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Genomic Analysis of familial Ovarian and Breast Cancer among Saudi Population

Maram Otaiby[1], Lamees Altamim[2]

[1]Assistantprofessor, pathology department, medical college, King Saud University
[2]Medical student, King Saud University

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Introduction

- Breast cancer is the most common cause of mortality of women at the age of 40-50 year old.
- In Saudi women the highest age of diagnosis was between 21 to 36 years which might suggests stronger link to hereditary factors like BRCA mutations.
- Ovarian cancer has the highest mortality rate among gynecological cancers with high morbidity rate estimated to be the seventh most common cancer among women.
- The Saudi population is estimated to have the highest rate of consanguinity worldwide, and the spread of unique genetic homogeneity and Mendelianism has resulted in a higher incidence of genetic disorders.
- There is not any local study that characterized ovarian cancer genetic mutation in Saudi women.

Introduction

- We aim to identify genetics predisposing factors of familial Ovarian and Breast cancers in Saudi Population.
- Founder mutation identification of breast and ovarian cancers will be of great value to expedite development of screening testing the target population.
- Identification of BRCA mutations in Saudi women will be an important intervention for the patient.
- we aim for (exome) genome sequencing to identify novel mutations in Saudi women and in patient with aggressive clinical features.

Objectives

- To Screen the patient samples for Epithelial Ovarian and Breast Cancer (excluding borderline tumors) genes which include BRCA1, BRCA2, MLH1, MSH2

- We further want to hypothesize to do whole exome sequencing on a subset of positive samples with evident mutations in BRCA1, BRCA2, MLH1, MLH2. This would enable to find other associated mutation in other physiologically related genes.

Materials and Methods

Inclusion Criteria

1. Female patient with confirmed cases epithelial ovarian cancer and breast cancer patients by histopathological examination

2. Saudi nationality at birth, aged 18-80.

Exclusion Criteria

Known case of BRCA mutation

Sample storage

Sample type: K3 – EDTA blood Sample

Sample Size: 2 EDTA tubes 3ml Each (one for testing on NGS) and one for confirmation using Sanger sequencing in case a mutation is found.

Sample Storage conditions: there is no limit on the time the sample should be stored, but the sample should be stored at around 4 degrees Celsius (not frozen).

Transport conditions: Samples should be transported at 4 degrees Celsius. (The containers arrangement)

Materials and Methods

Genetic Screening

Genomic DNA extracted from whole blood is used for genetic analysis. An average of 100-200ng genomic DNA will be utilized for the study. A set of specific mutations in the following genes will be studied –

1. Breast cancer 1 (BRCA1)
2. Breast cancer 2 (BRCA2)
3. mutL homologue 1, colon cancer, nonpolyposis type 2 (MLH1)
4. mutS homologue 2, colon cancer, nonpolyposis type 1 (MSH2)

Materials and Methods

DNA extraction and quantification

- The blood samples stored at -80°C is thawed to room temperature before use.

- The QIAamp DNA Mini kit is used for extraction.
- DNA extraction will be done after lysing the RBCs
- The extracted DNA is tested for quality and quantity on a Nanodrop spectrophotometer.
- The 100 nanogram quantity of DNA is used for PCR amplification.

Materials and Methods

Primer design, PCR optimization, amplification and purification

- The primers specific to the polymorphisms are designed using the UCSC Human Genome Browser.
- Primer pairs (forward and reverse) covering a minimum of 50 bases upstream and downstream are sent for commercial synthesis.
- A template of gene sequence with the aligned primers is prepared.
- The PCR program is finalized after several trails by a gradient PCR.
- The optimization is verified by analyzing the amplicon on a 2% agarose gel.

Materials and Methods

Primer design, PCR optimization, amplification and purification

- All the samples at 100 ng concentration are used for PCR amplification with Hotstar Taq PCR mastermix from Qiagen.
- The amplification is carried on a Veriti thermal cycler.
- A standard DNA ladder is also run alongside the samples. The separated bands are analyzed on a Biorad Gel Documentation unit.

Materials and Methods

Sequence PCR, Purification and DNA Sequencing

- The purified amplified products are used for setting up a sequence PCR. The Big Dye Terminator V3.1 kit from Applied Biosystems is utilized for this purpose.

- Specified volumes of SAM and X terminator solution are added to the sequence amplified products, vortexed at recommended speed for 30 minutes. Samples can be stored at 4°C at this stage for multiplexing on the DNA sequencer later.

Sequence Analysis and interpretation

A licensed sequence analysis software version 6 is used for the analysis

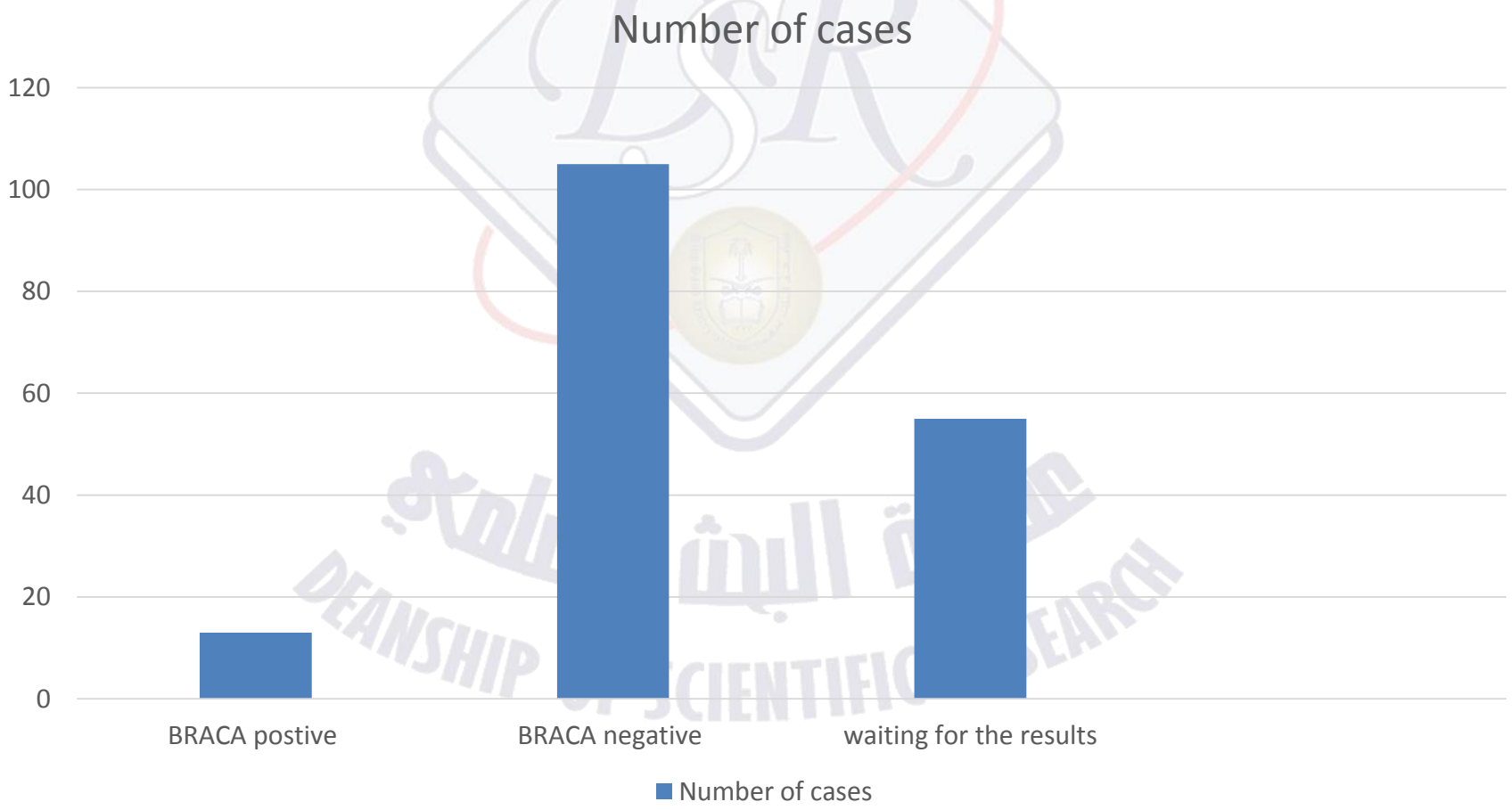
Results

- The total number of cases collected for BRACA genetic testing are 173 cases during last 2 years (2016-2018)
- 13 cases (7.5 %) was positive for BRACA, 105 (60.6%) was negative and 55 (31.8 %) are waiting for the results.
- Out of the 173 cases, 96 (55.49%) was diagnosed as breast cancer, 8(4.6%) was diagnosed as ovarian-adnexal mass.

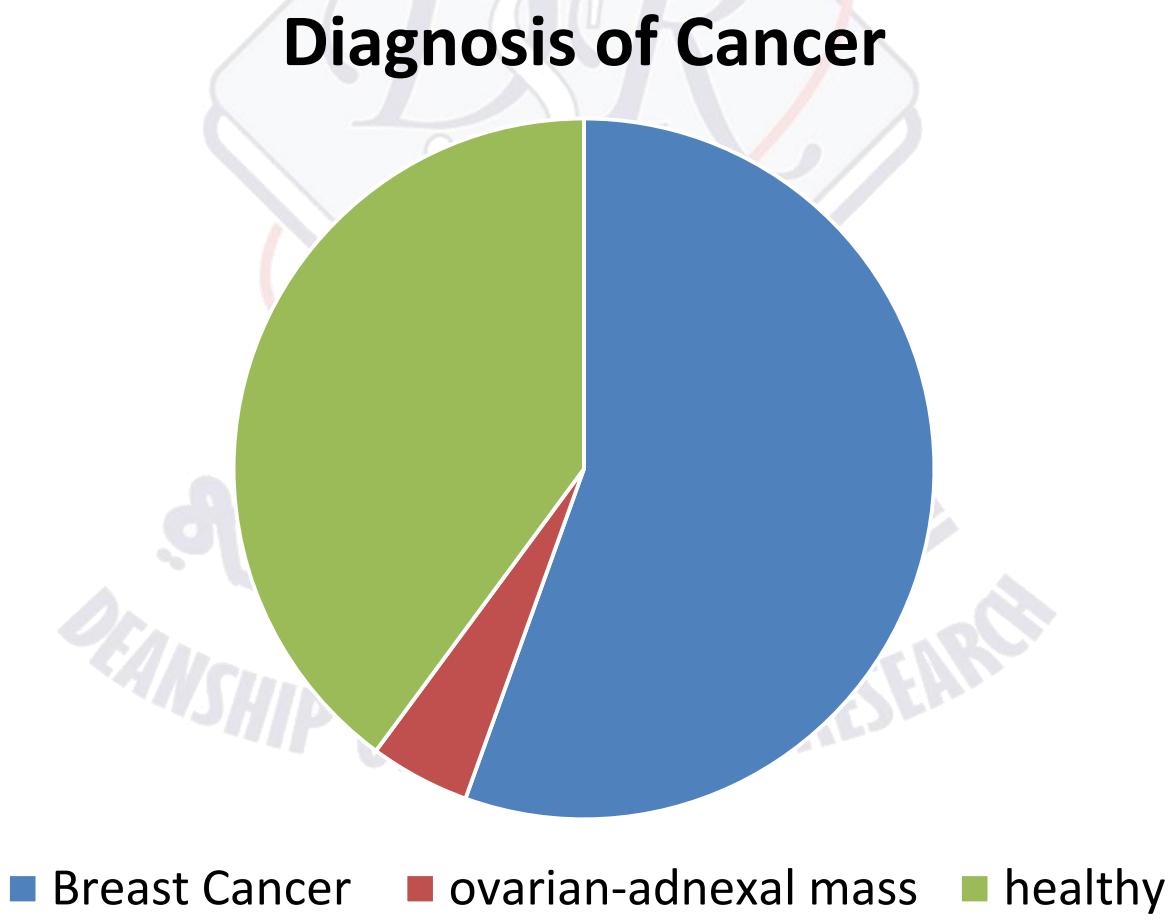
Results

- All of the cases are high risk patient for breast and ovarian cancer, 81 (46.8%) reported a history of breast cancer diagnosed at a young age (premenopausal or young than age 50), 10 (5.8%) has a personal history of triple negative breast cancer diagnosed at age 60 or younger, 6 (3.5%) has personal history of breast cancer affecting both breasts (bilateral breast cancer), 3 (1.73%) has a personal history of both breast and ovarian cancers. 9 (5.2%) had a personal history of ovarian-adnexal cancer.

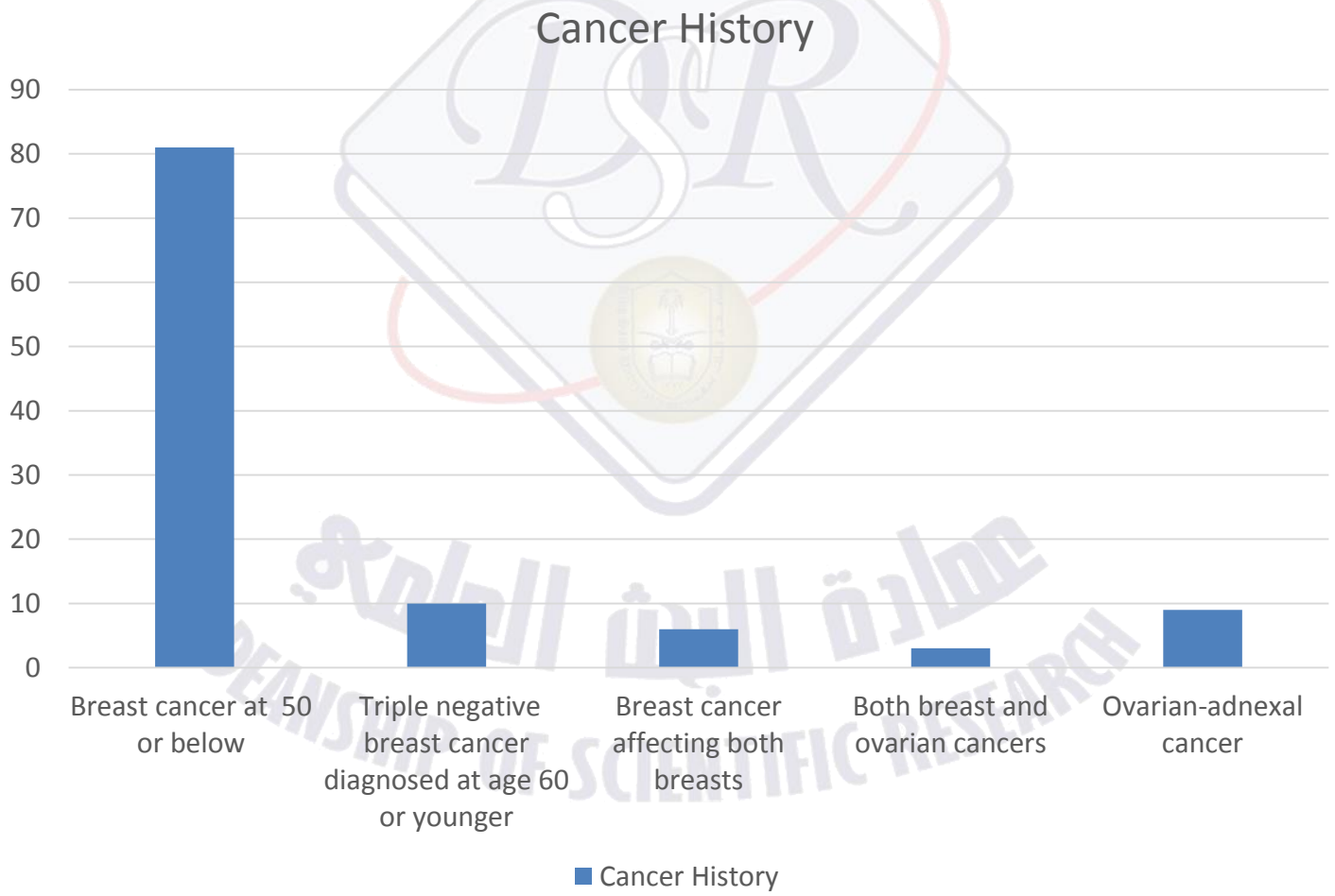
Results


Number of cases
120
100
80
60
40
20
0
BRACA postive
BRACA negative
waiting for the results
Number of cases

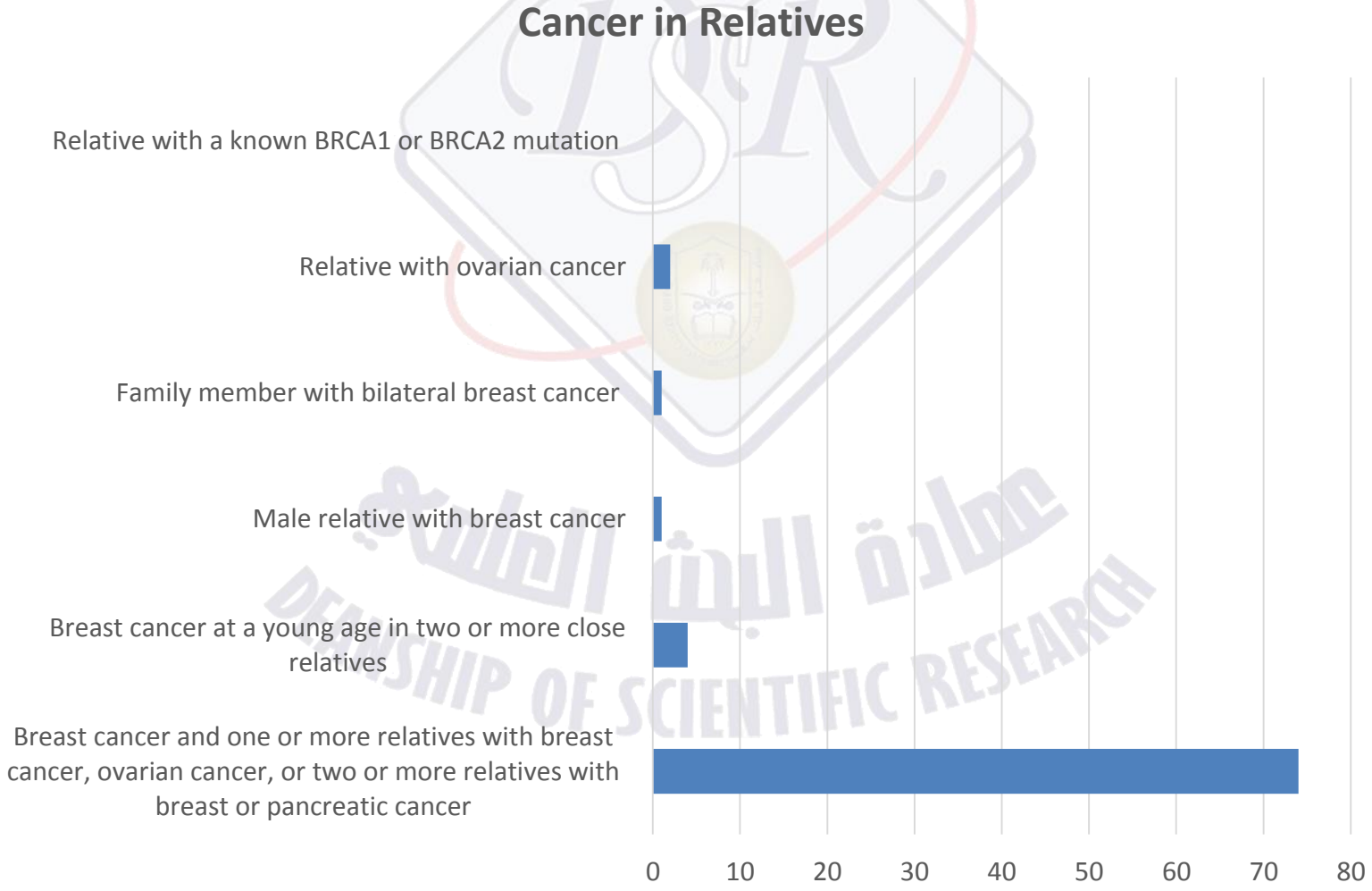
Results


Diagnosis of Cancer
Breast Cancer
ovarian-adnexal mass
healthy

Results


Cancer History
90
80
70
60
50
40
30
20
10
0
Breast cancer at 50 or below
Triple negative breast cancer diagnosed at age 60 or younger
Breast cancer affecting both breasts
Both breast and ovarian cancers
Ovarian-adnexal cancer
Cancer History

Results


Cancer in Relatives
Relative with a known BRCA1 or BRCA2 mutation
Relative with ovarian cancer
Family member with bilateral breast cancer
Male relative with breast cancer
Breast cancer at a young age in two or more close relatives
Breast cancer and one or more relatives with breast cancer, ovarian cancer, or two or more relatives with breast or pancreatic cancer
0
10
20
30
40
50
60
70
80

Conclusion

- The first phase of the study was concluded, all positive BRACA genes will be further studied for a set of specific genes :

1. mutL homologue 1, colon cancer, nonpolyposis type 2 (MLH1)
2. mutS homologue 2, colon cancer, nonpolyposis type 1 (MSH2)

- Whole (exome) genome sequencing will be conducted to identify novel mutations in Saudi women and in patient with aggressive clinical features.

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