (HBOC) in GCC countries have minimal contributions to *BRCA1/2* mutations, which is the most reported form of cancer in Oman, there is a lack of data about the *BRCA1/2* mutation role in breast cancer in the region [14].

The current population structure of the UAE is diverse; a result of a high percentage of expatriates (80-90%, residing in the country), compared to a relatively small but diverse indigenous population admixed with immigrants from Yemen, Oman, North Africa, Iran, Baluchistan, India, and other neighboring regions. Approximately 11 million residents in the UAE, however, around 10% only are Emirati citizens where endogamous marriage is common, making this segment of UAE population relatively homogenous [39-40].

The knowledge about the prevalence and types of *BRCA1* and *BRCA2* mutations involved in breast and ovarian cancer patients in UAE is unavailable. Given these considerations, this study aims to determine the prevalence of *BRCA1* and *BRCA2* mutations in patients with breast and/or ovarian cancer; leading to better care for patients in this region.

This study was conducted on patients from northern region of UAE including five emirates: Sharjah, Ajman, Ras Al Khaimah, Umm Al Qaiwain, and Fujairah. About one third of the population live in the Northern Emirates (Figure 4) [42].

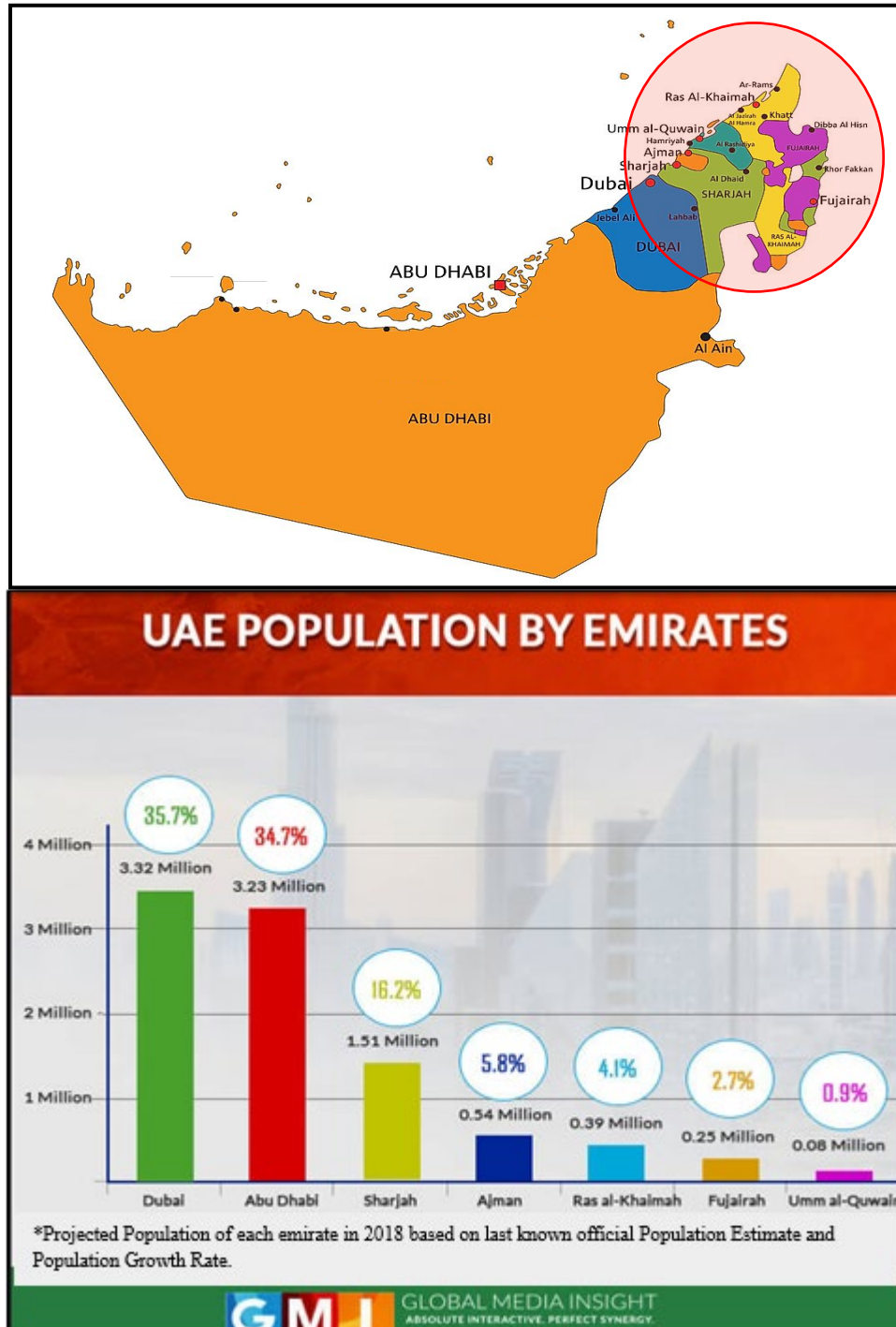


Figure 4: United Arab Emirates map (Up) showing the northern regions of the emirates and UAE population percent by emirate (Down) [41].

1.5 Hypothesis

BRCA1 and 2 mutations contribute to the incidence of breast and ovarian cancer among northern Emirates patients.

1.6 Objectives of the Study

The main aims of this study are to:

- 1- Estimate the frequency of *BRCA1/2* genes mutations among cases of breast and ovarian cancer in northern Emirates.
- 2- Determine the types of *BRCA1/2* genes mutations associated with breast and ovarian cancer patients diagnosed at the Oncology Department, Sheikh Khalifa Specialty Hospital, Ras Al Khaimah, UAE.
- 3- Investigate the potential relationship between *BRCA* status and TNBC.
- 4- Identify possible novel *BRCA1/2* mutations among UAE population.

Chapter 2: Materials and Methods

2.1 Study Design

This retrospective study was conducted on 262 patients who underwent *BRCA* genetic testing to determine hereditary breast/ovarian cancer. 262 unselected breast and ovarian cancer patients, diagnosed at any age from July 2015 to August 2020, from the Oncology Department, Sheikh Khalifa Specialty Hospital, Ras Al Khaimah, UAE. In total, 262 patients were approached in this study during their out-patient visit to the Oncology Department in the hospital. Before the test, all patients had been informed about the implications of genetic testing, and interviewed in person for their family history of cancer. The oncologist filled the genetic test request form with patient information, onset of symptoms, familial history of cancer with specific reference to a history of breast or ovarian cancer and family tree for at least three generation. Medical records for all patients included in this study were reviewed retrospectively. This study was approved by The Institutional Review Board (IRB) in Ministry of Health and Prevention in Dubai, UAE (MOHAP/DXB-REC/AAA/No.111/2020).

2.2 Blood Collection and DNA Extraction

For each *BRCA* gene analysis test request, a blood sample was obtained from each patient for DNA extraction. Approximately 6 mL of whole blood was collected in sterile tubes containing EDTA from all subjects enrolled in this study. Genomic DNA was extracted manually (following standard protocol) using the QIAamp Blood Mini Kit from QIAGEN manufacturer. The purity and concentration of the extracted DNA was assessed using Nanodrop 2000/2000c Spectrophotometer from

ThermoScientific. The ultimate concentration used was 25-50 ng/ μ L and the ratio of (A260/A280) was not less than 1.8 to assure the good quality of DNA.

2.3 *BRCA* Genes Analysis

2.3.1 Sanger Sequencing

BRCA1/2 gene analysis is based on the Gold Standard Sanger Sequencing. Human Genome build NCBI36 (*BRCA1*: Acc. nr: NM_007300, *BRCA2*:Acc. nr: NM_000059), M13 tailed PCR primers were designed and optimized for 100% coverage of the coding sequence of the *BRCA1* and *BRCA2* genes, including sections of approximately 50 bp up- and downstream of each exon (24 exons for *BRCA1* and 27 exons for *BRCA2*). All amplicons can be amplified and sequenced using one universal set of PCR conditions.

For sequencing, PCR using EasySeq™ PCR Plates (Figure 5). 96 well-plate containing dried down primer pairs in optimized concentrations, covering both *BRCA* genes. Columns 11 and 12 contain all amplicons, in a total of 15 PCR multiplexes, to be used as a No Template Control (NTC), to confirm all results are specific.

All primer sets have been optimized in concentration and design to be compatible with key chemistries [42].

Cycle Sequencing is performed with universal sequencing primers: Forward primers with -21M13 and reverse primers with M13Rev to enable cycle sequencing with universal primers (Figure 6).

Raw data of sequencing is analyzed with reference sequence using software (Sequencher v5).

	1	2	3	4	5	6	7	8	9	10	11	12
A	BRCA1 exon 1	BRCA1 exon 10	BRCA1 exon 11/8	BRCA1 exon 15	BRCA1 exon 23	BRCA2 exon 8	BRCA2 exon 11/3	BRCA2 exon 11/11	BRCA2 exon 13	BRCA2 exon 20	BRCA1 MP-1	BRCA1 MP-3
B	BRCA1 exon 2	BRCA1 exon 11/1	BRCA1 exon 11/9	BRCA1 exon 16	BRCA1 exon 24	BRCA2 exon 9	BRCA2 exon 11/4	BRCA2 exon 11/12	BRCA2 exon 14/1	BRCA2 exon 21	BRCA1 MP-2	BRCA2 MP-4
C	BRCA1 exon 3	BRCA1 exon 11/2	BRCA1 exon 11/10	BRCA1 exon 17	BRCA2 exon 1	BRCA2 exon 10/1	BRCA2 exon 11/5	BRCA2 exon 11/13	BRCA2 exon 14/2	BRCA2 exon 22	BRCA1 MP-3	BRCA2 MP-5
D	BRCA1 exon 5	BRCA1 exon 11/3	BRCA1 exon 11/11	BRCA1 exon 18	BRCA2 exon 2	BRCA2 exon 10/2	BRCA2 exon 11/6	BRCA2 exon 11/14	BRCA2 exon 15	BRCA2 exon 23&24	BRCA1 MP-4	BRCA2 MP-6
E	BRCA1 exon 6	BRCA1 exon 11/4	BRCA1 exon 11/12	BRCA1 exon 19	BRCA2 exon 3	BRCA2 exon 10/3	BRCA2 exon 11/7	BRCA2 exon 11/15	BRCA2 exon 16	BRCA2 exon 25	BRCA1 MP-5	BRCA2 MP-7
F	BRCA1 exon 7	BRCA1 exon 11/5	BRCA1 exon 12	BRCA1 exon 20	BRCA2 exon 4	BRCA2 exon 10/4	BRCA2 exon 11/8	BRCA2 exon 11/16	BRCA2 exon 17	BRCA2 exon 26	BRCA1 MP-6	BRCA2 MP-8
G	BRCA1 exon 8	BRCA1 exon 11/6	BRCA1 exon 13	BRCA1 exon 21	BRCA2 exon 5&6	BRCA2 exon 11/1	BRCA2 exon 11/9	BRCA2 exon 11/17	BRCA2 exon 18	BRCA2 exon 27/1	BRCA2 MP-1	BRCA2 MP-9
H	BRCA1 exon 9	BRCA1 exon 11/7	BRCA1 exon 14	BRCA1 exon 22	BRCA2 exon 7	BRCA2 exon 11/2	BRCA2 exon 11/10	BRCA2 exon 12	BRCA2 exon 19	BRCA2 exon 27/2	BRCA2 MP-2	EMPTY

Figure 5: EasySeq™ PCR plates for *BRCA1/2* Sequencing [42].

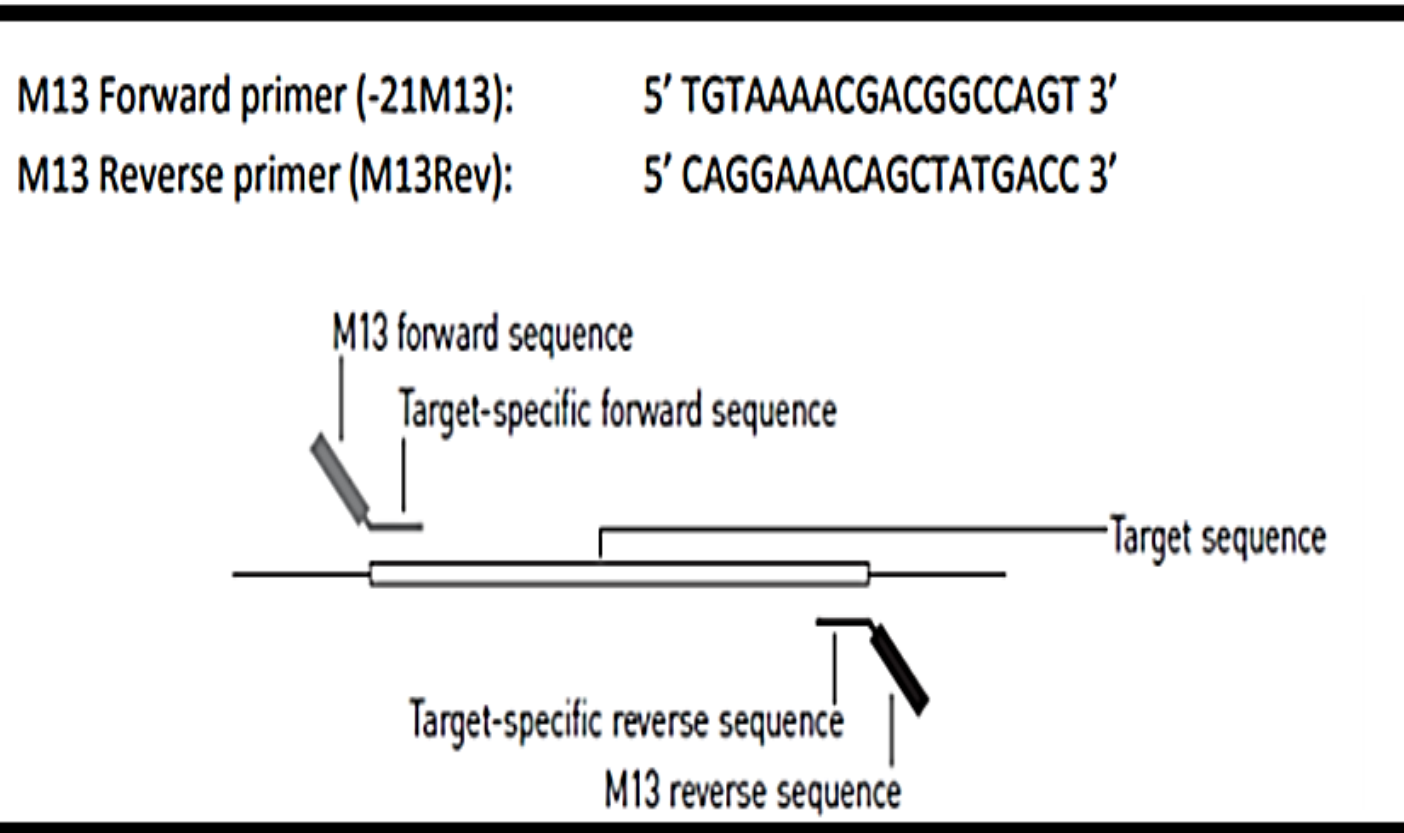


Figure 6: Forward primers with -21M13 and reverse primers with M13Rev.

2.3.2 Next Generation Sequencing (NGS)

The Ion Torrent system (<http://www.iontorrent.com>) is unique among Next Generation Sequencing technologies in that the detection for sequencing is not based upon fluorescent dyes but rather measuring the pH change as the result of the release of a H⁺ ion upon nucleotide incorporation using semiconductor technology (Ion semiconductor sequencing). By sequentially adding nucleotides, the machine is able to detect which nucleotide has been incorporated into the growing strand.

The Oncomine™ *BRCA* Research Assay consists of two pools of AmpliSeq™ oligonucleotide primers and associated reagents to generate amplicon libraries for next-generation sequencing (NGS) on Ion Torrent™ platforms. The assay is designed to provide sensitive and comprehensive sample amplification of all coding regions of the human *BRCA1* and *BRCA2* genes and by using the Ion Chef™ System where the library preparation is automated.

2.3.3 Multiple Ligation- dependent Probe Amplification Analysis (MLPA)

The MLPA protocol (MLPA®DNA protocol) was performed using the instructions provided by the company <http://www.mrc-holland.com>. The MLPA assay was performed using a set of probes for *BRCA1* (P002-D1 probe mix), for *BRCA2* (P045/CHEK2 probe mix) and the EK5 SALSA MLPA reagents kit purchased from MRC-Holland (Amsterdam, The Netherlands). These probes were used to detect the deletion or duplication of exons in the human *BRCA1/BRCA2* genes in order to determine genetic predisposition to hereditary breast and/or ovarian cancer. Briefly, 10 ng of genomic DNA in a volume of 5 µl was denatured for 5 min at 98°C, cooled and hybridized with MLPA probes (1.5 µl/sample) in the presence of SALSA MLPA buffer (1.5 µl/sample) for 16 h at 60°C. Ligation reaction was performed for 15 min at

54°C by adding 32 µl of ligation mixture (3 µl SALSA Ligase buffers A and B, respectively, 25 µl demineralized water, and 1 µl SALSA Ligase-65/sample) followed by heating to 95°C for 5 min. PCR amplification of the ligation product was performed according to the standard protocol. Briefly, 10 µl was added to the mixture consisting of (7.5 µl demineralized water, 2 µl SALSA PCR primers and 0.5 µl SALSA polymerase/sample) while the samples were left at 4°C or on ice. The samples were pre-heated to 60°C for 30 s, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1 min, and a final extension for 20 min at 72°C. From each PCR reaction, 0.7 µl product was mixed with 9 µl of Hidi Formamide (Applied Biosystems Foster City, CA, USA) and 0.2 µl of GeneScan™ 500 LIZ™ dye Size Standard (Applied Biosystems, Foster City, CA, USA) and all fragments were separated by capillary electrophoresis on the ABI 3500XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencer, SeqScape and GeneMarker, unique genotype analysis software's designed for mutation detection, were used in the analysis.

2.4 Immunohistochemistry

A total of 127 patients with invasive breast carcinoma who underwent mastectomy were tested for ER, PR and the HER-2 status. Briefly, tissue samples from each patient were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were deparaffinized in xylene, rehydrated in a graded ethanol series, and stained using a Benchmark XT (Roche, Basel, Switzerland). Immunohistochemical analysis was carried out with the following monoclonal antibodies: anti-estrogen receptor (clone SP1, commercially with appropriate dilution, Roche, Basel, Switzerland), anti-progesterone receptor (clone 2E2, commercially with appropriate dilution, Roche, Basel, Switzerland) and anti-HER-2 (clone 4B5, commercially with

appropriate dilution, Roche, Basel, Switzerland). Staining was assessed according to the ASCO/CAP guidelines.

2.5 Statistical Analysis

The data, either clinical or genetic findings, were statistically evaluated. Statistical significance was performed to find the association between BRCA1 gene mutation and other parameters using a two-sided significance level of 5% and 1%. The statistical analysis was interpreted and analyzed using the SPSS software version 16 and Microsoft Excel Data Analysis Tool.

Chapter 3: Results

3.1 Study Selection and Patient Characteristics

Total of 262 cases were tested for *BRCA1/2* mutation analysis. Patient's clinical characteristics are presented in Table 2. The mean age at the time of diagnosis was 46.5 years (range 21-90). 71 out of 262 patients (27%) had a positive family history for hereditary breast and ovarian cancer.

Of total 262, 248 (94.7%) patients were females and 14 (5.3%) were males. Total of 186 of the 262 patients (71%) diagnosed with breast cancer and 22/262 (8.4%) had ovarian cancer. Patients and relatives of cancer patients from Emirates citizen were the most common with total number 230 cases (87.8%), 10 cases from gulf countries (3.8%), 13 from Arab countries (5%) and 9 (3.4%) from other Asian, European, and African countries.

Table 2: Clinical Characteristics of 262 Patients.

	Wild type		Mutation				All			
	n=224	(85.5%)	All n=38	(14.5%)	<i>BRCA1</i> n=18	(6.9%)	<i>BRCA2</i> n=20	(7.6%)	n=262	(100%)
Mean age at diagnosis (Range)									46.5	(21-90)
45 or less	103	46	25	65.8	11	61.1	14	70	128	48.9
More than 45	121	54	13	34.2	7	38.9	6	30	134	51.1
Cancer type	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%
Breast Cancer	166	(74.1%)	20	(52.6%)	10	(55.6%)	10	(50%)	(186/262)	(71 %)
Ovarian Cancer	17	(7.6%)	5	(13.2%)	3	(16.7%)	2	(10%)	(22/262)	(8.4%)
Other	30	(13.4%)	3	(7.9 %)	0	(0.0%)	3	(15%)	(33/262)	(12.6%)
Relative of cancer patients	11	(4.9%)	10	(26.3%)	5	(27.8%)	5	(25%)	(21/262)	(8%)
Gender										
Female	214	(95.5%)	34	(89.5%)	17	(94.4%)	17	(85%)	(248/262)	(94.7%)
Male	10	(4.5%)	4	(10.5%)	1	(5.6%)	3	(15%)	(14/262)	(5.3%)
Family History										
Positive	50	(22.3%)	21	(55.3%)	13	(72.2%)	8	(40%)	(71/262)	(27%)
Negative	174	(77.7%)	17	(44.7%)	5	(27.8%)	12	(60%)	(191/262)	(73%)
Ethnicity										
Emirates Citizen	196	(87.5%)	34	(89.5%)	17	(94.4%)	17	(85%)	(230/262)	(87.8%)
Gulf Countries	7	(3.1%)	3	(7.9%)	1	(5.6 %)	2	(10%)	(10/262)	(3.8%)
Arabs	12	(5.4%)	1	(2.6%)	0	(0.0 %)	1	(5%)	(13/262)	(5%)
Others	9	(4.0%)	0	(0.0%)	0	(0.0 %)	0	(0%)	(9/262)	(3.4%)
ER, PR, HER2 receptor status										
TNBC	21	(9.4%)	7	(18.4%)	6	(33.3%)	1	(5%)	(28/127)	(22%)
Non-TNBC	90	(40.2%)	9	(23.7%)	2	(11.1%)	7	(35%)	(99/127)	(78%)
Unknown#	111	(49.6%)	22	(57.9%)	10	(55.6%)	12	(60%)	(133/262)	(50.7%)

#Unknown means did not underwent Immunohistochemistry test in SKSH and information is not available. **Abbreviations:** ER; Estrogen Receptor, PR; Progesterone Receptor, HER2; Human Epidermal Growth Factor 2 Receptors, TNBC; triple-negative breast cancer, *BRCA*; Breast Cancer genes

3.2 Prevalence of Germinal *BRCA* Mutation

Of total 262, 224 patients and relatives of cancer patients had no mutation in *BRCA* genes (85.5%). 38 mutations were identified in *BRCA1* and *BRCA2* genes (14.5%, 38/262). 18 out of 38 mutations were in *BRCA1* gene (6.9%) and 20 mutations were in *BRCA2* gene (7.6%) as shown in Table.2. All other patients had wild type *BRCA* genes with benign or likely benign mutations. All reported mutations revealed pathogenic mutations except three novel mutations of large exon rearrangements with one large duplication involving four exons (Exon 3, 5, 6 and 7) with more than 11,658bp in *BRCA1* gene and two deletions of either one or more exons in *BRCA2* gene. Duplication/ deletions mutations were detected by Deletion/Duplication Analysis (MLPA) method.

Six different mutations in *BRCA1* were identified in 18 patients diagnosed with BC or/and OC. The most common *BRCA1* mutation was frameshift in exon 10: c.4065_4068delTCAA p. (Asn1355Lysfs*10) which was detected in 5 females and 1 male of one family with a breast cancer history. Same mutation was found in other 6 unrelated patients, 3 with breast cancer and 3 with ovarian cancer. The mean age for those patients with this type of mutation was 39.7 years (Figure 7 and Table 3).

Another *BRCA1* frame shift mutation in exon 10 also c.2704delG (p. Glu902Asnfs*98) was detected in two members of one family (cousins) with BC family history. A frame shift mutation in exon 2 c.68_69delAG (p.Glu23Valfs*17) was detected in a woman with TNBC and positive family history. *BRCA1* uncertain significant mutation c.4186-10G>A was found in intron side of one BC female patient.

13/18 patients with mutation in *BRCA1* gene (~72%) had a family history of breast cancer with age range between 21 and 65 years old (Table 3).

Out of 38 *BRCA* positive cases, 20 patients revealed 12 different mutations in *BRCA2* gene. The most common mutation detected in *BRCA2* gene was the frame shift type. 6 *BRCA2* mutations revealed in exon 11. Out of 20 patients, 8 (40%) had a positive family history of breast cancer. The c.771_775delTCAA (p.Asn257Lysfs) mutation accounted for 15% of the cases with *BRCA2* gene mutation, and c.2808_2811delACAA (p.Ala938Profs*21) was reported also in other 3 patients (15%), one with BC and two with OC with average age 49 and positive hormone receptors. Also, the c.1593dupA (p.Glu532Argfs*3) mutation found in two members of one family (sister and brother) and one patient all diagnosed with BC (15%). In addition, two unreported mutations were identified, first a deletion of exon 5 in an ovarian cancer female patient with a positive family history and another deletion of four exons (Exons 10-13) in male patient with malignant neoplasm of axillary tail of left breast (Table 4).

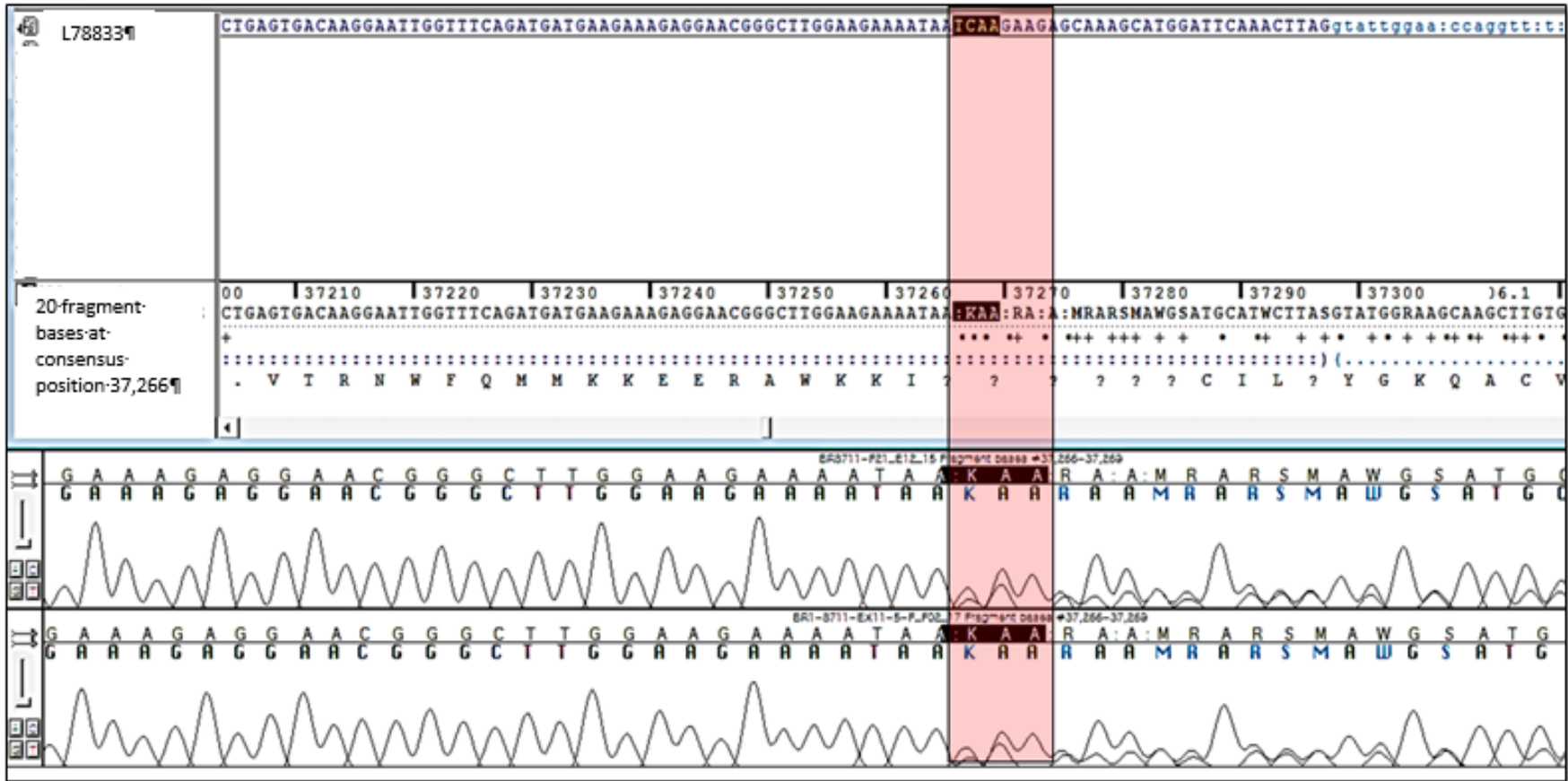


Figure 7: Sequencing chromatogram illustrating the most common mutations in *BRCA1* gene c.4065_4068delTCAA

Table 3: Characteristics of Patients with *BRCA1* Gene Mutation

<i>*BRCA1</i> Gene Mutation	RS ID	Exon Number	Type of Mutation	Cases with <i>BRCA1</i> Gene Mutation, Frequency of Disease Causing (n=18)		
				N. of Patients (n=18)	%	Characteristics
c.4065_4068delTCAA (p.Asn1355Lysfs*10)	rs80357508	10	Frameshift	12	67	9 with positive family history, 6 members of 1 family with BC, 6 unrelated patients with 3 BC and 3 OC, 3 TNBC, average age 39.7
c.68_69delAG (p.Glu23Valfs*17)	rs80357914	2	Frameshift	1	6	Positive family history and TNBC
c.2704delG (p.Glu902Asnfs*98)	rs886040064	10	Frameshift	2	11	2 members of 1 family (cousins) with BC, average age 48
c.(80+1_81-1)_ (441+1_442-1)dup (MLPA)		3,5,6,7	Frameshift \Duplication	1	6	Positive Family history, OC patient, Age ≤ 45
c.4186-10G>A (VUS)	rs80358172	11	IVS	1	6	TNBC, Age >45
c.5323G>C (p.Glu1775Gln)	rs80357432	20	Missense	1	6	TNBC, Age >45

**The description of nucleotide sequence is in accordance with HGVS nomenclature. DNA mutations are numerated according to NCBI reference sequence NM_007300.3 for *BRCA1* Bold: most common mutation. Highlighted: Not reported (Novel) mutation. VUS-variant of uncertain significance. MLPA-Multiplex ligation-dependent probe amplification analysis. IVS-Intron Variant Sequence.*

Table 4: Characteristics of Patients with *BRCA2* Gene Mutation

<i>*BRCA2</i> Gene Mutation	RS ID	Exon Number	Type of mutation	Cases with <i>BRCA2</i> Gene Mutation, Frequency of Disease Causing (n=20)		Characteristics
				No. of Patients		
				(n=20)	%	
c.4124delA (p.Glu1375fs)	rs886040512	11	Frameshift	2	10	Hormone receptors positive, average age 54, BC and OC patients
c.3649delA (p.Arg1217fs*11)	rs864622134	11	Frameshift	1	5	OC patient, age >45
c.771_775delTCAAA (p.Asn257Lysfs)	rs80359671	9	Frameshift	3	15	3 members of 1 family (2 sisters and aunty) age ≤ 45, all affected with BC
c.2808_2811delACAA (p.Ala938Profs*21)	rs80359351	11	Frameshift	3	15	Hormone receptors positive, average age 49, one BC and two OC patients
c.7007G>A (p.Arg2336His)	rs28897743	13	Missense	2	10	Hormone receptors positive, average age 54.5, Omani BC female and Prostate cancer male patient
Deletion in Exon 5 (MLPA)		5	Frameshift \ Deletion	1	5	Positive family history, age ≤ 45, OC patient
c.1593dupA (p.Glu532Argfs*3)	rs397507272	10	Frameshift	3	15	2 members of 1 family (brother and sister) and 1 patient, all with BC
**c.4574A>T (p.His1525Leu) (VUS)	rs397507336	11	Missense	1	5	Hormone receptors positive BC, Positive family history, age > 45
c.2654A>G (p.Asp885Gly)	rs398122750	11	Missense	1	5	BC patient , age ≤ 45
c.4415_4418delAGAA (p.Lys1472fs)	rs397507333	11	Frameshift	1	5	BC patient , age ≤ 45
c.9109C>A(p.Gln3037Lys)	rs397508037	23	Missense	1	5	TNBC, BC patient, age ≤ 45
Deletion in Exons 10-13 (MLPA)		10-13	Frameshift \ Deletion	1	5	Male patient with BC, age ≤ 45

**The description of nucleotide sequence variations is in accordance with HGVS nomenclature. DNA mutations are numerated according to NCBI reference sequence NM_000059.3 for BRCA2. Bold: most common mutation. Highlighted: Not reported (Novel) mutation. VUS-variant of uncertain significance. MLPA-Multiplex ligation-dependent probe amplification analysis. ** This mutation was classified as Pathogenic in Varsome and VUS in ClinVar.*

3.3 Association of *BRCA* Status and TNBC

Out of 262 cases enrolled in this study, 127 cases were tested for TNBC by immunohistochemistry test (Figure 8).

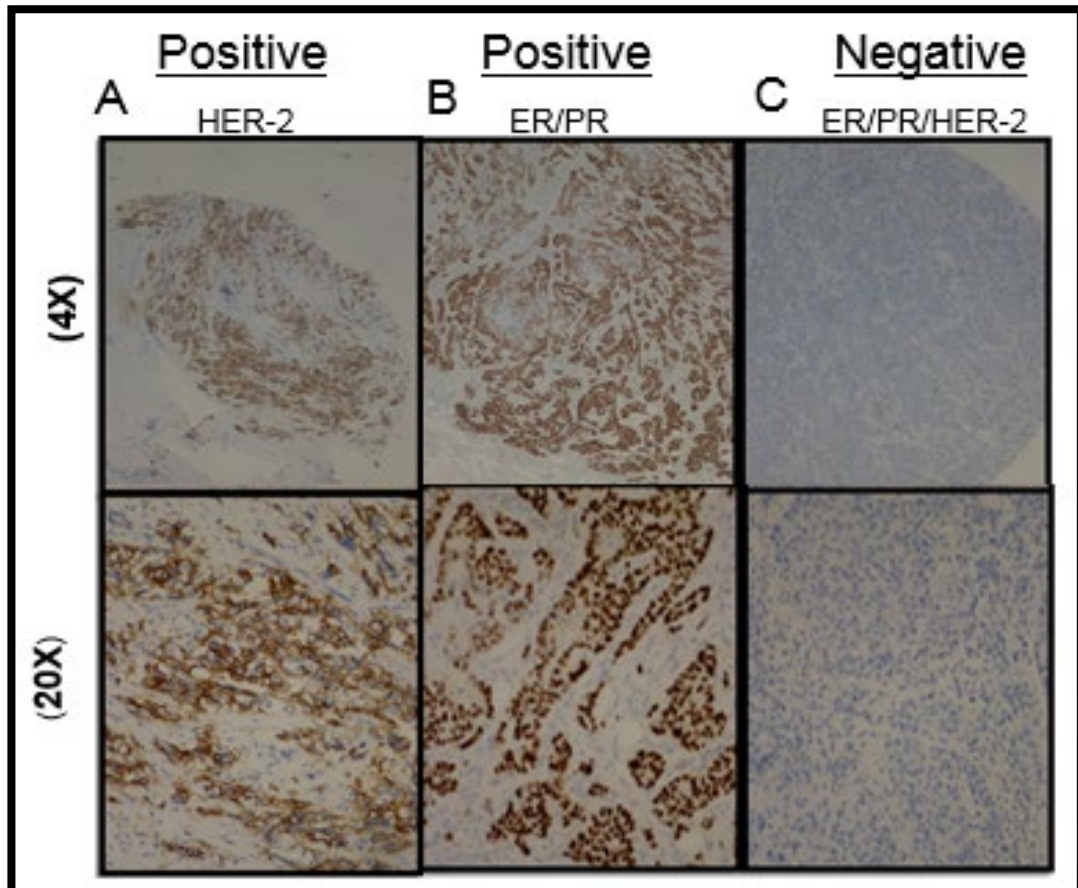


Figure 8: Immunohistochemical Staining. Representative examples of immunostaining for the protein markers ER, PR and HER-2. Negative or positive staining. All the slides were analyzed in x 4 and x 20 microscope objectives. Brown staining with light blue background refers to positive in ER, PR (B), and HER2 (A). Light blue background refers to negative (C) for ER, PR, HER-2 as shown alongside higher magnification.

Out of total 127 patients, 28 were TNBC (22%). 6 of these TNBC patients revealed a *BRCA1* gene mutations (21.4%) and one patient had a *BRCA2* mutation (3.6%). Other 21 patients didn't show any mutation either in *BRCA1* or *BRCA2* genes (75%). Taken together, *BRCA1* mutation carriers were more likely to have TNBC than those of *BRCA2* carriers (Figure 9).

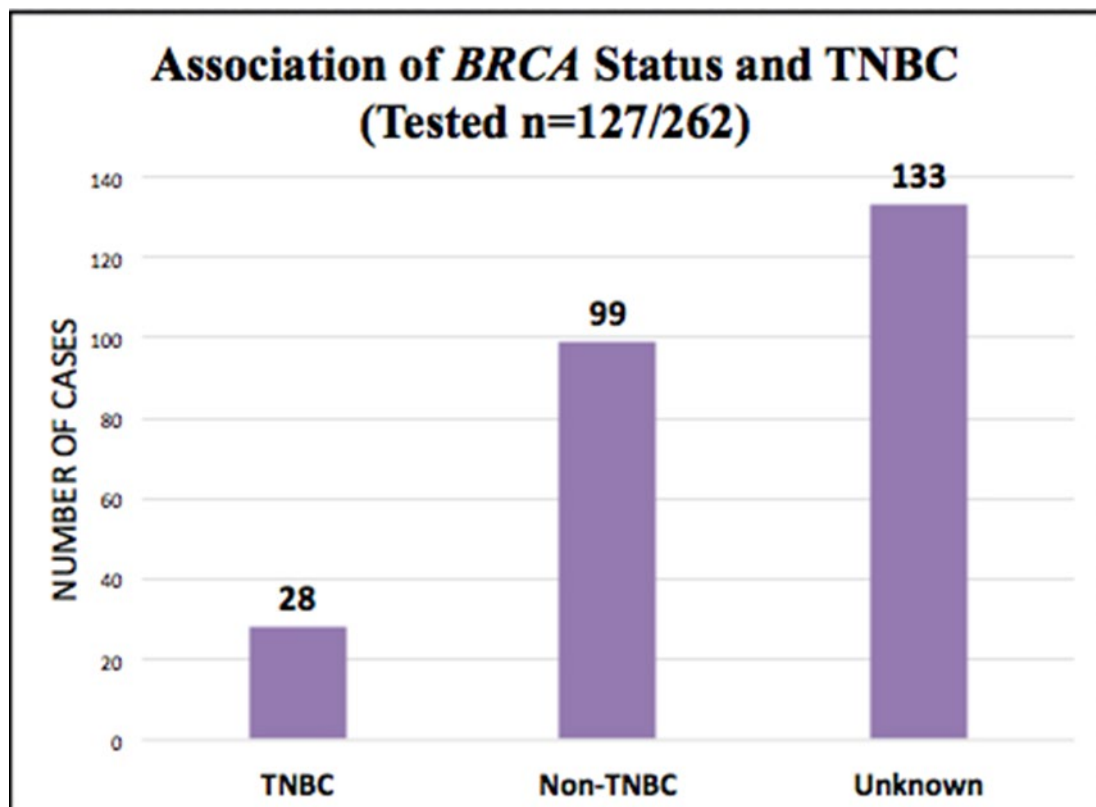


Figure 9: Association of *BRCA* status and TNBC. Unknown indicated patients who did not undergo immunohistochemistry test in SKSH.

3.4 Correlation of BRCA1 Gene Mutation with Different Factors

According to Chen et al. [31], *BRCA1* carriers were more likely to have TNBC than those of *BRCA2* carriers or non-*BRCA1/2* carriers among patients with breast cancer [31]. Also, the correlation of *BRCA1* gene mutation with different factors including TNBC, age and familial history was studied. The results showed that patients with *BRCA1* gene mutation is significantly correlated with young age ($p < 0.01$), and family history ($p < 0.05$) while TNBC ($p = 0.17$) alone was not associated with significant risk (Table 5).

Table 5: Correlation of *BRCA1* Gene Mutation with Different Parameters

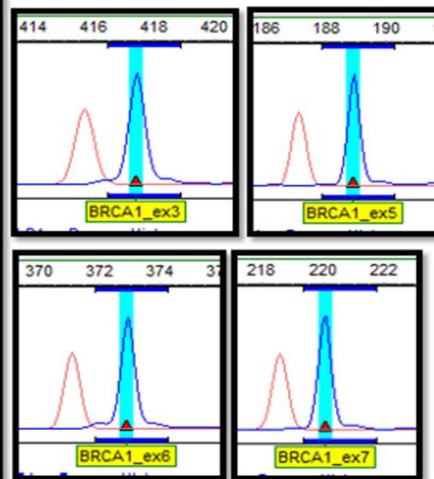
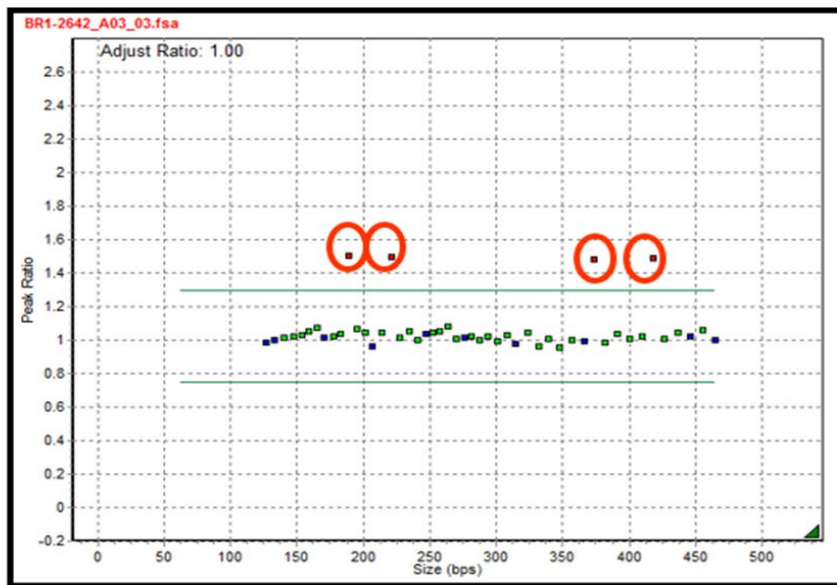
Factor	<i>BRCA1</i> Mutation				n	P
	Positive		Negative			
	No. of Patients	%	No. of Patients	%		
Age, years					262	0.000** (< 0.01)
≤ 45	11	(4.2%)	117	(44.7%)		
>45	7	(2.7%)	127	(48.5%)		
Family history of BC or OC					262	0.02* (< 0.05)
No	5	(1.9%)	186	(71.0%)		
Yes	13	(5.0%)	58	(22.1%)		
TNBC					127	
Yes	6	(4.7%)	22	(17.3%)		
No	2	(1.6%)	97	(76.4%)		
Abbreviations: BC: breast cancer; TNBC: triple-negative breast cancer, n: population size.						
*Two-sided significance level set at 5%.						
**Two-sided significance level set at 1%						

3.5 Novel *BRCA1/2* Mutations Among UAE Population in Northern Emirates

In Tables 3 & 4, 15 mutations were found and these mutations had been reported in different databases such as ClinVar, Breast Cancer Information Core (BIC), Varsome, AlAmut and/or HGMD2019. Three Novel mutations were detected by MLPA of a large exon rearrangements first, a duplication of 4 exons (Exon 3,5,6, and 7) in *BRCA1* gene of a 43 years old emirate female who have been diagnosed as an OC patient with a family history of BC. Other two novel mutations were found in *BRCA2* gene with a deletion of one exon (Exon 5) of a 39 years old female patient with family history of malignant neoplasm of ovary. Also large deletion of 4 exons (Exon 10-13) was detected in a 42 years old male patient with malignant neoplasm of axillary tail of left male breast (Figure 10).

A

***BRCA1* gene: Duplication of 4 exons (Exon 3,5,6 & 7)**



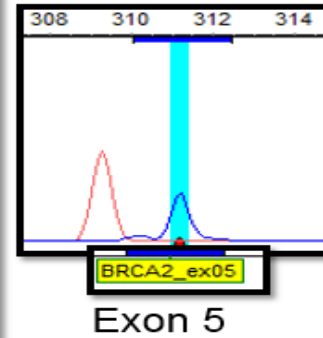
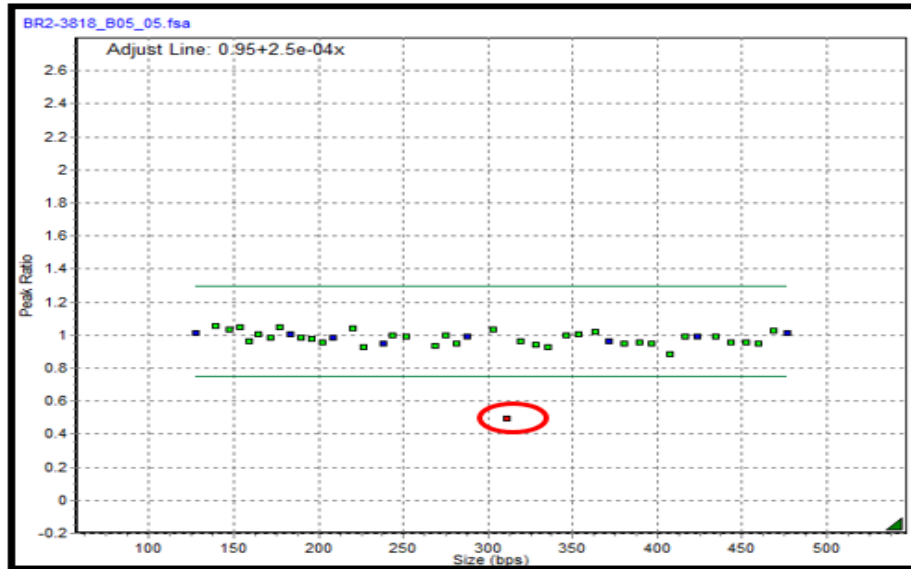
	REF - sj	BR1-2642	
1	*Ctrl1_5q31	127.2	
2	*Ctrl1_15q21	132.7	
3	BRCA1_ex11	140.3	
4	BRCA1_ex24	147.3	
5	BRCA1_ex1a	153.3	
6	BRCA1_ex16	159.2	
7	BRCA1_Upstrm	164.8	
8	*Ctrl1_18q21	170.7	
9	BRCA1_ex2	177.2	
10	BRCA1_ex23	183.1	
11	BRCA1_ex5	189.1	3
12	BRCA1_ex16_2	195.4	
13	BRCA1_ex13	201.5	
14	*Ctrl1_3q26	207.0	
15	BRCA1_ex19	214.0	
16	BRCA1_ex7	220.6	3
17	BRCA1_ex21	227.3	
18	BRCA1_ex11_2	234.1	
19	BRCA1_ex9	240.5	
20	*Ctrl1_13q14	246.7	
21	BRCA1_ex10	252.5	
22	BRCA1_ex18	257.6	
23	BRCA1_ex11_3	263.6	
24	BRCA1_ex14	269.6	
25	*Ctrl1_1p33	275.7	
26	BRCA1_ex11_4	281.1	
27	BRCA1_ex1b	287.5	
28	BRCA1_ex11_5	294.1	
29	BRCA1_ex13_2	301.0	
30	BRCA1_ex24_2	308.2	
31	*Ctrl1_6q16	314.8	
32	BRCA1_Upstrm_2	323.9	
33	BRCA1_ex15	331.8	
34	BRCA1_ex11_6	339.3	
35	BRCA1_ex17	347.1	
36	BRCA1_ex12	356.5	
37	*Ctrl1_8q12	365.6	
38	BRCA1_ex6	373.1	3
39	BRCA1_ex11_7	381.2	
40	BRCA1_ex20	390.6	
41	BRCA1_ex8	400.6	
42	BRCA1_ex22	410.1	
43	BRCA1_ex3	417.7	3
44	BRCA1_ex11_8	426.4	
45	BRCA1_ex24_3	436.7	
46	*Ctrl1_1q42	445.6	
47	BRCA1_ex13_3	455.1	
48	*Ctrl1_2q37	464.8	

Case: a 43 years old emirate female who have been diagnosed as an OC patient with a family history of BC

Figure 10: Novel BRCA1/2 mutations detected by MLPA analysis workflow in GeneMarker software in this study. Part of an MLPA analysis report (GeneMarker v3.0.1) of *BRCA1* in A and *BRCA2* in B and C. The resulting peak ratios are plotted in the ratio plot above, and are reported for each case in the Report Table to the right. (Upper: Four Duplications, middle: one deletion, and bottom: Four Deletions).

B

***BRCA2* gene: Deletion of Exon 5**



	REF - Syr	BR2-3818
1	*Cttrl_05q31	127.3
2	BRCA2_ex01A	139.1
3	BRCA2_ex01B	147.2
4	BRCA2_ex14	153.6
5	FRY	159.0
6	BRCA2_ex02	164.7
7	BRCA2_ex03B	171.6
8	BRCA2_ex03A	177.1
9	*Cttrl_04q	183.7
10	BRCA2_ex23	189.2
11	BRCA2_ex04	195.3
12	BRCA2_ex07	201.8
13	*Cttrl_09q	208.4
14	BRCA2_ex08	220.5
15	BRCA2_ex09	226.9
16	*Cttrl_02q	238.3
17	BRCA2_ex10	243.8
18	BRCA2_ex11A	252.2
19	HCS20	268.9
20	BRCA2_ex11B	275.1
21	BRCA2_ex12	282.5
22	*Cttrl_11q	288.3
23	BRCA2_ex13	302.6
24	BRCA2_ex05	311.2 1
25	BRCA2_ex27B	320.0
26	BRCA2_ex15	327.8
27	BRCA2_ex16	336.0
28	BRCA2_ex06	346.0
29	BRCA2_ex17	353.5
30	BRCA2_ex18	362.7
31	*Cttrl_11q	371.5
32	BRCA2_ex19	380.6
33	BRCA2_ex20	389.0
34	CHEK2	397.2
35	BRCA2_ex21	407.5
36	BRCA2_ex22	416.0
37	*Cttrl_08q	423.4
38	BRCA2_ex24	434.1
39	BRCA2_ex25	443.2
40	CG018	452.2
41	BRCA2_ex26	459.9
42	BRCA2_ex27A	468.7
43	*Cttrl_17q	476.8

Case: a 39 years old OC female patient with family history of malignant neoplasm of ovary.

Figure 10: Novel BRCA1/2 mutations detected by MLPA analysis workflow in GeneMarker software in this study. (Continued).

C

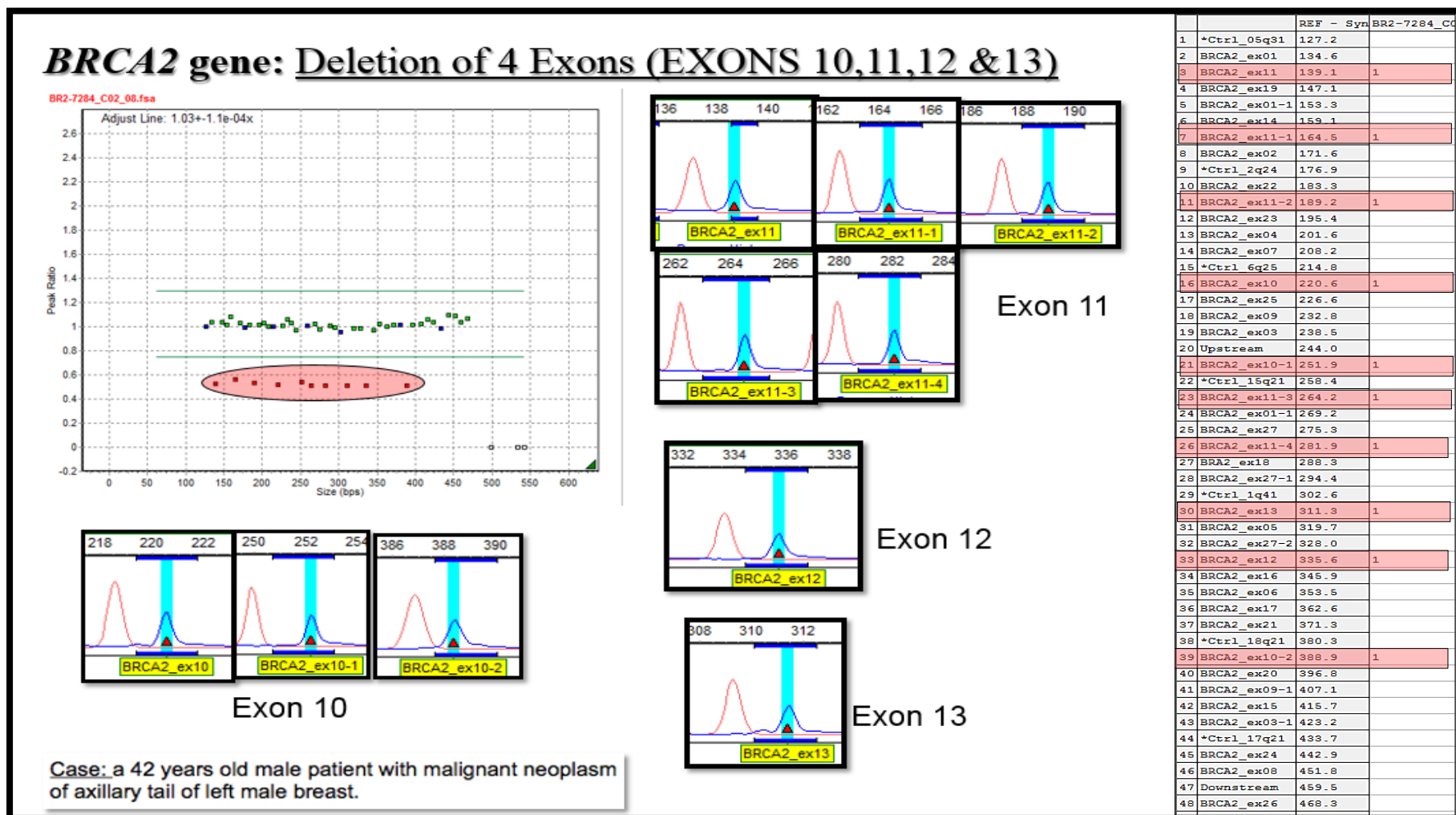


Figure 10: Novel BRCA1/2 mutations detected by MLPA analysis workflow in GeneMarker software in this study. (Continued).

Chapter 4: Discussion

Cancer poses one of the major health problem challenges worldwide. *BRCA1/2* germline mutations are responsible for genetic predisposition and may increase the risk for breast and ovarian cancer [43]. To date, few studies have been reported on *BRCA* associated breast cancer in the Arab world. In the present study, unselected breast or/and ovarian cancer patients with one of the following high-risk criteria were eligible for the *BRCA* mutation analysis: a first- degree relation with a known mutation in a cancer susceptibility gene, history of TNBC in family member, male breast cancer, first or second degree relative with breast or ovarian cancer at age ≤ 45 years.

According to several studies and reports across the globe, prevalence of *BRCA* mutations varies widely among different populations. This variation can be attributed to the effects of founder mutations and environmental factors as well [44-45]. In Western countries, the genetic testing for breast cancer have become standard clinical management for selected patients. However, in Arab countries it is still less managed and genetic testing is not well-established yet. Only few studies reported *BRCA* mutations among breast and ovarian cancer in the Eastern and Arab countries. According to these studies, the *BRCA1/2* mutation pooled estimate was about 20% among patients with both breast and ovarian cancer [12].

To date as per the documented literature, this is the first study in UAE that used genetic analysis methods to evaluate the prevalence of germline *BRCA* mutations in breast and ovarian cancer patients. The analysis was made on patients unselected for age, family history, gender or the expression of hormonal receptors status. This retrospective study enrolled patients between July 2015 and August 2020 at Sheikh Khalifa Specialty Hospital in Ras Al Khaimah, being the leading medical facility for

the Northern Emirates. The results show that the prevalence of the *BRCA1/2* mutation among the 262 cases enrolled in this study is 14.5% (38/262).

The results of this study were consistent and can be further ascertained with a recent publication (March 2019) by Abdulrashid et al who report *BRCA1/2* prevalence among HBOC /HBC based on 14 selected studies from different Arab countries. Eight studies from North Africa (3 Morocco, 2 Algeria, 2 Tunisia, 1 Egypt), four from Middle East (2 Lebanon, 1 Palestine, 1 Jordan) and two studies from GCC (1 Saudi Arabia and 1 Qatar) (Table. 1). In Middle East countries, the pooled prevalence of BRCA mutations was high ~ 28%, followed by the GCC countries with pooled prevalence ~22%, and the pooled prevalence was 16% in North Africa [18].

Globally, the prevalence of *BRCA1/2* mutations was also estimated in an international publication by Armstrong et al. [37], where a 70 large studies reported *BRCA1* and/or *BRCA2* mutation prevalence data in BC patients across the following 12 countries: (33 USA), (11 South Korea), (4 United Kingdom), (4 Spain), (3 Germany), (3 Italy), (2 Canada), (2 Australia) , (2 Russia), (2 France), (1 Japan) and (3 Israel). The prevalence of germline *BRCA* mutations enrolled in unselected population on any basis other than breast cancer was clearly shown in Table.1, where the highest prevalence was reported in United States study with 15.4% and the lowest prevalence was reported as 2.7% in a French study [37].

The prevalence of *BRCA1* and *BRCA2* in this study population were 6.9% and 7.6% respectively. Data from Arab region studies showed that *BRCA2* is the most common type mutation among HBOC patients with an estimated pooled prevalence of 17% [18]. Likewise, among Asian populations *BRCA2* mutations are more common. Western population showed a different pattern, where *BRCA1* mutations were more common. One study on Saudi patients reported the frequency of *BRCA1* mutations as

10.7% [27]. *BRCA* mutations prevalence could vary widely within demographic groups and over countries

In this study, 18 mutations were reported of both *BRCA* genes in 38 breast and/or ovarian cancer patients. In addition to sequencing method, MLPA was also performed for all patients' samples. The results showed a number of rearrangement of *BRCA* gene exons either deletion or duplication of one or more exons in *BRCA1/2* genes. Most of these mutations were frameshift deletions (10%) in *BRCA2*, duplications (5.6%) in *BRCA1* gene. Some common *BRCA1/2* mutations were found and reported previously in the GCC, Arab Countries and world populations. For example, the most abundant *BRCA1* mutation in this study c.4065_4068delTCAA (p.Asn1355Lysfs*10) was found twelve times in eight female patients and one male patient with strong family history of breast cancer and also this mutation is prevalent in GCC , Middle East, Europe populations [14, 36].

Another mutation was found in *BRCA1* gene, c.66_67AG (p.Glu23Valfs*17) which was detected in a migrant woman worker from India with breast cancer. Also, this mutation accounted for the 30.4% of the *BRCA1* mutations and is common in Spanish and Ashkenazi Jewish populations. Ovarian cancer patients are more likely to carry this type of mutation [36].

Three most common pathogenic frameshift mutations found in *BRCA2* gene are c.1593dupA (p.Glu532Argfs*3), c.771_775delTCAA (p.Asn257Lysfs) and c.2808_2811delACAA (p.Ala938Profs*21). All these mutations were detected in emirate families with breast cancer history. First c.1593dupA (p.Glu532Argfs*3), was found in one sister and brother, the second c.771_775delTCAA (p.Asn257Lysfs) in two sisters of another family and the third mutation c.2808_2811delACAA (p.Ala938Profs*21) found to be one of the common mutations in GCC populations

[31]. A missense mutation c.7007G>A (p.Arg2336His) was found twice in Omani female and male cancer patients and this mutation has been reported in populations of Middle East, Saudi Arabia and Qatar [14][36]. According to a review by Rahman, S., and Zayed, H. [14], ClinVar reports confirmed the pathogenicity for *BRCA2* c.7007G > A and that it can cause disease. Also, this mutation was found in Hong Kong [14].

Triple negative breast cancer is aggressive molecular subtype among other breast cancer cases because it lacks ER, PR and expression of HER2 receptors. Thus, no validated molecular targets are available for the treatment and therefore considered a high-risk breast cancer with overall poor prognosis. Of all breast cancers, TNBC accounts for about 15%. Reports showed that TNBCs are the predominant cancer subtype with a germline *BRCA* mutation in which the prevalence of the *BRCA* mutation is 10 to 30% [46-48]. *BRCA1* mutation shown also to occur more frequently at younger age [23]. In this study, *BRCA1/2* mutation in TNBC patients was evaluated. Among all 127 patients who underwent immunohistochemistry staining in Pathology Department of Sheikh Khalifa Specialty Hospital, 28 patients (22%) were identified with immunohistologically confirmed invasive, ER, PR, and HER2 negative BC.

A total of 7 (25%) *BRCA* mutations were identified in 28 patients who had TNBC. In the current screening, the prevalence of the *BRCA1* germline mutation was about 21.4% (6/28), and the prevalence of the *BRCA2* germline mutation was about 3.5% (1/28). These data revealed that the prevalence of *BRCA1* mutations is more common than *BRCA2* in TNBC patients, and *BRCA1* mutation is found more common among TNBC patients but not significantly correlated with the *BRCA1* mutation.

These findings are consistent with the data from international studies demonstrating that *BRCA1* gene mutation were more common (85.7%) than *BRCA2* gene mutation (14.3%) in TNBC [36]. Furthermore, literature revealed that TNBC is

the predominant molecular profile in patients with a germline *BRCA1* mutations [49-50]. Several studies have evaluated the prevalence of *BRCA1* mutations in patients with TNBC. Reports showed that the prevalence of *BRCA1* mutations in TNBC patients varies between populations and from one study to another. Most studies included patient populations selected for young age at diagnosis (< 50 years) and reported a prevalence ranging from 7.6 to 23% [51-54]. While other studies evaluated the frequency of *BRCA1* mutations in unselected TNBC patient populations. The *BRCA1* mutation frequency was 6.5% when unselected women with TNBC were evaluated [55]. When selected by age, the frequency of *BRCA1* mutations was 2.8% in patients diagnosed at age ≥ 50 years [55]. One study showed that *BRCA1* mutations were detected in 9% of unselected TNBC patients and in 4.8% of women diagnosed at age ≥ 50 years [56]. In one large study of unselected TNBC patients (n = 1824), the prevalence of *BRCA1* mutation was 8.5%. For the 50–59 age group, the prevalence was 7.4% [57]. In this study 21.4% (6/28) of TNBC patients, unselected for age and family history, were found to carry *BRCA1* mutation. These findings are with the agreement with previous reports for Asian populations [44-45, 58-59], where *BRCA1*-mutated tumors were ER- or PR-negative and had a higher histological grade, but exhibited less medullary carcinoma compared to the Western population [25]. Studies have shown that TNBC with *BRCA1* has a unique morphology and immunohistochemical phenotype that explains the susceptibility of breast cancer women with germline *BRCA1* mutations to be triple negative [30]. TNBC with family history may influence the *BRCA* mutation. Although several studies have evaluated the prognostic role of the *BRCA1/2* mutation in patients with TNBC, these studies have shown inconclusive results. The present study is a small scale screening analysis and not all patients underwent immunohistochemistry. Therefore, a possible association

between TNBC, *BRCA* carriers, and positive family history was not made involving all breast cancer patients enrolled in this study. Thus, a conclusion needs more data collection and further investigation.

Three novel large exons rearrangement of both *BRCA1* and *BRCA2* genes using MLPA were found in two female ovarian cancer patients with strong family history and one breast cancer male. One large *BRCA1* genomic rearrangements duplication in exon 3, 5, 6 and 7 (c.(80+1_81-1)_(441+1_442-1) dup) was detected in a 43 years old female patient with ovarian cancer and considered a novel mutation as it was not reported in any database yet. As reviewed by Rahman and Zayed [14], the genomic rearrangement in *BRCA1* that was found in a study of 43 Omani BC female patients which only exon 3,5,11,13 and 20 were tested by direct Sequencing and MLPA and they identified four large genomic rearrangement were revealed: two duplications and two deletions in exons 1 and 2 [14].

In *BRCA2*, one case with a deletion of exon 5 was detected in ovarian cancer female patient and there is no available data of the contribution of *BRCA2* mutations located on exon 5. Another large deletion of four exons in *BRCA2* gene was identified in breast cancer young male patient and similar to this mutation (deletion of 3 exons 10-12) was reported once in the literature [60].

Chapter 5: Conclusion and Future Recommendations

In conclusion, this study provides a baseline information on the prevalence of *BRCA* mutation in hospital based population in northern emirates. The current findings indicate that 14.5% of unselected patients enrolled in this study carried *BRCA* mutations. The present data provide an evident contribution of *BRCA1* and *BRCA2* mutation in patients of breast and ovarian cancer. One limitation of this study is the low number of patients who underwent TNBC testing. Furthermore, the results suggest to collect more data regarding the status of TNBC and *BRCA* mutations to make a conclusive data whether TNBC could be an effective threshold for *BRCA* genetic test and whether to be considered in genetic testing guidelines in UAE in the future. Moreover, this study could be extended further by investigating the possible association between patients with positive *BRCA* mutations and the clinical histopathology of the cancer.

To date, there is no large scale study conducted in Arab countries for *BRCA* mutations including UAE. Therefore, it is important to conduct a large scale investigation to better understand the contribution and the frequency of *BRCA* mutations in patients with TNBC. This will contribute to genetic counseling for mutation carriers, offer better disease management and will definitely play an important role in establishing local diagnostic frame for genetic testing.

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