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Title:

Molecular Identification of *BRCA1&BRCA2* Mutations in Unselected Breast Cancer Patients in Tumor Treatment & Cancer Research Center (*TTCRC*) - Shendi, River Nile State - Sudan

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M.S.c. Microbiology in Clinical Microbiology

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وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ كَذَٰلِكَ ۗ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ۗ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ ﴿٢٨﴾

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Declaration

I certify that this thesis submitted for the degree of PhD in molecular biology is the result of my own research, except where otherwise acknowledged, and that this thesis or any of its parts has not been submitted for higher degree in our university.

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Dedication

To my parents

To my husband

To my brothers

To my sisters

To my sons

To my daughter

And to my all friends

Acknowledgment

***All thanks be to Allah* و ما توفيقى الا بالله**

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Abstract

Background:

Breast cancer is the most commonly diagnosed cancer in women worldwide. Up to every eighth woman will develop it during lifetime. Most cases are sporadic, but an estimated (7%) are due to dominantly inherited predisposition, with germline mutations in *BRCA1* and *BRCA2*, predisposing genes.

Objectives:

The objectives of this study were to determine the role of *BRAC1/BRCA2* mutations in breast cancer patients attending Tumor Treatment and Cancer Research Center in Shendi using Sanger Sequencing, to identify the most clinical risk factors and also histological types associated with positive *BRCA1*&*BRCA2* mutations.

Design and setting:

The study is a cross sectional, hospital based study. The patient's group size was (52) patients age (30-71) years from Tumor Treatment & Cancer Research Center in Shendi. The control sample size was (30) healthy women matched with their age.

(52) Unselected females diagnosed with breast cancer during the period December 2013 to January 2015 and (30) healthy one as control, were interviewed face to face using a validated questionnaire. In addition to their demographic characteristics and risk factors a blood samples (3-5 ml) was taken from each patient and control, genomic *DNA* was extracted from peripheral blood. Polymerase chain reaction was used for the analysis of exons 11 (two fragments) and 20 of *BRCA1* and exon 11(one fragment) of *BRCA2*. PCR products with specific bands were sending for sequencing at Macrogen - Korea. All data were

analyzed using different bioinformatics programs and Statistical Package for the Social Sciences (SPSS Inc.) version 20.

Results:

The study results found that; invasive ductal carcinoma was seen to be the most histological types 21(45.7%) within age (51-60) years and there was a highly significant statistical correlation linkage between *IDC* and cancer grade II; (P 0.000). Moreover non- hereditary breast cancer (sporadic) type was revealed to be the most presented 29 (55.8%) compared to hereditary one, second degree relatives was observed to be the most type of cancer occurrence among hereditary types 16 (69.6%) and the breast cancer was the most common malignant cancer type diagnosed among patient's family relatives17 (73.9%).

Results revealed presence of three types of mutations all were presented in *BRCA1* in a total of 11 (21.2%) in patients and 8 (26.7%) of control. Two were novel, one of them was point mutation in exon 20 in which (G >A 160980) and was appeared in (8) of each patient and control with frequency (15.4%) and (26.7%) respectively. The second was a missense occurred in exon 11 (**S768T**) and was appeared in three patients with frequency (5.8%). the third one was silent mutation (**rs16940**) presented also in exon 11 (**L771L**) and also was appeared in two patients with frequency (3.8%). There was no mutation presented in *BRCA2*.

Moreover, *BRCA1* was found to play a role in both types (hereditary and sporadic) with frequency of (54.5 %) and (45.5 %) respectively.

In regards to risk factors associated with mutations, there was statistically significant difference with mutation and tribal / ethnicity (Jaalia) (P= 0.047).

But there were no statistically significant differences among mutated patients in related to age (P= 0.233), parity (P= 0.855), family history (P= 0.438), age at

menarche (P= 0.775), obesity (P= 0.625), oral contraceptive (P= 0.327), exposure to radiation (P= 0.666) and menopause (P= 0.989).

On the other hand, the results fount that; there was strong relation between the breast cancer mutation and the invasive ductal carcinoma histological type in comparison with others, 11(100%), but there was no statistically significant value observed (P= 0.611).

Results of the study performed found that; breast cancer mutations were shown to be more occurring with cancer grade II 6 (54.5%), at the same time there was no statistical significant differences seen.

Finally, the results indicated that; breast cancer mutations were observed to be more associated with *ER, PR, and HER-2* receptors negative with frequency (54.5%), (63.6%), and (90.9%) respectively. There was no statistical significant differences related to *ER* and *PR* receptors, but there was statistically significant relation with *HER-2* was observed.

Conclusions:

BRCA1 mutations are responsible for a significant proportion of both types of breast cancer in the study population, *BRCA2 was not contributed to hereditary breast cancer in this study, and* Ethnicity plays a role as risk factor for mutation inheritance.

Key word: *BRCA1*, *BRCA2*, Hereditary, Sporadic, Ethnic، *TTCRT*, Shendi

ملخص الدراسة:

مدخل:

يعتبر سرطان الثدى من اكثر السرطانات شيوعا بين النساء عالمياً، واحدة من بين تسعة نساء تصاب بسرطان الثدى خلال فترة حياتها ، 7% من الحالات تحدث بسبب توريث الطفرات الجينية الشائعة لجينى البركا1 والبركا2.

أهداف الدراسة:

الهدف الأساسى من الدراسة هو معرفة ما اذا كانت للطفرات الوراثية لجينى البركا 1 والبركا2 دور فى حدوث سرطان الثدى بنوعيه الوراثى والأكثر شيوعا لدى النساء المصابات بسرطان الثدى بمركز معالجة الأورام وأبحاث السرطان بشندى وكذلك تحديد عوامل الخطر وتحديد أكتر الأنواع النسيجية حدوثا بين الطفرات.

منهجية الدراسة:

دراسة مقطعية معتمدة على المستشفى، تتكون من 52 مصابة بسرطان الثدى غير مختارة تتراوح أعمارهم بين (71- 30) سنة فى الفترة من ديسمبر 2013 الى يناير 2015 وعينة ضابطة تتكون من 30 إمرأة سليمة تشترك مع العينة المصابة فى العمر.تم الحصول على المعلومات الديمغرافية وعوامل الخطر من خلال إستبانة كما تم جمع 3-5 مل من الدم، تم إستخلاص الحمض النووى الدنا كما تمت مضاعفته بإختبار البلمرة لإكسون 20 وجزيئين من إكسون 11 للبركا1 وجزء من إكسون 11للبركا2 كما تم إرسال العينات لإختبار تسلسل الحمض النووى لشركة ماكروجين بدولة كوريا. تم تحليل المعلومات من خلال إستخدام برمجيات مختلفة للمعلوماتية الحيوية وبرنامج الحزم الإحصائية للعلوم الإجتماعية للتحليل الاحصائى.

النتائج:

أوضحت الدراسة النتائج التالية: أن 21 (45.7%) عبارة عن سرطان الأغنية الغازى وهو أكثر الأنواع النسيجية شيوعاً بين المرضى كما توجد علاقة قوية ذات دلالة إحصائية بينه وبين الدرجة الثانية للورم (P= 0.000) .بالإضافة الى ذلك وجد أن النوع الشائع من سرطان الثدى أكثر حدوثاً من النوع الوراثى بنسبة 29(55.8%) بين المرضى. كما يعتبر الأقرباء من الدرجة الثانية الأكثر تاتيراً على المرضى المصابين بسرطان الثدى الوراثى 16 (69.6%) كما وجد أن سرطان الثدى هو الأكثر شيوعاً بين أقرباء المرضى بنسبة (73.9%) .

كذلك أظهرت الدراسة وجود ثلاثة أنواع من الطفرات الوراثية تركزت جميعها فى جين البركا 1 بنسبة (21.2%) للمرضى و (26.7%) للعينة الضابطة . نوعين من الطفرات تظهر لأول مرة بين مجموعة الدراسة، إحداهما (G>A 160980) فى إكسون 20 وقد وجدت بين المرضى والعينة الضابطة بنسبة (15.4%) و (26.7%) على التوالى ، والأخرى فى إكسون 11 (**S768T**) عند ثلاثة من المرضى (5.8%) . أما الثالثة فقد وجدت أيضا فى أكسون 11 (**L771L**) عند إثنين من المرضى (3.8%) .

أما بالنسبة لعوامل الخطر المرتبطة بالطفرات الجينية فقد أوضحت النتائج فرق ذا دلالة إحصائية بين الطفرات الجينية وقبيلة الجعليين (P= 0.047) .

على العكس من ذلك أوضحت النتائج أنه لاتوجد فروقات ذات دلالة إحصائية بين الطفرات الجينية من حيث العمر (P= 0.233) ، عدد الولادات (P= 0.855) ، التاريخ العائلى (P= 0.438) ، العمر عند أول دورة شهرية (P= 0.775) ، السمنة (P= 0.625) ، إستخدام حبوب منع الحمل (P= 0.327) ، التعرض للإشعاع (P= 0.666) ، وإنقطاع الطمث (P= 0.989) .

من جانب آخر أظهرت الدراسة علاقة قوية بين الطفرات المسببة لسرطان الثدى والنوع الغدى الغازى (100%) كما لوحظ أن هذه العلاقة ليست ذا دلالة إحصائية (P = 0.611) .

كما وجد أيضاً أن الطفرات الجينية أكثر حدوثا مع الدرجة الثانية للمرض و أنها أيضاً ليست ذات دلالة إحصائية (54.5%) .

وزيادة على ذلك أشارت الدراسة أن الطفرات المسببة لسرطان الثدى أكثر إرتباطاً مع المستقبلات السالبة لهرمونى الإستروجين والبروجسترون و HER-2 بنسبة 54.5 %، 63.6 %، و 90.9 % على التوالى. وهذة العلاقة ليست ذات دلالة إحصائية مع مستقبلات هرمونى الإستروجين والبروجسترون لكنها ذات دلالة إحصائية مع HER-2.

الملخص:

أخيراً أثبتت الدراسة أن الطفرات الوراثية الموجودة على جين البركا 1 هى المسئولة عن سرطان الثدى وأن جين البركا2 غير مرتبط بتوريث المرض لعينة الدراسة. كما بيّنت أن للعرق أو القبيلة كإحدى عوامل الخطر المرتبطة بسرطان الثدى دور فى توريث المرض .

الكلمات المفتاحية: جينى سرطان االثدى، النوع الوراثى ، النوع الأكثر شيوعاً ، العرق أو القبيلة ، مركز علاج الأورام وأبحاث السرطان ، شندى

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Abbreviations, Definitions & Synonyms

a. a	Amino acid.
ABRA 1	Tumor suppressor gene = (Abraxas) = CCDC98 = FAM175A.
ADP	Adenosine diphosphate.
AJ	Ashkenazi Jewish.
ASO	Allele specific oligonucleotides hybridization.
AT – rich	(Adenine, Thymine) -rich.
AT	Ataxia- telangiectasia syndrome.
ATM	Ataxia- telangiectasia mutated.
ATP	Adenine triphosphate.
ATR	Ataxia - telangiectasia and Rad 53 - related.
BACH	BRCA1- associated C-terminal helicase = BRIP1 = FANCJ.
BAP1	BRCA1- associated protein -1
BARD1	BRCA1- associated ring domain gene-1.
BASC	BRCA1- associated (genome) surveillance complex
BAT	BRCA - associated tumor.
BBD	Benign breast disease.
BC	Breast cancer.
BLAST	Basic local alignment search tool.
BCL	B - Cell leukemia/ lymphoma.
BCLC	Breast cancer linkage consortium.
BCT	Breast conserving therapy.
BER	Base Excision repair.
BIC	Breast Cancer Information Core.
BLM	Bloom syndrome gene.
BMI	Body Mass Index.

BP	Benzopyrene.
bp	Base pairs.
BRCA – PRO	BRCA- probability.
BRCA1	Breast cancer susceptibility gene-1.
BRCA2	Breast cancer susceptibility gene-2.
BRCT	BRCA1 carboxyl terminus.
BRIP 1	BRCA1 interacting protein (c-terminal) helicase 1.
CCDC 98	Coiled - coil domain containing protein 98 = Abra1=FAM175A.
CCND1	Cyclin (protein) D1. *Proto-oncogene.*
CDC 2	Cell division cycle 2.
CHIP	Chromatin immunoprecipitation.
CHK 1	Checkpoint kinase 1gene.
CHK 2	Checkpoint kinase 2 gene.
CDH 1	Cadherin I, type 1 gene.
cDNA	Complementary deoxyribonucleic acid.
CDK 1	Cyclin dependant kinase 1.
CDK N2A	Cyclin dependant kinase inhibitor 2A.
CI	Confidence interval.
CIP2A	Cancerous inhibitor of (PP) 2A.
CK	Cytokeratin.
CN	Contortrostatin.
CpG	Cytosine & guanine linked by phosphate.
CtIP	Carboxy terminal interacting/binding protein.
CYTO P450	Cytochrome P450 subfamily enzymes.
dbSNP	Database single nucleotide polymorphism.
ddNTPs	Dideoxy nucleotides triphosphate.

DDR	DNA damage response.
dNTP	Deoxynucleotides triphosphate (N= A, G, C, T).
dsDNA	Double stranded DNA.
DEL	Deletion (chromosomal mutation).
DCIS	Ductal carcinoma in-situ.
DG	(ΔG) = Gibbs free energy (thermodynamics function).
DGGE	Denaturing Gradient Gel Electrophoresis.
DNA	Deoxyribonucleic acid.
DSB	Double stranded break.
DSBR	Double stranded break repair.
DSS1	Deleted in split –Hand/Foot1.
dup	Duplication (chromosomal mutation).
EBV	Epstein Bar virus.
E – Cadherin	Epithelial cadherin.
EDTA	Ethylene diaminetetraacetic acid.
ER	Estrogen receptor.
ERBB2	Erb-B2 receptor tyrosine kinase 2.
ERCC 1	Excision repair cross complementing (protein) 1.
ERT	Estrogen receptor therapy.
FA	Fanconi anemia.
FDRs	First degree relatives.
FAM175A	Family with sequence similarity 175, member A.
FANCD	Fanconi Anemia complementation group D protein (/C/G/A).
FGFR	Fibroblast growth factor receptor.
G0 – phase	Resting phase.
G- phase	Gap phase.

G1	Gap between mitosis & the onset of DNA replication.
G2	Gap between DNA synthesis & the onset of mitosis.
GC–AT	Guanine, cytosine – Adenine, thymine.
GWAS	Genome wide association studies.
HBOC	Hereditary breast cancer and/or ovarian cancer.
HDA	Heteroduplex analysis.
HER-2	Human epidermal growth factor receptor 2 = erbB2.
HER-2/neu	Human epidermal growth factor receptor2/neural tumor.
HNPCC	Hereditary non- polyposis colon cancer.
HPV	Human papilloma virus.
HR	Homologous recombination.
HRT	Hormone replacement therapy.
IARC	International Agency for Research on Cancer.
IBC	Inflammatory breast cancer.
ID4	Inhibitor of DNA binding/ differentiation protein 4.
INK4	Inhibitor of kinase/ or inhibitor of cdk 4.
ins	Insertion (chromosomal mutation).
IR	Ionizing radiation.
JFA	Fanconi anemia group (J).
KAP 1	KRAB-associated protein 1.
Ki 67	(Kiel) University, nuclear factor/index of cell proliferation.
KI 67	Tumor proliferative index protein. 67= No of clone.
KIP	Kinase inhibiting protein/ cdk inhibitor protein.
KRAB	Krüppel associated box.
LCIS	Lobular carcinoma in-situ.
LFS	Li-Fraumeni syndrome.

LKB	Liver kinase B1.
LOH	Loss of heterozygosity.
LSP 1	Lymphocyte- specific protein 1.
MAP	Mitogen activated protein.
MAP	Microtubule associated protein.
MAP3K1	Mitogen activated protein - 3(kinase).
M- phase	Mitosis phase.
MET	Mesenchymal-Epithelial transition.
MHS	Multiple Hamartoma syndromes.
MLH 1	Mutator L Homolog 1.
MRE 11A	Meiotic recombination 11 homolog A.
MRN	Mre11- Rad50-NBS1 complex. DNA repair protein complex.
m-RNA	Messenger ribonucleic.
MSH 1	Mutator S homolog 1.
MTS	Muir-Torre Syndrome.
Myc	Myelocytomatosis viral oncogene homolog. Proto-oncogene.
NBR	Neighboring of BRCA1 gene 2.
NBS	Nijmegen breakage syndrome.
NBS 1	Nijmegen breakage syndrome gene 1= NIBRIN.
NCBI	National Center for Biotechnology Information.
NER	Nucleotide excision repair.
NFA	Fanconi anemia group (N).
NGS	Next-generation sequencing.
NHEJ	Non homologous end joining.
NLS	Nuclear localization signal.
OC	Ovarian cancer.

P	(Petit), short arm of the chromosome.
PACB	Pure apocrine carcinoma B.
P value	Probability value (statistical)
P21	Cyclin dependant kinase 1.
P27	Cyclin dependant kinase 1β.
PAH	Polycyclic aromatic hydrocarbons.
PALB 2	Partner & localizer of BRCA 2.
PARP	Poly (ADP- ribose) polymerase.
PCNA	Proliferation cell nuclear antigen (elongation factor).
PgR	Progesterone receptor.
PI3K	Phosphoinositide-3(kinase).
PIG	P53-induced gene.
PJS	Peutz Jegherz Syndrome.
PMS	Post meiotic segregation.
PP2A	Protein phosphatase 2A.
PPT	Precipitation.
PS	Peptide sequence.
PSIC	Position- specific-independent count.
PTEN	Phosphatase and tension homolog.
PTT	Protein truncation test.
q	(*Grand*), long arm of the chromosome.
R	Restriction point
RAD	Radicicol (homologous) protein (enzyme), Radiation sensitive.
*RAD 51*A	Recombination (protein) A 51 domains (*recombinase*).
RAD 21	Radiation sensitive mutated 21.
RAP 80	Receptor associated protein 80.

RBBP 8	Retinoblastoma binding protein 8 = *CtIP*.
RBC	Red blood cell.
RET	Rearranged During Transfection.
RFC	Replicating factor C (elongation factor).
RFLP	Restriction fragment length polymorphism.
RI	Reliability index.
RICK	Radiation & Isotope Centre of Khartoum.
RING	Really interesting New Gene.
RTK	Receptor tyrosine kinase.
RR	Relative risk.
rRNA	Ribosomal ribonucleic acid.
RNA	Ribonucleic acid.
RNase	Ribonuclease enzyme.
RZFD	Ring zinc finger domain.
SSA	Single strand annealing.
SERM	Selective estrogen receptor modulator.
SSB	Single strand break.
SNPs	Single nucleotide polymorphisms.
SPSS	Statistical package for social science.
SSDNA	Single stranded DNA.
SDS	Sodium dodecyl sulphate.
SSCP	Single Strand Conformational Polymorphism.
STK	Serine-threonine kinase = *LKB.*
Tag DNA	Thermus aquaticus DNA, (polymerase DNA).
TBE	Tris Borate EDTA.
TGF	Transforming growth factor.

TNF	Tumor necrosis factor ά (alpha).
TNM	Tumor lymph node metastases.
TOX	Thermocyte selected association HMG- BOX.
TOX3	TOX HMG-BOX family member 3.
TP53	Tumor protein 53.
TPA	Tetra decoaoylphorboacetate.
TRAIL	TNF related apoptosis – inducing ligand.
tRNA-	Transfer ribonucleic acid.
TSGs	Tumor Suppressor genes.
TTCRC	Tumor Treatment &Cancer Research Center.
UICC	Union international center cancer.
USA	United States of America.
UV	Ultraviolet.
VUC	Variants of unknown significance.
WES	Whole exome sequencing.
WHI	Women's Health Initiative.
WHO	World Health Organization.
WGS	Wide genome sequencing.
WT	Wild - type.
XRCC 1	X- Ray Cross complementing (Protein) 1.
ZBRKI	(Zinc binding) & BRCA 1, interacting protein with KRAB Domain. Transcription repressor
UNITS	
Am	Ampere.
Cm	Centimeter.
mL	Milliliter.

µL	Microliter.
µg	Microgram.
Kb	Kilobase.
Kcl	Kilocalorie.
KDa	Kilodalton.
Kg	Kilogram.
M	Molar.
Mb	Megabase.
min	Minute.
g	Gram.
rpm	Revolution per minute.
V	Volt.

Glossary

Abraxas: New *BC* susceptible gene (tumor suppressor gene), central organizer of a large *BRCA1* holoenzyme complex, its insufficiency (haploinsufficient tumor suppressor gene) causes defective *DNA* repair and genomic instability; (Abraxas – *BRCA1*) interaction is crucial for *DNA* repair and genomic stability and maintenance in response to *IR*. Abbreviated as: (*ABRA1*, *FAM175A*, and *CCDC98*).

Allele: One alternative among different versions of a gene that may be defined by the phenotype that it creates, by the protein that it specifies, or by its nucleotide sequence.

Alternative splicing: Process whereby a pre-m*RNA* may be spliced in several alternative ways, resulting in *mRNAs* composed of different combinations of exons.

***Alu* repeat:** Sequence block of about (300) bp that is found in almost 1 million copies scattered throughout the human genome.

Amplicon: A defined stretch of (chromosomal) *DNA* that undergoes amplification.

Amplification: Genetic mechanism by which the copy number of a gene is increased above its normal level in the diploid genome.

Apoptosis: Complex program of cellular self-destruction, triggered by a variety of stimuli and involving the activation of caspase enzymes, that results in rapid fragmentation of a cell and phagocytosis of resulting cell fragments by neighboring cells.

Autosomal dominant: A pattern of inheritance in which an affected individual has one copy of a mutant gene and one normal gene on a pair of autosomal chromosomes. Individuals with autosomal dominant diseases have a (50-50)

chance of passing the mutant gene and therefore the disorder on to each of their children.

Autosomal recessive: A genetic condition that appears only in individuals who have received two copies of an autosomal gene, one copy from each parent. The gene is on an autosome, a non sex chromosome. The parents are carriers who have only one copy of the gene and do not exhibit the trait because the gene is recessive to its normal counterpart gene.

Autosome: Any chromosome other than the sex chromosomes

Base - excision repair: A form of *DNA* repair that initially involves cleavage by a repair enzyme of the glycosidic bond between a base and a deoxyribose, leaving behind a basic nucleotide.

Benign: (1) Describing a growth that is confined to a specific site within a tissue and gives no evidence of invading adjacent tissue. (2) Referring to an epithelial growth that has not penetrated through the basement membrane.

Biallelic: Referring to a state in which both copies of a gene are expressed or exert effects on phenotype.

Bioinformatics: The science of using computational methods for analyzing biological information, notably complex sets of biological data.

Biomarker: A measurable property or parameter of a cell, tissue, or organism that provides information about the biological state of the entity being analyzed; biomarkers can be used for stratification of disease subtypes and, in the clinic, for disease diagnosis or prognosis.

BRCA1: A gene on chromosome 17 that normally helps to suppress cell growth.

BRCA2: A gene on chromosome 13 that normally helps to suppress cell growth.

Breast cancer: Cancer that forms in tissues of the breast.

Cancer: (1) A clinical condition that is manifested by the presence of one or another type of neoplastic growth. (2) A malignant tumor.

Carcinogen: An agent that contributes to the formation of a tumor.

Carcinogenic: Capable of causing or contributing to the causation of cancer.

Carcinoma: A cancer arising from epithelial cells.

Caretaker: A gene that encodes a protein that maintains the integrity of the genome and thereby prevents the formation of neoplastic cells.

Carrier: An individual who is heterozygous for a recessive allele.

cDNA: *DNA* that has been enzymatically synthesized from an *RNA* template (i.e., "complementary" *DNA*).

Cell cycle: The sequence of changes in a cell from the moment when it is created by cell division, continuing through a period in which its contents including chromosomal *DNA* are doubled, and ending with the subsequent cell division and formation of daughter cells.

Centromere: A chromosomal region to which the spindle fibers attach during mitosis and meiosis.

Checkpoint: Control mechanism that ensures that the next step in the cell cycle does not proceed until a series of preconditions have been fulfilled including the completion of all previous steps.

Chemoprevention: The use of drugs, vitamins, or other agents to try to reduce the risk of, or delay the development or recurrence of, cancer.

Chi-square test: A statistical test for measuring the significance of the discrepancy between observed and expected results; also written $\chi 2$.

Chromatid: A strand of a replicated chromosome that is attached by a centromere to an identical sister strand (arms of the chromosome).

Chromatin: The complex of protein and nucleic acids that makes up the eukaryotic chromosome, which may change in state as a result of changes in the division cycle or developmental programming.

Codon (Triplet codon): A section of m*RNA*, three nitrogenous bases (A- adenine, G- guanine, C- cytosine, U- uracil, T- thymine) in length, that code for an amino acid.

Colorectal: Referring to the lower gastrointestinal tract including the colon and rectum.

Complementary *DNA*: The *DNA* strand that is produced on an *RNA* template by reverse transcriptase.

Computed tomography (CT) scans: A procedure in which imaging generated by successive X-ray scans of a tissue or of the entire body is processed digitally to generate imaging slices of a tissue or of the entire body; sometimes termed computed axial tomography.

Cowden syndrome: An inherited disorder marked by the formation of many non cancerous growths called hematomas.

CpG Island: A cluster of (*CpG*) dinucleotide sequences located in the vicinity of a gene promoter; the state of methylation of these (CpGs) may lead to transcriptional repression of the nearby gene.

C- terminus: End of a protein chain that is synthesized last.

Cyclin: A protein that associates with a cyclin- dependent kinase and serves as a regulatory subunit of this kinase by activating its catalytic activity and directing it to appropriate substrates.

Cyclin-dependent kinase: Type of serine/threonine kinase deployed by the cell cycle machinery that depends on an associated cyclin protein for proper functioning.

Cycloheximide: A drug able to prevent the movement of ribosomes down an mRNA template, thereby blocking protein synthesis.

Cytokines: (1) Growth factors that stimulate one or several of the cell types constituting the hematopoietic system. (2) Regulatory factors of the immune

system, including interferons and interleukins that, like mitogenic growth factors, convey signals between cells.

de novo: (1) Arising or formed anew. (2) Occurring for the first time.

Deletion: A loss of a part of a chromosome.

Denaturation: Process that causes a molecule, such as a macromolecule (*DNA*, *RNA*, or protein), to lose its natural three-dimensional structure.

Deoxyribonucleic acid (*DNA*): Organic molecule that is the basis of heredity. A double-stranded structure composed of nucleotides (phosphate group, 5- carbon Deoxyribose sugar and purine or pyrimidine base).

Dideoxyribonucleotides: Analogues of the normal *DNA* precursor molecules that lack 3-hydroxyl residues. Incorporation of dideoxyribonucleotides into *DNA* terminates chain elongation and is the basis for the *DNA* sequencing technology developed by Frederick Sanger.

Diploid: Having two sets of chromosomes; found in the somatic cells of higher organisms; represented as 2*n*.

***DNA* sequencing:** A technique that permits the base-by-base determination of the linear array of nucleotides within a *DNA* chain.

***DNA*:** The molecules inside cells that carry genetic information and pass it from one generation to the next.

Dominant: An allele is dominant when it is expressed in the heterozygous state (i.e., when it is present on one of the two homologous chromosomes); carrier of dominant disorder inherits x mutation from one parent, unless it is a new mutation; each child of an affected parent may inherit a normal or an abnormal gene; probability that a child will be affected is one in two.

Duplication: A part of a chromosome in which the genetic material is repeated; also the process of forming a duplication.

Electrophoresis: A technique used to detect variation in proteins, involving the use of an electric field to cause the proteins to migrate along a gel and then observing their relative positions on the gel by protein- specific staining reactions.

Endonuclease: An enzyme that produces an internal cut in the sugar-phosphate backbone of *DNA* (for example, in repair of thymine dimmers, in which a specific endonuclease cuts the backbone at a nucleotide adjacent to a thymine dimmer).

Epigenetics: (1) The study of heritable changes in gene expression those are not due to alteration in the primary *DNA* sequence. (2) More broadly, the study of changes in phenotype that reflect non genetic alterations in the cell or organism.

Estrogen: A steroid hormone that controls development of a variety of tissues including those involved in female development and reproductive function.

Etiology: (1) Mechanism or agent that is responsible for causing a specific pathological state. (2) The study of causative mechanisms of pathology.

Exons: The coding regions of a gene that are separated from each other by intervening sequences (*intones*).

Exonuclease: An enzyme that removes the terminal nucleotide of nucleic acids

Familial cancer: Cancer that occurs in families more often than would be expected by chance.

Family history: A record of the relationships among family members along with their medical histories. This includes current and past illnesses. A family history may show a pattern of certain diseases in a family.

Fanconi anemia: A rare inherited disorder in which the bone marrow does not make blood cells.

First-degree relative: A parent, sibling, or offspring of an individual (all of whom share (50%) of their genome with the individual).

Frameshift: A mutation caused by deletion or addition of nucleotides in *DNA*, which has the effect of changing the reading frame of codons, causing the wrong sequence of amino acids to be produced.

Gatekeeper: A gene that operates to hinder cell multiplication or to further cell differentiation or cell death and in this way prevents the appearance of populations of neoplastic cells.

Gene amplification: Increase in the number of copies of a gene normally present in the diploid genome.

Gene family: A Group of genes all of which are descended from a common ancestral gene. The members of a gene family often encode distinct, structurally related proteins.

Gene: The functional and physical unit of heredity passed from parents to offspring. Genes are pieces of *DNA*.

Genetic counseling: A communication process between a specially trained health professional and a person concerned about the genetic risk of disease.

Genetic polymorphism: A variant sequence element in an organism's genome that has no effect on phenotype yet is transmitted genetically as a Mendelian determinant.

Genetic testing: Analyzing *DNA* to look for a genetic alteration that may indicate an increased risk for developing a specific disease or disorder.

Genetic: (1) Involving the action of genes and the information that they carry. (2) Depending directly on the *DNA* and the nucleotide sequences that it contains.

Genotype: the total genetic information contained in an organism or the genetic constitution of an individual with respect to the genes under consideration.

Germline: (1) The collection of genes that is transmitted from one organism generation to the next. (2) The cells within a multicellular organism that is

responsible for carrying and transmitting genes from one organism generation to its offspring.

Grade: Degree or extent to which a tumor has advanced toward a highly aggressive state, as assessed by a pathologist usually on the basis of its histopathologic appearance; high grade tumors are more progressed and generally carry worse prognoses.

Growth factor: Protein that is able to stimulate the growth and/or proliferation of a cell by binding to a specific cell surface receptor displayed by that cell.

Haploid: Having one set of unpaired chromosomes; found in cells such as gametes; the haploid chromosome number (1*n*) is the same as the number of linkage groups; also termed *monoploid.*

Haploinsufficiency: State in which the presence of only a single functional copy of a gene yields a mutant or partially mutant phenotype.

Haplotype*:* Group of alleles from closely linked loci that are usually inherited as a unit.

Herceptin: Chimeric anti-*HER2*/Neu monoclonal antibody bearing murine antigen- combining (variable) domains and a human constant domain (*also called* trastuzumab).

Heteroduplex: A double strand of *DNA* in which the bases within the helix are not totally complementary; may be the result of recombination between single strands of the homologues.

Heterozygosity: Genotypic situation in which two homologous loci in a given chromosome pair each carry a different allele.

Heterozygote: An organism having different alleles at a given locus on homologous Chromosomes.

Heterozygous: Referring to the configuration of a genetic locus in which the two copies of the associated gene carry different versions (alleles) of the gene.

Homologous: (1) Referring to the relationship between a pair of chromosomes that carry the same set of genes within a diploid cell or organism. (2) Referring to genes or characteristics that are similar in related organisms because of shared descent from a common precursor. (3) Referring to two nucleic acids having similar nucleotide sequences.

Homozygosity: Presence of identical alleles on both chromosomes in a given pair; this term may apply to the genotype of individuals who have inherited a double dose of an abnormal allele, whether the mutated version is the same or different on each chromosome.

Homozygote: An organism having the same allele at a given locus on homologous chromosomes.

Homozygous: Referring to the configuration of a genetic locus in which the two copies of the gene carry identical versions (alleles) of the gene.

Hydrophilic molecules: Molecules that interact with water.

Hydrophobic molecules: Molecules those are insoluble in water.

Immunohistochemistry: Procedure in which expression of an antigen is localized in a histological section through the use of an antibody that has been coupled to an enzyme (e.g., peroxidase) capable of generating a product that is visible in the light microscope.

In situ: (1) Occurring in the site of origin. (2) In the case of carcinomas, confined to the epithelial side of the basement membrane.

Incidence: Frequency with which a condition or a disease occurs or is diagnosed in a population.

Incomplete penetrance: Situation in which a dominant allele fails to dictate phenotype because of the actions of other genes present in an organism’s genome.

Inflammation: A process in which certain cellular components of the immune system are involved in the remodeling of a tissue in response to wounding, irritation, or infection.

Inherited: Passing of genetic information from parent to child through the genes in sperm and egg cells.

Initiation: (1) Process of changing a cell, usually in a stable fashion, so that it is able to respond subsequently to the growth-stimulatory actions of a tumor-promoting agent. (2) Such a process, with the implication that the change involves a mutation. (3) The first step in multi-step tumorigenesis.

Intron: Portion of a primary *RNA* transcript that is deleted during the process of splicing. An intervening sequence.

Invasive: (1) Referring to the increased aggressiveness of a tumor or its associated cells. (2) Referring to a procedure that involves the insertion of medical instruments into the body.

Inversion: A change in gene order within a chromosome, involving two breaks and turning around of the segment between the breaks is aiding the characterization of multifactorial traits.

Kinase: Enzyme that covalently attaches phosphate groups to substrate molecules, often proteins.

Lifetime risk: A measure of the risk that a certain event will happen during a person's lifetime.

Li-Fraumeni syndrome: A rare, inherited predisposition to multiple cancers, caused by an alteration in the p53 tumor suppressor gene.

Locus: the physical position of a gene on a chromosome.

Loss of heterozygosity: A genetic event in which one of two alleles at a heterozygous locus is lost; the lost allele may simply be discarded or be replaced with a duplicated copy of the surviving allele.

Low-grade: Referring to a tumor that has progressed minimally and is still relatively benign.

Lumpectomy: Surgical procedure in which a tumor is removed together with immediately surrounding normal tissue while leaving the bulk of the affected organ intact; usually used in the context of breast cancer surgery.

Mammary: Referring to the breast and its milk-producing glands.

Mammogram: An x-ray of the breast.

Menarche: Time in life when menstrual cycling begins.

Messenger *RNA* (mRNA): The *RNA* that carries the code sequence for polypeptide chains.

Metastasis: Malignant growth forming at one site in the body, the cells of which derive from a malignancy located elsewhere in the body. (2) The process leading to the formation of metastases.

Microarray: A collection of sequence-specific *DNA* probes that are attached at specific sites to a solid substrate, such as a glass microscope slide; these probes may derive from specific segments scattered throughout a cell genome or from a particular subset of chosen genes.

Missense mutation: Mutation causing an amino acid substitution.

Mortality: A term used for death rate, or the number of deaths in a certain group of people in a certain period of time.

MRI (Magnetic Resonance Image): A procedure in which radio waves and a powerful magnet linked to a computer is used to create detailed pictures/images of areas inside the body.

Mutation: Any detectable and heritable change in the genetic material that is not caused by segregation or recombination.

Non disjunction: The irregular distribution of chromosomes or chromatids during anaphase of either mitosis or meiosis; results in either addition or loss of chromosomes.

Non homologous recombination: Process of recombination between two *DNA* molecules in which the two participating molecules do not share significant sequence identity.

Nonsense mutation: Mutation causing premature termination of a growing polypeptide chain.

Nucleotide: The monomeric unit that makes up *DNA* or *RNA*, formed by the addition of a phosphate group to a nucleoside.

Nucleotide-excision repair: A type of *DNA* repair in which the initial step involves the excision of nucleotides (rather than bases).

Nulliparous: A female who has never given birth.

Oncogene: (1) A cancer-inducing gene. (2) A gene that can transform cells.

Oral contraceptive: A pill used to prevent pregnancy.

Ovarian cancer: Cancer that forms in tissues of the ovary.

***P* arm:** the term for the short arm of a human chromosome.

Parity: (1) The condition of having given birth. (2) The number of times that a female has given birth.

Parous: A female who has given birth at least once.

Penetrance: the frequency with which a gene manifests itself in the phenotype of the heterozygote (if dominant) or of the homozygote (if recessive)

Perutz-Jeghers syndrome: A genetic disorder in which polyps form in the intestine and dark spots appear on the mouth and fingers.

Phenotype: (1) A measurable or observable trait of an organism. (2) The sum of all such traits of an organism.

Point mutation: Substitution of a single base for another in a *DNA* sequence.

Polymerase chain reaction (*PCR*): the amplification of a unique segment of *DNA*, often from a heterogeneous *DNA* population, through progressive cycles of primer annealing, *DNA* polymerization, and strand denaturation. The specificity of the amplification is controlled by using two oligonucleotides primers complementary to the *DNA* flanking the target *DNA* sequence.

Polymerases: enzymes that catalyze the assembly of nucleotides into *RNA* or *DNA*

Polymorphism: A variant germ-line allele that does not appear to be associated with any pathology and, by implication, is a reflection of normal intraspecies genetic variability.

Primer: A *DNA* or *RNA* molecule whose 3′ end serves as the initiation point of *DNA* synthesis by a *DNA* polymerase.

Prognosis: A prediction about the future clinical course of a disease often influenced by detailed analyses of its existing attributes, such as histopathology and biochemical markers.

Proofreading: (1) Process whereby an already-assembled text is read in order to detect and eliminate errors in its assembly. (2) Process by which a DNA polymerase scans the deoxyribonucleotide segment that it has just synthesized in order to ensure that the sequence of this segment is precisely complementary to that of the template strand.

Prophylactic: Preventative.

Proto - oncogene: A normal cellular gene that, upon alteration by *DNA-damaging* agents or viral genomes, can acquire the ability to function as an oncogene.

Purine: An adenine or guanine nitrogenous base found in *RNA* and *DNA* nucleotides

Pyrimidine: Nitrogenous base found in *RNA* and *DNA* nucleotides; in *DNA* the two pyrimidines are cytosine and thymine; in *RNA* they are cytosine and uracil.

***q* Arm:** the term for the longer arm of a human chromosome.

Radiotherapy: Treatment of a disease, notably cancer, through ionizing irradiation.

Recombination: A process occurring during normal meiosis that gives rise to linked combinations of alleles that differ from those found in the parents.

Replication: the process by which the two strands of *DNA* separate and each serves as a template for the synthesis of a new complementary strand.

Restriction endonuclease: an enzyme that internally cleaves double-stranded *DNA* molecule after binding to a specific nucleotide recognition sequence.

Restriction fragment length polymorphism: Variation in *DNA* sequence that can be detected through its effect of allowing or preventing cleavage of a chromosomal *DNA* segment by a restriction enzyme.

Reverse transcriptase: Enzyme capable of making a *DNA* complimentary copy of an *RNA* molecule using the *RNA* molecule as template.

Ribosomal *RNA* (rRNA): Any of several forms of *RNA* that are a part of the structural unit called a *ribosome.*

Risk factor: Something that increases the chance of developing a disease.

Salpingo-oophorectomy: Surgical removal of the fallopian tubes and ovaries.

Screening: Checking for disease when there are no symptoms.

Second-degree relatives: grandparents, grandchildren, aunts, uncles, nieces, nephews, half sisters, and half-brothers.

Silent mutation: is a type of mutation in the coding region of a gene that doesn't actually change the amino acid sequence of the protein that is made.

Single nucleotide polymorphism (SNP): *DNA* marker where a single base pair difference at a particular site in the genome is sufficient to distinguish different individuals; the development of high-density S*NP* maps in the human genome

Somatic cells: are all cells in the body except germ cells, which are egg and sperm.

Sporadic: Describing a disease or condition that occurs randomly in a large population without any apparent predisposition, such as one caused by a heritable genetic susceptibility.

Stage: The extent of a cancer in the body. Staging is usually based on the size of the tumor.

Syndrome: Collection of symptoms that together define a specific disease condition.

Synonymous: A variant affecting 1 or more nucleotides that does not change the amino acid sequence.

Telomere: Region found at the tips of chromosomes; unique in being "non sticky" that is, chromosome pieces will not attach to it.

Third-degree relatives: Great-grandparents, great-grandchildren, great-aunts, great- uncles, first cousins, grand-nephews, and grand-nieces.

Thymine dimmer: Two adjacent thymine bases connected by a double covalent bond; often results when *DNA* is hit by ultraviolet radiation.

Transcription factor: Any of a large number of proteins that bind to *DNA* regions upstream or in the termination region of a gene and influence transcription.

Transcription: The process by which *RNA* is produced from a *DNA* strand through the action of DNA- directed RNA polymerase.

Transfer *RNA* (*tRNA*): The *RNA* molecules that carry amino acids to the ribosome.

Transition: A base - pair substitution in which the purine - pyrimidine orientation is preserved; that is, a purine is replaced by a purine or a pyrimidine by a pyrimidine.

Translation: The process by which an *mRNA* sequence is used to produce a polypeptide chain.

Transversion: Point mutation in which a purine base replaces a pyrimidine or vice versa.

Triple-negative breast cancer: Breast cancer cells that do not have estrogen receptors, progesterone receptors, or large amounts of *HER2*/neu protein.

Tumor suppressor gene: A type of gene that makes a protein that helps control cell growth.

Wild type: The allele of a gene that is commonly present in the great majority of individuals in a species.

X- ray: A type of radiation used in the diagnosis and treatment of cancer and other diseases (All glossary from (Weinberg, 2013)).

List of contents

Chapter One

(Introduction, Justification, Objectives)

1. Introduction

1.1. Cancer:

Cancer is a genetic disease that arises from an accumulation of mutations in critical genes. This allows a cell to escape the normal growth control and proliferate until it becomes a clinically evident tumor. Six acquired capabilities common to human tumor cells have been identified: they keep proliferating even in the absence of growth signals, disregard growth-inhibitory signals, evade apoptosis, have limitless replicative potential, sustain angiogenesis to guarantee a continuous supply of nutrients and oxygen, and are capable of invading tissues and form metastasis (Hanahan &Weinberg, 2000). These properties give the cancer cells a growth advantage over the general cell population. Based on epidemiological and *in vitro* experiments, it has been estimated that between (4) and (8) successive mutations are needed for the normal human cell to turn malignant (Renan, 1993; Hahn *et al*, 1999). Given the multiple defense mechanisms that protect the cells from cancer development, this leads to a very low likelihood that a single cell will accumulate the requisite number of independent mutations needed for neoplastic transformation. Therefore, it has been suggested that tumor cell genomes must acquire increased mutability in order for the tumorigenesis to reach completion during a human life span (Loeb, 1991). Indeed, the mutation frequency has been estimated to be Up to (1000) - fold higher in tumor cells than in normal cells (Seshadri *et al*, 1987).

According to *GLOBOCAN 2012*, an estimated (14.1) million new cancer cases and (8.2) million cancer-related deaths occurred in (2012), compared with (12.7) million and (7.6) million, respectively, in 2008. Prevalence estimated for 2012 showed that there were (32.6) million people (over the age of 15 years) alive who had had a cancer diagnosed in the previous (5) years (Ferlay, 2013).

The most commonly diagnosed cancers worldwide were those of the lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%), and colorectum

(1.4 million, 9.7%). The most common causes of cancer deaths were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%).

Projections based on the *GLOBOCAN (2012)* estimated a predictive substantial increase to (19.3) million new cancer cases per year by (2025), due to the growth and ageing of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in (2012) occurred in less developed regions of the world, and these proportions will increase further by (2025) (Ferlay, 2013).

1.2. Breast cancer:

Breast cancer is the most common malignancy affecting women worldwide. Up to every eighth woman will develop it during lifetime. Most cases are sporadic, but an estimated (7%) are due to dominantly inherited predisposition, with germline mutations in predisposing genes (Claus *et al*, 1996). Thus far,

Two highly penetrant susceptibility genes, *BRCA1* and *BRCA2*, have been identified (Miki *et al*, 1994; Wooster *et al*, 1995). Germline mutations in *BRCA1* and *BRCA2* explain the majority of families with both breast and ovarian cancer, whereas vast majority of familial aggregation of site-specific breast cancer appears to be caused by other, as yet unidentified genes (Ford *et al*, 1998). Identification and characterization of genes predisposing to hereditary cancer may help to unravel the genetic background, etiology, and pathogenesis of cancer in question, and eventually lead to better diagnostics and patient management.

In (2012), (1.7) million women were diagnosed with breast cancer and there were (6.3) million women alive who had been diagnosed with breast cancer in the previous five years. Since the (2008) estimated, breast cancer incidence has increased by more than (20%), while mortality has increased by (14%). Breast cancer is also the most common cause of cancer deaths among women (522, 000

deaths in 2012) and the most frequently diagnosed cancer among women in (140) of (184) countries worldwide. Recently it represents one in four of all cancers in women (Ferlay, 2013).

Despite the common occurrence, the exact etiology of breast cancer is still unknown. It is believed that breast cancer is a multifactorial disease and it is a result of the interaction of genetic and environmental factors (Ponder, 1988).

In Sudan, cancer of breast is the most commonly diagnosed type of cancer. According to a statistical report from the Radiation and Isotopes center in Khartoum (*RICK*), approximately (836) women developed breast cancer in (2007) and (895) in (2008). This accounts for more than (30%) of all cancers in women in Sudan and is estimated as (17.2%) of all types of cancers in (2007) and (17.9%) in (2008). In (2009) were (938) cases and (1068) cases in (2010) (RICK, 2009).

However, Sudan is a very large country, inhibited by many different heterogeneous communities, which greatly varying in their life style and exposure to environmental factors that might be potent risk factors for the development of breast cancer. What is more, no epidemiological study has been undertaken this issue before (Gadkarim, 2010).

Therefore, this study is an attempt to identify the genetic mutation that occurs in *BRCA1* and *BRCA2* genes, which might contribute to the etiology of breast cancer in patients attending the Tumor Treatment & Cancer Research Center (*TTCRC*) in Shendi-Northern Sudan.

An even better understanding of the genetic mechanisms underlying the development and progression of breast cancer would be a major advance for improved prevention, detection and treatment strategies.

1.3. Justification:

The identification of a genetic defect in *BRCA1/2* is of major importance as biomarker for hereditary breast cancer, and has value as predictive genetic testing for relatives, which lead to reduce the breast cancer in high-risk individuals.

Accurate inclusion criteria to select those patients who will benefit from genetic testing are extremely important to gain further insights into the prevalence of *BRCA1/2* mutations in sporadic breast cancer patients in this study population, and to evaluate if the genetic testing of patients without any family history for breast cancer is worthwhile.

The broad range of associated risks reported in literature is consistent with the hypothesis that risks in *BRCA1* or *BRCA2* mutation carriers can vary substantially due to the presence of additional risk factors for breast cancer, including genetic modifiers.

The contribution of *BRCA1* and *BRCA2* to breast cancer incidence remains largely unknown, as most studies have concentrated on families with multiple affected family members, on isolated populations, or on young patients. Results from such studies may not be used to assess the contribution of *BRCA1* and *BRCA2* in other populations, in patients without such a strong family history of cancer, or in patients with later-onset disease.

For all these reasons, this research attempted to evaluate the role of *BRCA1* &*BRCA2* genes and to identify the most risk factors contributed to disease in unselected breast cancer patients attending newly establish center for tumor treatment and cancer research in Shendi as the first study tasked this problem, to be a current one as there is no recent knowledge or information handled the problem.

1.4. Objectives

1.4.1. General objective:

- To determine the role of *BRAC1/ BRCA2* mutation in breast cancer patients attending Tumor Treatment and Cancer Research Center (*TTCRC*) in Shendi - Northern Sudan.

1.4.2. Specific objectives:

1. To evaluate the frequency and distribution of mutations in *BRCA1/BRCA2*.
2. To identify the most common histological types of breast cancer related with mutation in *BRCA1/BRCA2*.
3. To determine the most clinical risk factors associated with a positive *BRCA1/2* mutations.
4. To assess the role of *BRCA1/2* in sporadic breast cancer patients, and implications for genetic testing.

2. Literature Review:

2.1. The normal breast:

The female breast is made up mainly of *lobules* (milk-producing glands), *ducts* (tiny tubes that carry the milk from the lobules to the nipple), and *stroma* (fatty tissue and connective tissue surrounding the ducts and lobules, blood vessels, and lymphatic vessels) **(Figure 2.1)**.

Most breast cancers begin in the cells that line the ducts (*ductal* cancers). Some begin in the cells that line the lobules (*lobular* cancers), while a small number start in other tissues (Moya *et al*, 2004).

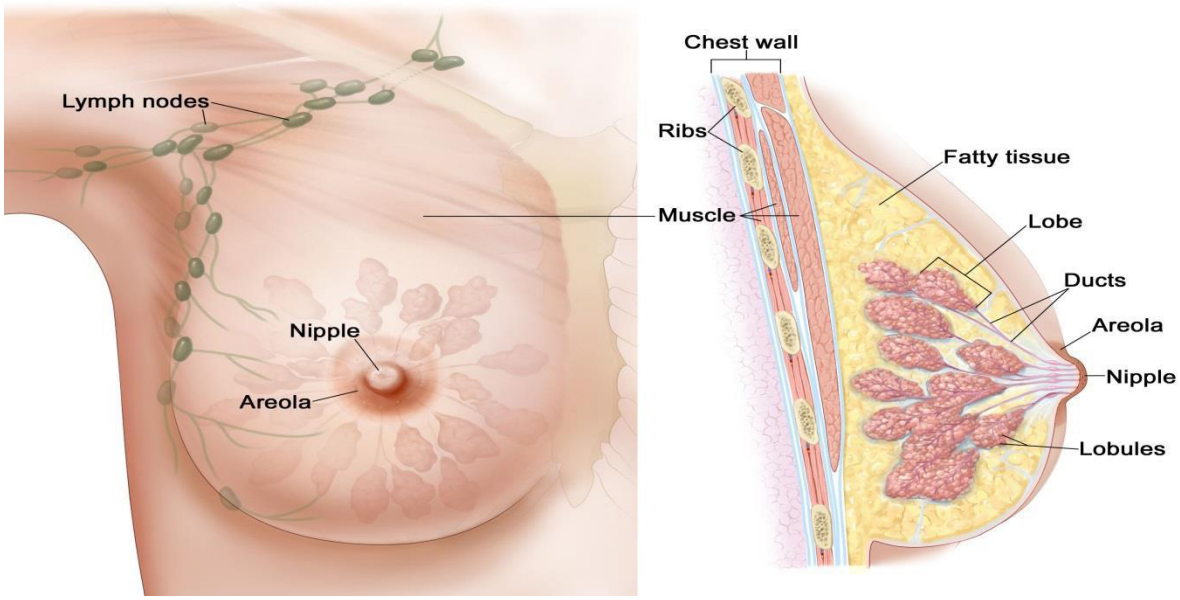

Figure 2.1: Anatomy of the breast

(Resource: Moya *et al*, 2004).

2.2. The lymph (lymphatic) system of the breast:

This system has several parts. Lymph nodes are small, bean-shaped collections of immune system cells (cells that are important in fighting infections) that are connected by lymphatic vessels. Lymphatic vessels are like small veins, except that they carry a clear fluid called *lymph* (instead of blood) away from the breast. Lymph contains tissue fluid and waste products, as well as immune system cells. Breast cancer cells can enter lymphatic vessels and begin to grow in lymph nodes.

Most lymphatic vessels in the breast connect to lymph nodes under the arm (*axillary nodes*). Some lymphatic vessels connect to lymph nodes inside the chest (*internal mammary nodes*) and those either above or below the collar-bone (*supraclavicular* or *infraclavicular nodes*) as in **(Figure 2.2**). If the cancer cells have spread to lymph nodes, there is a higher chance that the cells could have also gotten into the bloodstream and spread (*Metastasized*) to other sites in the body. The more lymph nodes that have breast cancer, the more likely it is that the cancer may be found in other organs as well. Because of this, finding cancer in one or more lymph nodes often affects the treatment plan. Still, not all women with cancer cells in their lymph nodes develop metastases, and some women can have no cancer cells in their lymph nodes and later develop metastases (Georgia *et al*, 2008).

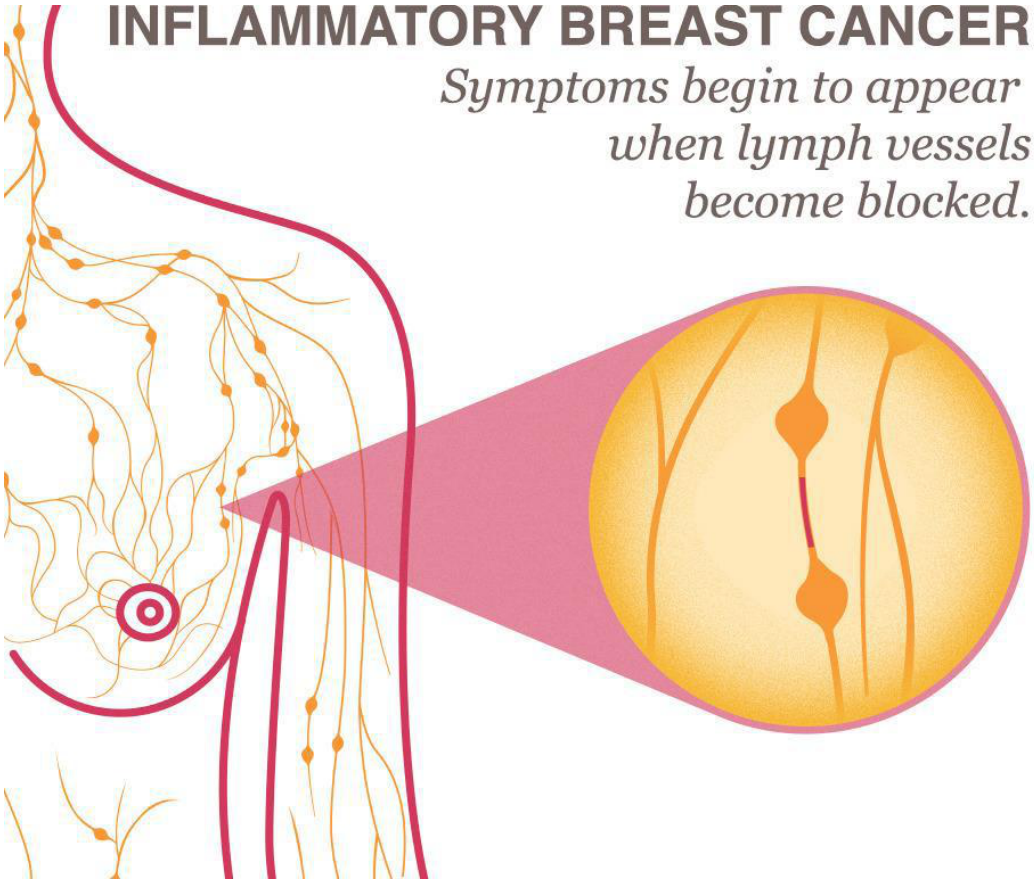

Figure 2.2: Lymph system of the breast

(From Georgia *et al*, 2008)

2.3. Breast cancer types:

Breast cancer originates from the terminal ductolobular unit of breast tissue. Breast cancer that has not invaded the basement membrane and thus confined within the terminal ducto-lobular units is termed *carcinoma in-situ*. Mainly, there are two types of in-situ cancers; *lobular carcinoma in-situ and ductal carcinoma in-situ.*

On the other hand, breast cancers that invade the basement membrane are called invasive cancers. The two main types are synonymously called *invasive lobular and ductal carcinoma*. The main difference between in-situ and invasive cancers is the ability of the invasive form to spread through the lymphatic and vascular vessels located under basement membrane leading to regional lymphatic and distant organ metastases (**Figure 2.3**).

2.3.1. Lobular carcinoma in- situ:

Lobular carcinoma in-situ (*LCIS*) originates from the lobular elements of the breast. Thus, it is only observed in women since men have no lobular units in their breasts. (*LCIS)* is usually not detectable macroscopically during physical examination and is frequently an incidental microscopic finding in the breast tissue removed for another reason. In the biopsy specimens done for benign breast abnormalities, it was found that only (0.5%) to (3.6%) is (*LCIS*) (Winer *et al*, 2001). It is noted to be more common in younger women with (80%) to (90%) of cases of (*LCIS)* occurring in premenopausal women (Winer *et al*, 2001). Besides, (*LCIS)* is accepted as a premalignant lesion of the breast since an invasive cancer could develop in any part of the breast and even in the contralateral breast after the diagnosis of (*LCIS)*. Bilaterality and multicentricity are common features of (*LCIS).*

2.3.2. Ductal carcinoma in- situ:

Ductal carcinoma in-situ (*DCIS*) is an entity distinct in both its clinical presentation and its biologic potential from (*LCIS)*. (*DCIS)* originates from the ductal epithelium in the breast and could be diagnosed in both males and females. These are the precursor lesions for invasive ductal carcinoma and during its natural course; it changes into its invasive form. (*DCIS*) is characterized pathologically by a proliferation of presumably malignant epithelial cells within the mammary ductal-lobular system without light microscopic evidence of invasion into the surrounding stroma. However, (*DCIS)* encompasses a heterogeneous group of pathologic lesions that differ in their growth pattern and cytological features. The traditional system for classifying (*DCIS)* is based primarily on architectural pattern and grouped into (5) major subtypes: comedo, cribriform, micro papillary, papillary, and solid (Winer *et al*, 2001). The comedo type usually appears more malignant cytologically and is more often associated with invasion than are the other types.

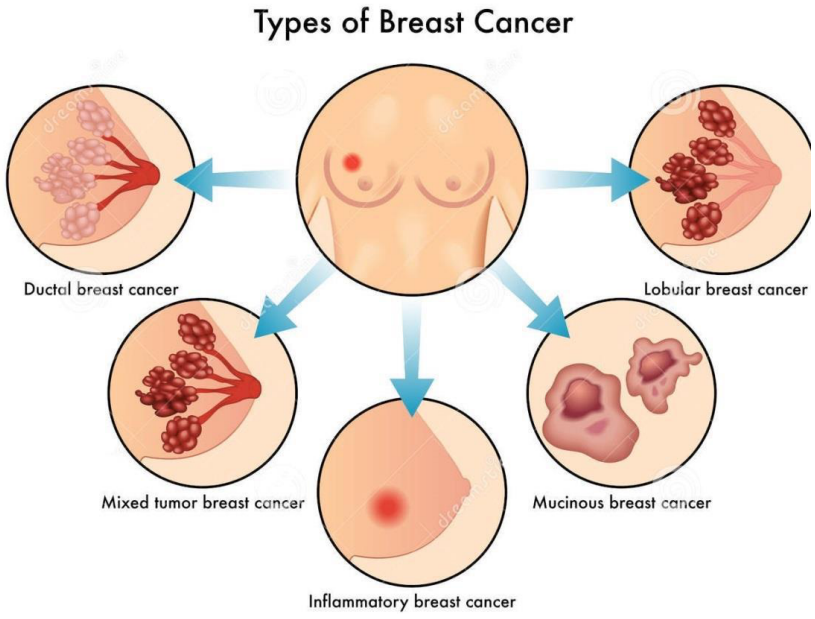

Figure 2.3: Types of breast cancer

(From Agarwal *et al*, 2007).

2.3.3. Invasive lobular carcinoma:

These carcinomas originate in terminal ductules of the lobule and possess characteristic features that distinguish them from the lesions of larger ducts. Invasive lobular carcinoma constitutes approximately (10%) of breast cancers. When compared to invasive ductal carcinoma, they usually have a propensity for bilaterality, multicentricity, and multifocality.

The treatment strategies utilized are similar to those used for invasive ductal carcinoma. Furthermore, the stage of the disease is the major determinant of outcome rather than the histological type of the tumor. Thus, at the same stage both invasive ductal and lobular carcinomas have similar prognosis (Winer *et al*, 2001).

2.3.4. Invasive ductal carcinoma:

Invasive ductal carcinoma is the most common type of invasive breast cancer accounting for (75-80%) of cases. These lesions are usually single and unilateral. Invasive ductal carcinomas have similar prognosis as invasive lobular carcinoma when diagnosed at the same stage.

2.3.5. Other invasive types:

Besides the above mentioned common types of invasive breast cancers, there are other rare forms such as medullary, papillary, mucinous, tubular, apocrine and adenoid cystic carcinoma. These histological types usually have better biological properties rendering them to be known as less aggressive types of breast cancer. The slower progression of disease in these types results in a better prognosis for patients (Winer *et al*, 2001).

2.3.6. Inflammatory breast carcinoma:

This is a rare but very aggressive form of breast cancer. Inflamed cells actually block the lymphatic channels in the skin of the breast. In this type of breast

cancer the organ appears red, swollen and is termed as inflammatory breast cancer (*IBC*)**.** About (1-3%) of breast cancers are inflammatory breast cancers. High metastasizing tendency has been observed in inflammatory breast cancer as compared to other forms. Inflammatory breast cancers are always staged as stage IIIB, unless it has been spread to other organs (Singletary *et al*, 1994).

2.3.7. Paget's disease:

It contributes to only (1%) of all types of breast cancer and is generally associated with bleeding, or crusting/scariness of the nipple or areola. The primary symptoms include eczema like rash accompanied by a burning sensation which may further lead to fluid discharge, crusting and a sore that does not heal. Prognosis is better when compared with other types of tumors (Noel *et al*, 2010).

2.4. Male breast cancer:

It is a rare disease which does exist in men. Less than (1%) of all breast cancers occurs in males. Some characteristic signs include a lump felt in the breast, nipple discharge, sores on the nipple and areola, enlarged lymph nodes under the arm. Simultaneous enlargement of both breasts is not cancer but is usually observed in gynecomastia (hyperplasia and hypertrophy of both glandular and stromal cells). Sometimes breasts enlargement may also be attributed to medications, heavy alcohol use, weight gain, or marijuana use (Pant & Dutta, 2008).

2.5. Stages of breast cancer:

The staging systems currently in use for breast cancer are based on the clinical size and extent of invasion of the primary tumor (*T*), the clinical absence or presence of palpable axillary lymph nodes and evidence of their local invasion (*N*), together with the clinical and imaging evidence of distant metastases (*M*).

This is then translated into the (*TNM*) classification which has been subdivided into Stage 0 called carcinoma in situ (lobular carcinoma in situ (*LCIS*) and ductal carcinoma in situ (*DCIS*) and four broad categories by the Union International Center Cancer (*UICC*), which are the following.

Stage I - early stage breast cancer where the tumor is less than (2) cm across and hasn't spread beyond the breast.

Stage II - early stage breast cancer where the tumor is either less than (2) cm across and has spread to the lymph nodes under the arm; or the tumor is between (2) and (5) cm (with or without spread to the lymph nodes under the arm); or the tumor is greater than (5) cm and hasn't spread outside the breast.

Stage III - locally advanced breast cancer where the tumor is greater than (5) cm across and has spread to the lymph nodes under the arm; or the cancer is extensive in the underarm lymph nodes; or the cancer has spread to lymph nodes near the breastbone or to other tissues near the breast.

Stage IV- metastatic breast cancer where the cancer has spread outside the breast to other organs in the body (Gore, De Gregori and porter, 2013).

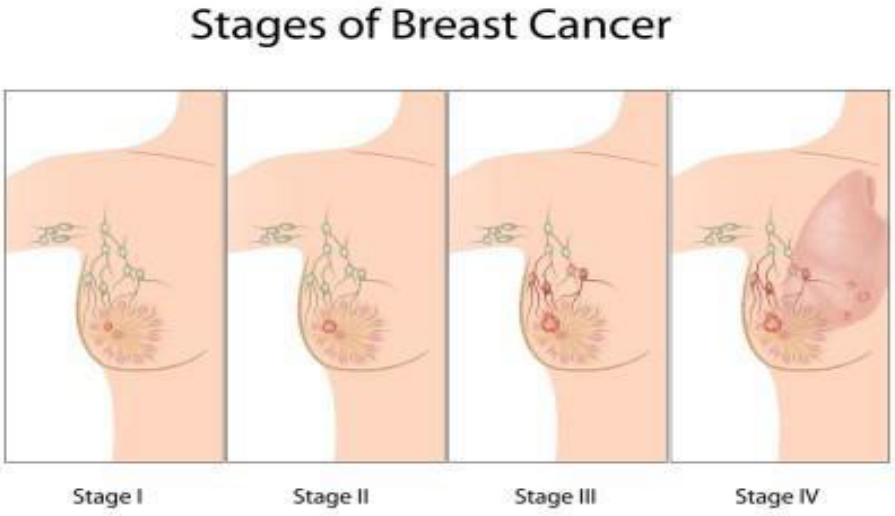

Figure 2.4: Stages of breast cancer

(From Gore, De Gregori & Porter, 2013).

2.6. Breast cancer symptoms:

Breast cancer may be accompanied by many of symptoms. Abnormal lumps persisting in the breast are perhaps one of the most commonly associated symptoms. Skin dimpling, unusual changes in texture or skin color on the breast, change in the shape of the nipple, and discharge of blood through the nipple may be symptoms of breast cancer as shown in **(Figure 2.5).** In some cases of breast cancer, however, there are no noticeable symptoms. In fact (50%) of women who get breast cancer experience no obvious symptoms and discover their breast cancer only after undergoing a medical examination (American cancer society, 2006). Therefore, it is important for women to have periodic screenings for breast cancer.

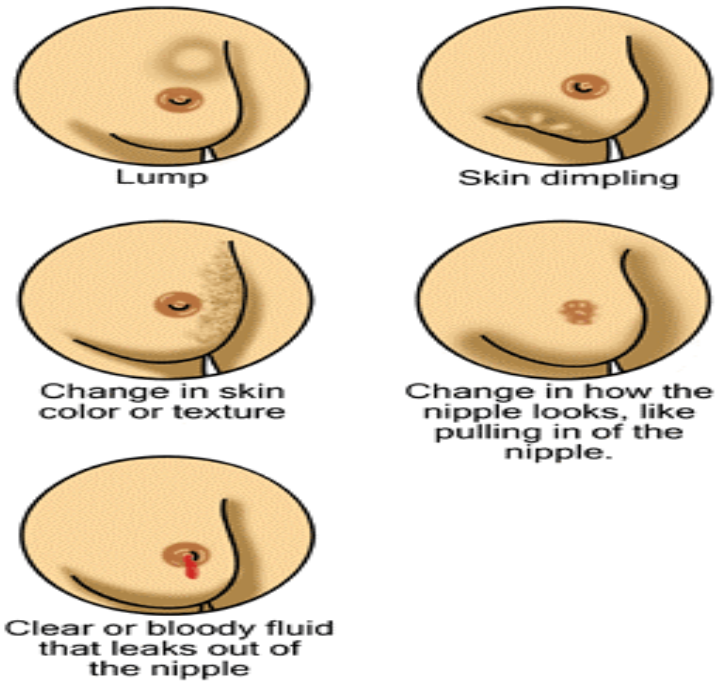

Figure 2.5: Illustrations of early signs of breast cancer

(Image originally from the National Institutes for Health).

2.7. Etiology of cancer:

There are number of etiologically associated factors classified as carcinogenesis. Some of the salient contributory factors include:

2.7.1. Chemical carcinogenesis:

Association of chemicals and environmental agents with cancer was firstly established by Percival Pott in (1775). He observed that personnel working in chimney sweeps were affected with scrotal cancer (Pott, 1775). Later on with the discovery of benzopyrene (*BP*) a member of polycyclic aromatic hydrocarbons (*PAH*), special attention was given to the chemicals responsible for provoking such havoc disease in different industry related workers (Cook *et al*, 1933). After extensive research, it was established that most of the synthetic chemical carcinogens fall under two main categories as organic and inorganic compounds. Organic compounds are further subcategorized into three classes as; alkylating agents, aryl alkylating agent and aryl hydroxylamine. These compounds vary on structural grounds in the type of residue transfer to create *DNA* adduct, classified as follows:

2.7.1.1. Organic compounds alkylating agents:

These compounds, as evident from their name, transfer alkyl (methyl or ethyl) groups to *DNA* creating adduct. N-nitroso compounds (nitrosamines) are among the most insidious and potentially hazardous of the various carcinogens included in this group (Kimbrough, 1983). Activation of these compounds sometimes need biotransformation either enzymatically by oxidation (as N-nitrosaminedimethlyamine) or by hydrolysis (as methyl nitrosourea). These compounds are ubiquitously distributed within the environment and are synthesized endogenously. Under acidic conditions formation of N-nitroso compounds by the nitrosation of secondary amines in stomach and in non-acidic environment like gut through the aid bacteria, are examples of its endogenous

production. There are also several chemicals which are synthesized naturally by plants and fungi exhibiting carcinogenic activity (Ames *et al*, 1987). Aflatoxin B1, a toxin naturally produced from *Aspergillus flavus*, is initially thought to be hepatotoxic compound (Blount, 1961). Its carcinogenic potential was confirmed after evaluating higher incidence of liver tumors in African populations (Hsu *et al*, 1991). Metabolic activation of Aflatoxin B1 via (*P450*) leads to the synthesis of an intermediate which reacts with cyclic nitrogen in guanine, forming adduct 8, 9- dihydro-8- (N7- guanyl)-9-hydroxyaflatoxin B. Thus alkylating agents either directly or after enzymatic activation induce damage to human genome which later provoke in the form of cancer.

2.7.1.2. Aryl alkylating agents:

These chemicals generally transfer aromatic or multi-ringed compounds to a nucleotide to form adduct. Polycyclic aromatic hydrocarbons (*PAH*) are the principal group which has raised serious concerns among industry related workers. Hydrocarbons exposure in chimney sweep and crude oils in cotton spinning have been found responsible for induction of scrotal cancers in workers (Heller, 1930; Waldron *et al*, 1984). Reactive metabolites of the (*PAHs*) has been investigated extensively and found to be highly reactive with *DNA* (Jerina *et al*, 1991).

2.7.1.3. Aryl hydroxylamine agents:

The chemicals included in this class are those which deliver aromatic amines to nucleotides to form adducts. These compounds were discovered after conducting a number of studies on the workers related to dyestuff industry. High incidence of bladder cancer was reported in aniline dye exposed workers which led to the identification of 2-naphthylamine and benzidine (Rehn 1895; Case, 1969). Like (*PAH*), aryl hydroxylamines also require (*P450*) for forming intermediate

compounds which are responsible for creating irreversible damage (Kadluar *et al*, 1977).

2.7.1.4. Inorganic compounds and asbestos:

A wide range of inorganic metals and minerals exhibit increased risk association with cancer. Arsenic, nickel and chromium metals are included in this group. Asbestos which is an extensively used compound in cement industry has been found hazardous for lung as well as peritoneum cancer. About (20-25%) of heavily exposed workers in this industry develop lung cancer (Lemen *et al*, 1980).

2.7.2. Radiation:

Chronic exposure to ultraviolet light increases the risk of skin cancer. (*UV*) radiations catalyze the formation of cyclobutane dimmers and also cause *GC-AT* bp transitions (Beukers & Berends, 1960). (*UV*) radiation wave length ranges between (200 - 400) nm and is divided into three categories namely UV- A (320- 400 nm), UV-B (280–320 nm) and UV- C (200-280nm). Harmful effects mostly induced by UV-B radiations include acute symptoms of sunburn and skin thickening as a result of adoptive response (Urbach, 1993). Similarly harmful effects of ionizing radiations from X-ray machine had also been traced after frequent induction of skin cancer by earlier radiologists (Ron *et al*, 1988). Ionizing radiations have also been linked to excessive cancer cases in populations exposed to nuclear detonations, as increased incidences of leukemia cases along with significant contribution of solid tumors of digestive, reproductive system had been revealed among survivors of Hiroshima and Nagasaki in several studies (Shimizu *et al*, 1989; Shimizu *et al*, 1990).

2.7.3. Viral carcinogenesis:

Association of viruses with carcinogenesis was initially considered as a revolutionary milestone in cancer research. Some of the well known viruses include human papilloma virus (*HPV*) for warts, Epstein Bar virus (*EBV*) for Burkett's lymphoma and Hepatitis B Virus (*HBV*) for liver cancer. Epidemiological studies have shown the involvement of virus in venereal cancers. Out of (65) different strains of (*HPVs*), (10) are categorized as highly risk (*HPV*) sequences, which are also found in (85%) of cervical cancer cases (Riou *et al*, 1990). Breast cancer association with virus is a subject of controversy and still requires further experimentation.

2.7.4. Endogenous carcinogenesis:

Apart from chemicals and viral infestations, certain endogenous factors also contribute in carcinogenesis. Despite several highly accurate proofreading mechanisms, there are still chances of error either during *DNA* synthesis or in repair mechanism. Genomic instability associated with tumor progression and carcinogenesis itself can occur with exogenous factors involvement (Loeb, 1989; Cheng & Loeb, 1993). Apurinic sites are created by repair enzymes that recognize an altered base or adduct and eliminates it from *DNA* strand (Lindahl & Nyberg, 1972). Respiration and phagocytosis are among the other potential sources for oxygen free radical production, which can induce damage to *DNA* strands. An estimated (10,000) alterations in *DNA* per human cell per day is expected as a result of these free radicals (Richter *et al*, 1988).

2.8. Cascade of carcinogenesis:

Carcinogenesis may be considered by the process of inherent adulterations in cells, but it does not mimic the contribution of exogenous chemicals at all. Majority of the carcinogenesis mechanism may complete within the body in the following prescribed order:

2.8.1. Tumor initiation:

Aforementioned factors are responsible for initiating disequilibrium inside the body for initiating tumorigenesis. They are mainly attributed for bringing several defects at cellular and molecular levels. These events further aggravate the process of chromosomal aberration, lesions and adulterations in normal cell signaling cascade.

2.8.2. Tumor promotion:

Repetitive exposures of the compounds, included in this class, bring an increase in tumor incidence and are categorized as promoters. They show relatively little if any genotoxic effect and stimulate growth or block differentiation preferentially of initiated cells. A well known example is (12-O-tetradecanoylphorbol 13 acetate). After initial exposure of (*PAHs*), a relatively few skin tumors are observed, while after repetitive applications of croton oils (source of *TPA*) marked increase in tumor incidence is observed (Hecker, 1967). Apart from exogenous compounds, a wide range of endogenous elements have also been observed. These may include epidermal growth factor and transforming growth factors even certain hormones. Reduced testosterone levels, due to orchidectomy, also mimic the incidence of prostate cancer tumor growth by (90%). Though detailed biological mechanism is yet to be discovered, yet it is evident that hyperplasia is an absolute prerequisite for promotion (Loury *et al*, 1987). Promoters thus facilitate initiated cells to grow either by their selective proliferation or by minimizing normal cells interaction with initiated cells for growth restrictions.

2.8.3. Tumor progression:

After initiation and tumor hyperplasia as a result of promotion, tumor either regresses following promoter removal or proceed to further proliferation. Details of these specific events at cellular scale is still lacking because of the cellular

heterogeneity. Multiple changes in cellular signaling pathways are also another limiting factor to establish a cause or consequence relationship in this process. However, genome becomes highly unstable at this phase of carcinogenesis with several chromosomal translocations evident on microscopic level. Specific primary chromosomal alterations have been found to be associated with different solid tumors while secondary chromosomal changes also influence cell metastasis and treatment response (Kreeger & Lauffenburger, 2010). As the progressive phase ends, tumor cells are found invasive, metastatic and highly autonomous. They can penetrate in near vicinity of the tissues and can also escape both physical or growth regulatory restrains.

2.9. Risk factors:

The etiology of breast cancer is multi-factorial involving both genetic and environmental influences. Some of the most evidential factors are as follows:

2.9.1. Family history as risk factor in breast cancer:

It has been observed in several studies that around (5-10%) of women suffering from breast cancer already have a history of mammary tumor in maternal or parental lineage (Hoffman & Johnson, 1995). An estimated relative risk (*RR*) of breast cancer among females having a familial history cancer in first degree relatives was observed as 2.1% (with 95% confidence interval (CI 2-2.2) (Pharoah *et al*, 1997). Risk estimation may vary with age at diagnosis of the affected relative, number of relatives involved and closeness to affected personnel on pedigree basis. Family history of other types of cancers like ovarian cancer also poses a threat for breast cancer. Contrary to several published reports, it has been observed that first degree relatives (*FDRs*) of ovarian cancer patients had a modest risk of breast cancer of around 1.27 (95% CI 0.91-177) in Utah Cancer Registry (Kerber & Slattery, 1997).

2.9.2. Inheritance of breast cancer predisposition:

Autosomal dominant pattern of breast cancer is vertical transmission and has been observed following pedigree analysis in breast cancer affected families (Newman *et al*, 1998). Thus both male and female contribute (50%) of the inheriting predisposing genetic alterations and susceptibility may equally be contributed by mother or father sides, as incomplete dominance and gender restricted expression patterns do restrict cancer initiation. Hence this (50%) predisposition of inheritance does not mean that everyone included in their off springs will develop cancer.

2.9.3. Non-genetically associated risk factors for breast cancer:

Apart from genetic susceptibility there are also several contributory factors involved in breast cancer anomalies. Age, age at menstrual cycle initiation, reproductive history, hormone therapy, radiation exposure, mammography, breast density, life style factors and history of breast cancer are among the main associated factors.

2.9.3.1. Age:

A direct correlation of age with breast cancer incidence has been observed. Women after (50) years of age are more prone to develop mammary tumors (Feuer *et al*, 1993). Tumors tend to occur at an earlier age among women carrying germline mutations either in *BRCA1* or *BRCA2* genes (Ford *et al*, 1998). However, majority of the cancer affected women have no prior familial history of cancer (sporadic).

2.9.3.2. Menstrual and reproductive history:

Early menarche and late menopause also increases the chances of breast tumors. These risk factors are also largely reduced by early full-term pregnancy. Women having an early puberty (menstrual cycle start) before the age of (12) years are

twice at risk as compared to those who mature after (13) years of age. Similarly menopause after (55) years of age also doubles the risk among the females when compared with women having an early menopause around the age of (40) years (Handerson *et al*, 1992).

2.9.3.3. Oral contraceptive:

Prolonged uses of oral contraceptives also slightly increase breast cancer risk. No difference in relative risks (*RR*) is observed among current users and (10) or more years's earlier using females after stopping oral contraceptives intake (Collaborative Group on Hormonal factors in Breast Cancer, 1996). However, use of oral contraceptives in *BRCA1* mutation carriers as prevention against ovarian cancer may increase breast cancer risk up to study, no statistical significant value has been observed in *BRCA1* mutation carriers after using oral contraceptives for (1) year (Haile *et al*, 2006). Association of oral contraceptive usage with *BRCA1* mutation carriers is still an area that requires further research. A quite recent publication has shown a quite opposite story which indicates that *BRCA* mutation carriers as well as women with strong familial history are more prone to exogenous hormones present in oral contraceptives (Pasanisi *et al*, 2009).

2.9.3.4. Hormonal therapy:

A meta- analysis of (51) studies concluded a positive correlation of relative risk of breast cancer with postmenopausal hormone replacement therapy (*HRT*). RR value calculated was 1.35% (95% CI 1.21-1.49) for women who had used (*HRT*) for (5) or more years after menopause (Collaborative Group on Hormonal Factors in Breast Cancer, 1996). Similar findings were also noted by Women's Health Initiative (*WHI*) controlled on postmenopausal women with (*RR*) value of 1.24% (95% CI 1.02-1.5) (Chlebowski *et al*, 2003). Controversial reports were observed regarding hormone replacement therapy (*HRT*) and breast cancer

risk among women with a family history of breast cancer (Steinberg *et al*, 1991; Gorsky *et al*, 1994; Stanford *et al*, 1995; Schuurman *et al*, 1995).

2.9.3.5. Radiation exposure:

Survivors of atomic bomb in Hiroshima and Nagasaki have shown an increased prevalence of breast cancer as a result of radiation exposure (Tokunaga *et al*, 1994; Morishita *et al*, 2005). *DNA repair* mechanism has been found lacking among these persons. *BRCA1* and *BRCA2* have a potential role in radiations induced *DNA breaks* and *DNA repair* mechanism (Scully *et al*, 1997). A cohort study of *BRCAs* mutation carriers treated with Breast Conserving Therapy (*BCT)* showed no evidence of increased radiation sensitivity either in breast or any other organ of the body (Pierce *et al*, 2000).

3.9.3.6. Life style contributory role:

Life style factors include several parameters as weight gain, obesity, fat intake and physical activity. It is generally observed that overweight women are more prone to cancer after menopause. Sedentary life style may also be considered as a risk factor. Alcoholism, smoking and other drug abuse are further considered as contributory factors for mammary tumors (Moore &Sobue, 2010). Significant correlation of health food choice (low fat intake) with survival rates among patients has also been observed (Thomson & Thompson, 2009).

2.9.3.7. Benign breast disease:

Benign breast disease (*BBD*) is also a risk factor for breast cancer, independent of the effects of other major risk factors for breast cancer (age, age at menarche, age at first live birth, family history). Women suffering from atypical hyperplasia have (2.5) to (5.3) times more risk developing cancer than non-proliferative benign breast disease. Women with proliferative disease of breast growth without atypia are at (1.6-1.9) fold risk of developing mammary tumors

(Carter *et al*, 1988; London *et al*, 1992). Tumors of connective tissue also raise the chances of mammary tumors by (20- 40%) as observed among fibroadenoma diagnosed females (DuPont *et al*, 1994). Similarly, it has also been observed that breast density also directly has influence on the relative risk of tumor formation in both pre and postmenopausal females. Breast density of (75%) or more, increase risk of disease up to five folds (95% CI 3.6 -7.1) as compared to low breast density (Pankow *et al*, 1997; Boyd *et al*, 1999; Vachon *et al*, 1999).

2.9.3.8. Ethnicity:

Initially, association of breast cancer prevalence with respect to different ethnic groups was lacking, but this space is now largely been filled by extensive research on polymorphism and genetic mutations. So far wide ranges of founder mutations on various genes have been observed in different populations. In a study, overall increase trends regarding high incidence and mortality rates for all cancer sites were observed more frequent in black people (Hayat *et al*, 2007). Further investigations regarding the use of genetic markers in epidemiologic studies may help to clarify associations with purposed risk factors for cancers.

2.9.4. Rare affiliated breast cancer syndromes:

Many syndromes also act as risk factors for breast cancer, include the following:

2.9.4.1. Li-Fraumeni (*LFS)*:

This malignancy includes tumors of soft tissues sarcomas, brain tumors, adrenocortical carcinoma and leukemia (Li & Fraumeni 1969; Strong *et al*, 1987; Li, 1988). Tumors in (*LFS*) families usually occur at very young age along with multiple primary cancers in the same individual. Evidence supports a genotype - phenotype correlation associated with the location of mutation and the kind of cancer that develops along with the age of onset of disease (Olivier *et al*, 2003). More than (50%) of the families show a germline mutation in (*p53)*

gene. Several reports narrated that mutations are clustered in the conserved sequences exon (5) through (9) of the gene (Law *et al*, 1991; Santibanez-Koref *et al*, 1991; Srivastava *et al*, 1992; Sameshima *et al*, 1992; Brugieres *et al*, 1993). Alterations of (*p53*) gene account for a relative less proportion of larger breast cancer prevalence in general population.

2.9.4.2. Cowden's Syndrome:

It is also termed as Multiple Hamartoma syndrome (*MHS*) with autosomal dominant pattern of inheritance. This syndrome is characterized by an excess of breast as well as gastrointestinal malignancies and thyroid disease of both benign and malignant orientation (Tsou *et al*, 1997). The pathgonomonic and formidable symptoms of this syndrome are mucocutaneous lesions, including trichilemmomas, papillomatosis of lips and oral mucosa and acral keratosis (Starink, 1984). Gene responsible for this syndrome has been identified as (*PTEN)* localized on (*10q22-23*) Chromosome. Women with Cowden syndrome are at (25-50%) increased risk of developing breast cancer (Pilarski & Eng, 2004).

2.9.4.3. Peutz Jegherz Syndrome (*PJS)*:

This is characterized by gastrointestinal hamartomas and macular melanotic pigmentation of the muscoa, lips, fingers and toes (Nakagawa *et al*, 1998). (*PJS*) usually predisposes the individuals to cancers of gastrointestinal tract, respiratory tract, urinary tract, female genital tract, breast and ovary (Sommerhaug & Mason, 1970; Jancu, 1971; Utsunomiya *et al*, 1975; Cheney *et al*, 1986; Foley *et al*, 1988). Relative involvements of female genital tract tumors are rare and also not well recognized (Cantu *et al*, 1980). (*PJS)* locus was mapped on chromosome (*19p13.3)* by comparative genomic hybridization and loss of heterozygosity helped in identifying (*LKB1*/*STK11)* gene (Hemminki *et al*, 1997, 1998). Somatic mutation of *STK11* (serine-threonine kinase) result

in premature truncated protein product leading (*STK11)* as first kinase gene associated with hereditary cancer. No mutation has been observed in sporadic breast, colon and testicular carcinoma (Bignell *et al*, 1998; Wang *et al*, 1998; Avizienyte *et al*, 1998) suggesting its involvement to inherited predisposition. No linkage of (*STK11*) in small number of (*PJS)* families also indicates the potential involvement of other (*PJS)* gene elsewhere in the genome (Olschwang *et al*, 2001).

2.9.4.4. Ataxia Telangiectasia (*AT*):

Autosomal recessive inheritance pattern has been observed in this syndrome. Cerebella ataxia, oculocutaneous telangiectasia, radiation hypersensitivity and increased incidence of malignancy are characteristic features of this syndrome. Chromosomal abnormalities and *DNA rearrangements* are thought to result from genetic defects that underlie the clinical syndrome (*AT*). Cancers observed in association with (*AT*) include non-Hodgkin lymphoma and significant but lower risk of developing breast cancer, ovarian cancer, leukemia, malignancies of oral cavity, stomach, pancreas and bladder (Morrell *et al*, 1986). Gene found responsible for this syndrome has been mapped on chromosome (*11q22*) and termed as (*AT*) (Savitsky *et al*, 1995).

2.9.4.5. Muir-Torre Syndrome (*MTS*):

This given cancer includes association among multiple skin tumors and multiple benign and malignant tumors of upper and lower gastro-intestinal and genitourinary tract (Muir *et al*, 1967; Hall *et al*, 1994). Many lesions including basal cell carcinoma, keratoacanthomas, and colonic diverticula occur at younger age but are similar in distribution. Autosomal dominant inheritance pattern has been observed in this syndrome. Females with the syndrome have increased risk of developing breast cancer particularly after menopause (Anderson, 1980). It has been observed that genes involved in hereditary non-

polyposis colon cancer (*HNPCC*) *MSH2*, *PMS1*, *PMS2*, *MLH1* and MSH6 are also involved in pathogenesis of Muir-Torre syndrome (*MTS*) (Kolodner *et al*, 1995). Truncating mutations of *MSH2* and *MLH1* leading to impairment in *DNA* repairs, accumulation of replication errors and genome instability have also been detected in this syndrome affected families (Bapat *et al*, 1996).

2.10. Hereditary predisposition to cancer:

The vast majority of genetic alterations in cancer are somatic, and thus found only in the tumors of the affected individuals. In hereditary cancer, on the other hand, the gene defects are in the germline, and therefore present in every cell of the body. Although additional somatic genetic aberrations are needed for cancer development, the presence of a germline mutation greatly speeds up the process of accumulation of mutations needed for malignant conversion. Individuals with such mutations are not only at higher risk at developing cancer, but they are more likely to do so at younger age than the general population, and also their risk for developing multiple primary tumors is greatly increased. Therefore, the possibility of an inherited cancer syndrome should be considered when numerous family members develop cancer at an especially young age or affected individuals develop multiple primary tumors (Fearon, 1997). Also congenital abnormalities or other rare conditions in families with multiple cancer patients should arouse suspicion of a cancer syndrome, and some cancers such as retinoblastoma are so rare that the diagnosis itself warrants further evaluation for the possibility of inherited predisposing mutation (Fearon, 1997). Some pathological features may further suggest the possibility of hereditary cancer. For example, breast carcinomas associated with mutations in the *BRCA1* gene are more likely to be of medullary histology and less likely to express estrogen receptor than sporadic tumors (Johansson *et al*, 1997). On the other hand, the recognition of hereditary cancer may be difficult in some families as the family history may be obscured (Fearon, 1997). This may be due to a small family size,

uncertain family history, and unknown or poorly documented medical records, or incomplete penetrance. Germline mutations may also arise spontaneously leading to so called *de novo* mutations. Additionally, sporadic cancers of the same type, especially common carcinomas such as breast or colon, may arise in individuals who do not carry germline mutations, making it difficult to distinguish true hereditary cancer syndromes from familial clustering formed by chance.

The lifetime risk of cancer for individuals carrying a mutation in a cancer predisposition gene is high and ranges between (50%) and (80%) (Ponder, 1988). The likelihood of developing cancer depends on the actual gene and the mutant allele as well as on other modifying risk factors, both genetic and non-genetic. Furthermore, it also depends on the complex gene environment interactions which are currently under intense investigation, but at the moment remain poorly understood (Ponder, 1988).

2.11. Cancer genetics:

Cancer is the general name for over (100) medical conditions involving uncontrolled and dangerous cell growth. A cancer generally derives from a single cell that is changed dramatically by a series of genetic alterations.

A healthy cell has a well-defined shape and fits nearly within the ordered array of cells surrounding it. It responds to the environment, giving rise to daughter cells solely when the balance of stimulatory and inhibitory signals from the outside favors cell division. But the process of replication carries the constant hazard of random genetic mutations which can impair the regulatory circuits of a cell (Hanahan & Weinberg, 2000).

Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer promoting “oncogenes” are typically activated in cancer cells, giving those cells new properties (gain of function). “Tumor suppressor genes”

are then inactivated in cancer cells, resulting in the loss of normal functions in those cells.

2.12. Genes involved in hereditary cancer:

2.12.1. Oncogenes:

Under normal conditions, proto-oncogenes stimulate cell growth and differentiation required e.g. for the continuous renewal of epithelial cells. When inappropriately activated, they may turn into cancerous oncogenes and continue to grow - or refuse to die - even in the absence of growth signals. At the cellular level oncogenes are dominant, meaning that mutation in one allele alone can promote uncontrolled cell proliferation. At present, over (100) oncogenes have been identified. Inherited mutations in those are, however, relatively rare, presumably because such mutations remove normal control over cell growth, thereby being lethal during embryogenesis and leading to spontaneous termination of pregnancy (Frank, 2001). Only three oncogenes have been shown to be involved in hereditary cancer syndromes, namely *RET* in thyroid cancer, *CDK4* in melanoma, and *MET* in papillary renal cell carcinoma (Mulligan *et al*, 1993; Zuo *et al*, 1996; Schmidt *et al*, 1997).

2.12.2. Tumor suppressor genes:

Tumor suppressors negatively regulate cell proliferation either through controlling the cell division or by promoting programmed cell death. According to Knudson's "two-hit" hypothesis both copies of the tumor suppressor gene have to be inactivated in order for the cell to turn malignant (Knudson, 1971). In hereditary cancer one defective allele is inherited and the function of the remaining allele is lost by a somatic mutation. Traditionally, the second hit is considered to be a large deletion that can be visualized by the loss of heterozygosity (*LOH*) in the corresponding tumor (Ponder, 1988).

However, recent evidence suggests haplo-insufficiency in a growing number of tumor suppressor loci (Fero *et al*, 1998 ; Tang *et al*, 1998 ; Venkatachalam *et al*, 1998; Wetmore *et al*, 2000; Xu *et al*, 2000; Kwabi-Addo *et al*, 2001; Buchholz *et al*, 2002), indicating that bi-allelic inactivation of these genes may not be necessary at least for the initiation of tumorigenesis. According to function, tumor suppressors have been further divided into *gatekeepers* and *caretakers* (Kinzler & Vogelstein, 1997). Gatekeepers prevent malignant transformation directly by regulating proliferation, promoting differentiation, or by inducing cell death, whereas caretakers prevent neoplasia indirectly by maintaining genomic integrity through *DNA repair* and *replication*. In a third class of tumor suppressors, *landscapers*, and the genetic defect is not in the transforming cell population itself but rather in adjacent stromal cells which promote malignancy of the associated cells due to an abnormal intercellular signaling (Kinzler &Vogelstein, 1998). Recently, a new class of tumor suppressors was proposed (Liu *et al*, 2000). These genes encode proteins that are essential for the cell survival and only modestly enhance tumorigenesis in a heterozygous form (e.g. *CHEK1* and *ATR*). So far, more than (30) tumor suppressor genes have been identified, and inactivating mutations in them cause most of the inherited cancer syndromes.

2.12.3. Proto- oncogenes vs tumor suppressor genes:

Oncogenes are the altered forms of proto-oncogenes. Proto- oncogenes are found in normal cells and encode proteins involved in the control of replication, apoptosis (cell death) or both. They are involved in the normal function of the cell, but can turn a cell into a cancer cell when activated. Activation of proto - oncogenes by chromosomal rearrangements, mutations, or gene amplification confers a growth advantage or increased survival of cells carrying such alterations. All (3) mechanisms cause either an alteration in the oncogenes structure or an increase in or deregulation of its expression (Bishop, 1991).

Tumor suppressor genes (*TSGs*) are targeted by genetic alterations in the opposite way as proto-oncogenes. The affected cell loses one of its functions like accurate (*DNA)* replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system. According to Knudson's two hit hypothesis (Knudson *et al*, 1975), inactivation of both (*TSG)* alleles is necessary for tumor development **(Figure 2.6)**. Knudson suggested that multiple "hits" are necessary to cause cancer. The first inactivation is inherited and any second mutation would rapidly lead to cancer. In non-inherited cancer, (2) "hits" need to take place before tumor development, explaining the higher age of onset compared to inherited cancer.

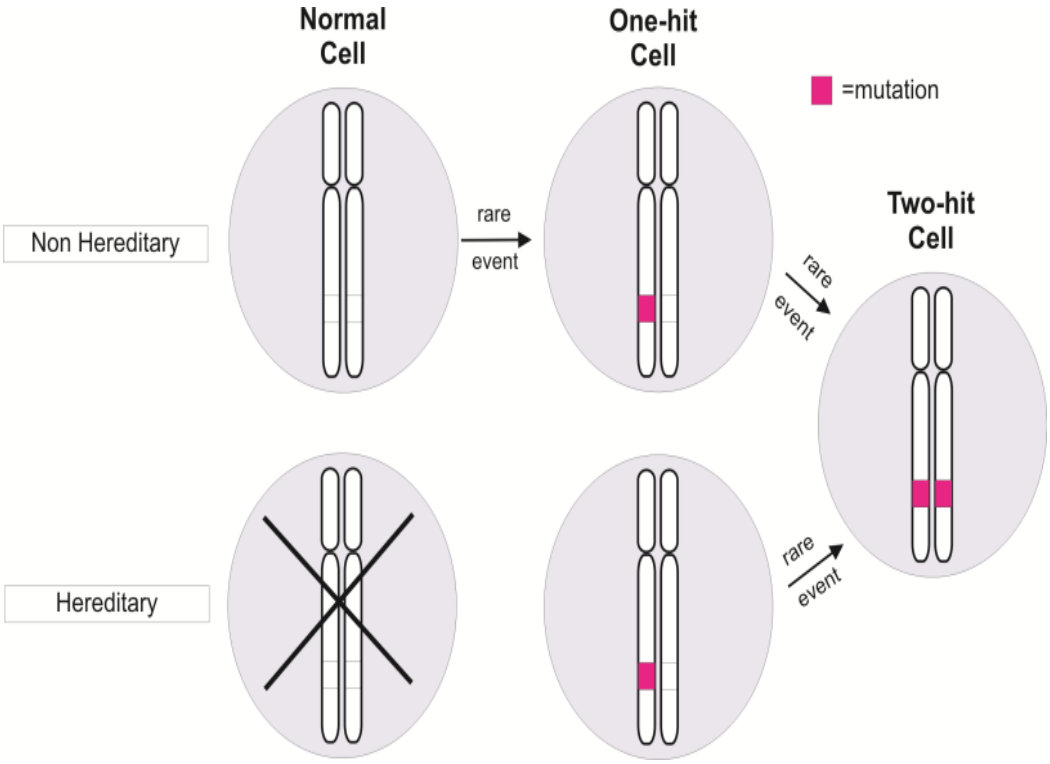

Figure 2.6: Knudson's two - hit hypothesis

In hereditary tumor syndromes, the initial inactivation of one allele is present in the germ cells. To start tumorigenesis an additional "hit" or somatic inactivation of the second allele is required. Somatic inactivation events include subchromosomal deletions, mitotic recombination, and nondisjunctional chromosome loss with or without reduplication of the chromosome carrying the affected TSG, intragenic mutation or an epigenetic event. In sporadic tumors, the initial and second inactivating event occurs in the same somatic cell of an individual.

(TSGs) can be subdivided into several classes according to their normal gene function, i.e. gatekeepers, caretakers and landscapers (Kinzler & Vogelstein, 1998*;* Kinzler & Vogelstein, 1997). Gatekeepers (e.g. *p53*) act directly by inhibiting cell growth. Caretakers are involved in maintaining (*DNA) integrity* and repairing (*DNA) damage.* Mutations in these caretakers have no direct effect on the proliferation, but they result in an accelerated accumulation of other mutations and will eventually lead to genomic instability. The land scapers, the (3^{rd}) subgroup of *TSGs* are genes, which act by modulating the micro-environment rather than the tumor itself.

Recently, a new class of tumor suppressors was proposed (Liu *et al*, 2000). These genes encode proteins that are essential for the cell survival and only modestly enhance tumorigenesis in a heterozygous form (e.g. *CHEK1* and *ATR*). So far, more than (30) tumor suppressor genes have been identified, and inactivating mutations in them cause most of the inherited cancer syndromes.

2.13. Genetic susceptibility to breast cancer:

Generally, a distinction is made between; high and moderate risk families with breast and/or ovarian cancer. In families with a clear autosomal dominant inheritance pattern for breast cancer, the diagnosis of hereditary breast and/or ovarian cancer (*HBOC*) is proposed.

Members of such families *a priori* have a highly increased risk to develop breast cancer. The age at onset is considerably lower compared to sporadic patients and the prevalence of bilateral or multiple primary breast cancers are higher. (*HBOC)* represents (5- 8%) of all breast cancer cases.

In families where breast cancer is overrepresented compared to the incidence in the general population, but not fulfilling the criteria for hereditary breast cancer. Approximately (15%) of all breast cancer patients have a (1^{st}) degree or in case of paternal inheritance a (2^{nd}) degree relative.

2.13.1. High-penetrance breast cancer predisposition genes:

Mutations in (3) high- penetrance breast cancer predisposition genes confer a higher than (10) - fold relative risk of breast cancer *BRCA, BRCA2*, and *P53*. Germline mutations in *P5*3 lead to Li-Fraumeni syndrome and are associated with other phenotypic manifestations as well.

BRCA1 and *BRCA2* are the (2) major breast cancer genes. Genetic testing for these genes is widely available in a clinical context. As mutation detection in these genes is challenging and expensive, a number of models and scoring systems have been proposed to estimate the probability of the presence of a *BRCA1* or *BRCA2* mutation in a given individual based on family history and age at onset. Important and widely used examples are the Claus, Tyrer- Cuzick and *BRCAPRO* model (Fasching *et al*, 2007) but these cannot be reliably used for patients without a family history for breast or ovarian cancer. Hence, in many labs worldwide easily applicable, descriptive inclusion criteria based on age of onset and family history are applied.

The *BRCA1* gene was identified by positional cloning in (1994) (Miki *et al*, 1994). It is located on Chromosome (*17q21*) and spans approximately (81) kb on the minus strand. *BRCA1* contains (22) coding exon, including a large exon (11) which covers almost (50%) of the coding sequence. *BRCA1* spans a region with an unusually high density of (*Alu) repetitive DNA* (41.5%), but a relatively low density (4.8%) of other repetitive sequences **(Figure 2.7)**. *BRCA1* intron lengths range in size from (403) bp to (9.2) kb and contain (3) intragenic microsatellite markers located in intron (12, 19, 20) (Smith *et al*, 1996). The (5) prime end of the *BRCA1* gene lies within a duplicated region on chromosome (*17q21*). This region contains *BRCA1* exon (*1A, 1B, 2)* and their surrounding intron; as a result, a *BRCA1* pseudo gene lies upstream of *BRCA1* (Puget *et al*, 2002).

BRCA2 was identified by (Wooster *et al*, 1995) on chromosome (*13q12*). *BRCA2* contains (27) exon (26 coding) and spans (84) kb of the genome. Similar to *BRCA1*, *BRCA2* is very *AT-rich*, has a large exon (11) and a translational start site in exon (2) (**Figure 2.7)** (Tavtigian *et al*, 1996).

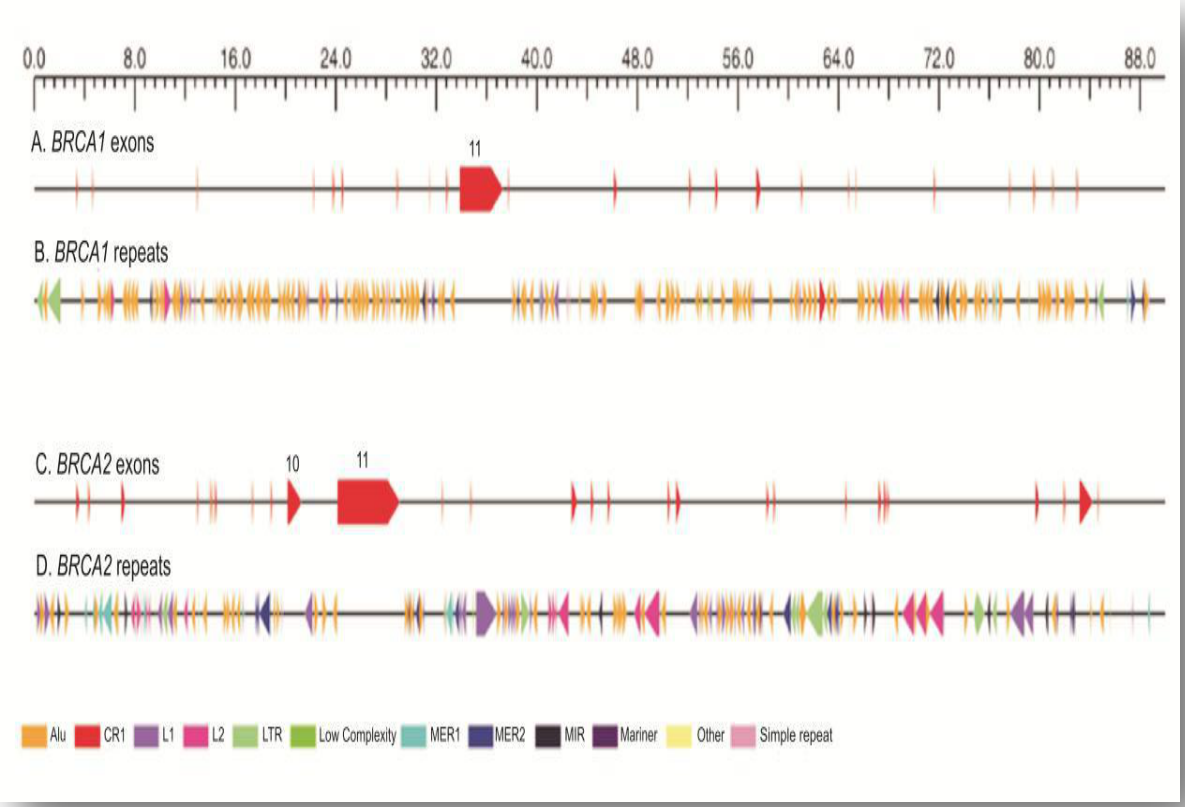

Figure 2.7: ***BRCA1* and *BRCA2* genes**

Genomic structure of the BRCA1 and BRCA2 genes. BRCA1 has 22 coding exon including a large exon 11 (panel A). Panel B shows repetitive elements in BRCA1. BRCA2 has 26 coding exon, including a large exon 10 and 11 (panel C).Panel D shows the repetitive elements in BRCA2 (modified from (*Welcsh & King, 2001)).*

The *BRCA1* gene encodes a protein of (1863) amino acids (Miki *et al*, 1994) (**Figure 2.7)**. Most of the coding region shows no sequence similarity to previously described proteins apart from the presence of a (*RING*) zinc finger domain *(RZFD)* (Saurin *et al*,1996) at the amino terminus of the protein and two *BRCT'* (*BRCA1* carboxyl terminus) repeats at the carboxyl terminus (Koonin,1996; Callebaut, 1997). The (*RING*) finger domain is involved in mediating protein-protein interactions. The (*BRCT)* repeat is a poorly conserved domain found in a range of proteins many of which are involved in either *DNA repair* or metabolism (Callebaut, 1997). A (2)-hybrid screen with the *RING* finger domain region of *BRCA1* resulted in the isolation of a novel gene, (*BARD1)* the encoded product of which contains both a (*RING*) finger and a (*BRCT)* domain but no other similarity to *BRCA1* (Wu *et al*,1996). Furthermore, a nuclear localization signal (*NLS*) domain was identified in exon (11), which is absent in different alternative spliced (*mRNA*s) leading to the hypothesis that splicing regulates the *BRCA1* function and its expression in different tissues (Thakur *et al*, 2002).

BRCA2 encodes a protein of (3418) amino acids with an estimated molecular weight of (384) *kDa* (Wooster *et al*, 1995; *Tavtigian et al,* 1996) **(Figure 2.7)**. Although *BRCA2* shows no strong similarity to known proteins, a significant feature is the presence of (8) copies of a (30-80) amino acid repeat (*BRC repeats*) that are present in the part of the protein encoded by exon (11) (Bork & Nilges, 1996). Comparison of the sequence of exon (11) from (6) mammalian species reveals that many of the repeats appear to be retained within the generally poorly conserved context of exon (11) (Bignell *et al*, 1997).

The majority of *BRCA1* and *BRCA2* breast cancer susceptibility mutations (~ 70%) are known to result in a truncated, and therefore inactive, gene product. This class of mutations can be readily identified using currently available genetic screening methods, and are likely all clinically significant (*BIC*). The remaining *BRCA1* and *BRCA2* cancer-predisposing mutations do not interrupt

the open reading frame of the genes, but instead are found to be missense, or in-frame splicing mutations. The current number of mutations falling within this class cumulatively numbers over (1400) to date (*BIC*). Unlike truncating mutations, establishing the clinical relevance of this subset of mutations remains a major challenge, and these mutations are hence termed-Variants of Unknown Significance (*VUS*). Current estimates place individuals who inherit a single germline mutation in either *BRCA1* or *BRCA2* at a lifetime risk of approximately (80%) of developing breast or ovarian cancer by age (70) years (Wooster &Weber, 2003) however the reasons why these mutations contribute to the development of breast and ovarian cancer remain poorly understood. Elucidation of the precise molecular mechanisms of these genes could potentially improve our understanding of the way in which these mutations contribute to the development of the disease as well as offer new options for diagnosis and treatment.

2.13.1.1. *BRCA1* Structure & its relationship to breast cancer:

The *BRCA1* gene encodes a large (1863) amino acid protein with multiple functional domains (**Figure 2.8**). The well characterized *BRCA1* domains consist of an N-terminal RING domain, a globular zinc- binding motif that has been implicated in ubiquitination pathways (Lorrick *et al*, 1999) and (2) C-terminal tandem repeat globular domains termed (*BRCT)* (Koonin *et al*, 1996) now known to be a common structural feature of proteins involved in the DNA *damage response (DDR)* (Bork *et al*, 1997). Mounting evidence implicates *BRCA1* in all phases of the cell cycle and in the regulation of orderly events during cell cycle progression. Consequently, *BRCA1* deficiency results in defects in the *G1/S-phase checkpoint* (indirectly), the *S-phase checkpoint* and the *G2/M checkpoint* (Deng & Brodie, 2000; Venkitaraman, 2000). *BRCA1* interacts with a large number of diverse molecules including tumor suppressors, oncogenes, *DNA damage repair proteins*, cell cycle regulators, and

transcriptional activators and repressors (Deng &Brodie, 2000). Consistent with this expansive pattern of interaction, loss of function mutations in *BRCA)* result in pleiotrophic phenotypes including growth retardation, increased apoptosis, defective *DNA damage repair*, defective *G2/M cell cycle checkpoint*, chromosome damage, and aneuploidy (reviewed in (Venkitaraman, 2000)). *BRCA1* cancer-predisposing truncating and missense mutations, validated through functional analysis, are commonly found within the (2) C-terminal (*BRCT*) motifs and to a lesser extent in the critical (Zn^{2+}) binding residues within the N-terminal *RING* finger, indicating that these regions are critical for tumor suppressor function (Freidman *et al*, 1994). It is proposed that mutations in *BRCA1* do not directly result in tumor formation, but instead cause genetic instability thereby subjecting cells to a high risk of malignant transformation (Kinzler & Vogelstein, 1997).

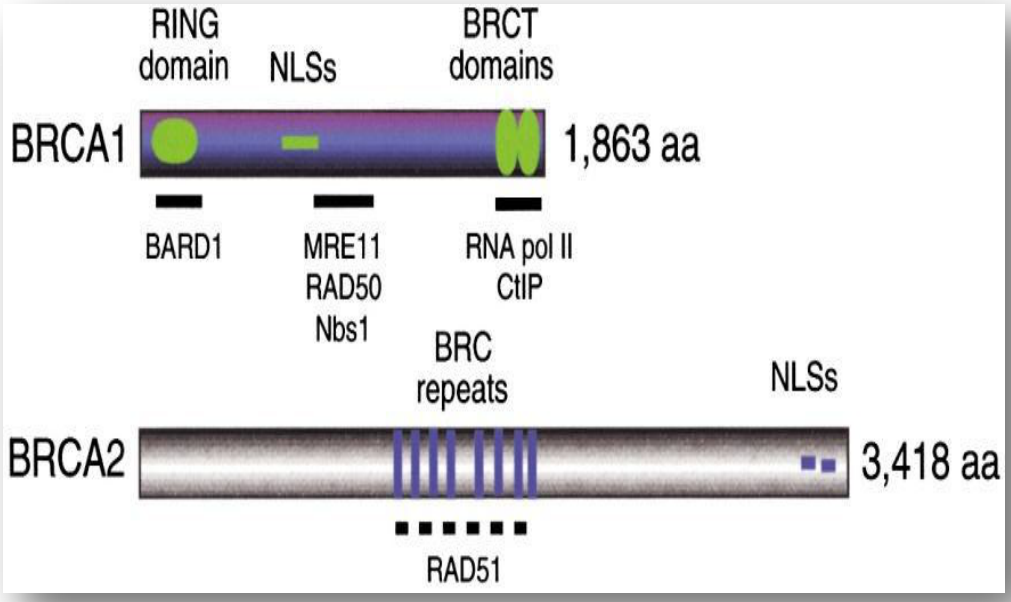

Figure 2.8: Features of the Human *BRCA* Proteins

BRCA1 contains an N-terminal RING domain, nuclear localization signals (NLSs), and two C terminal BRCT domains of 110 residues (also found in several proteins with functions in DNA repair or cell cycle control). Examples of interacting proteins are shown below approximate regions of binding. BRCA2 contains eight repeats of the 40 residue BRC motifs. (Adapted from Venkitaraman, 2002).

2.13.1.2. Abnormal product:

Most mutations lead to frame shifts resulting in missing or non-functional protein. In all cancers that have been studied from individuals with a disease-causing mutation, the wild-type allele is deleted, strongly suggesting that is in the class of tumor suppressor genes, i.e. genes whose loss of function can result in neoplastic growth (Smith *et al*, 1992). Additional evidence that is a tumor suppressor gene is that over expression of the *BRCA1* protein leads to growth suppression similar to the paradigmatic tumor suppressors *(p53)* and the retinoblastoma gene (Holt *et al* 1996).

2.13.1.3. Pathologic variants:

More than (600) different mutations have been identified in *BRCA1*. While a small number of these mutations have been found repeatedly in unrelated families, the vast majority have not been reported in more than a few families. Although some research studies have suggested differences in cancer risk associated with different *BRCA* mutations, no definitive data on this point are yet available. About a third of mutations identified in *BRCA1* and *BRCA2* sequencing studies are of uncertain clinical significance (Shattuck-Eidens *et al*, 1997). As research proceeds, some of these mutations will likely be proven to be normal variants without clinical significance, while others may be associated with an increased cancer risk.

2.13.1.2. *BRCA2* Structure & its relationship to breast cancer:

The human *BRCA2* gene encodes a (3418) amino acid protein that is one of the largest polypeptides in the human proteome (**Figure 2.8) (**Wooster *et al*, 1995). Well characterized domains of the *BRCA2* in *protein* include a repeated motif termed the (*BRC)* domain, located centrally, which consists of a series of (8) repeated (*BRC)* sequences (Bork *et al*, 1996). It is these (*BRC)* domains, in concert with the C-terminal region of the protein, that mediate direct binding of

BRCA2 to (*Rad51)*. Of the (8) (*BRC)* repeats, (*Rad51)* has been shown to bind to (6) of them *in vivo*, showing the highest affinity for (*BRC3, 4)* and very low affinity for (*BRC5, 6)* (Wong *et al*, 1997; Davies *et al*, 2001). This interaction highlights the importance of the (*BRC)* domains in facilitating *BRCA2's* role in *DNA* double-stranded break (*DSB*) *repair* as the physical interaction of *BRCA2* and (*Rad51)* is essential for error-free homologous recombination (*HR*) to take place. Another domain important to *BRCA2* function in (*HR*) is the C-terminal nuclear localization signal (*NLS*). It is thought that the (*NLS)* on *BRCA2* facilitates the transport of (*Rad51)* into the nucleus to sites of *DNA* damage as a (*NLS)* has yet to be identified on (*Rad51*) and in *BRCA2* deficient cells nuclear transport of (*Rad51)* is found to be impaired (Davies *et al*, 2001). As in the case of *BRCA1*, the majority of known carcinogenic *BRCA2* mutations result in a truncated and therefore non-functional protein (*BIC*). Thus it is proposed that mutations in *BRCA2* cause increased chromosomal aberrations and genetic instability through the loss of error-free (*DNA DSB)* repair via (*HR)* thereby subjecting cells to a higher than normal risk of malignant transformation (Gundmundsdottir & Ashworth, 2006).

2.13.2. Breast cancer predisposition genes of uncertain penetrance:

Three genetic syndromes have clearly been associated with an increased risk of breast cancer but the magnitude of the associated risk for each remains uncertain: examples are Cowden disease associated with mutations in (*PTEN)* (Rustad *et al*, 2006), Peutz-Jeghers syndrome with germline mutations in (*LKB1)* (Chen & Lindblom, 2000) and Hereditary diffuse gastric cancer syndrome (caused by *CDH1* mutations) (Pharoah *et al*, 2001). All these syndromes are associated with other phenotypic manifestations and in site specific breast cancer families the contribution of mutations in (*PTEN)*, (*LKB1)* or (*CDH1)* is at most infrequent (Ripperger *et al*, 2009).

It is not expected that these genes are acting as breast cancer susceptibility genes outside the context of these syndromes. Together, they may cause less than (1%) of all breast cancers **(Figure 2.9)**.

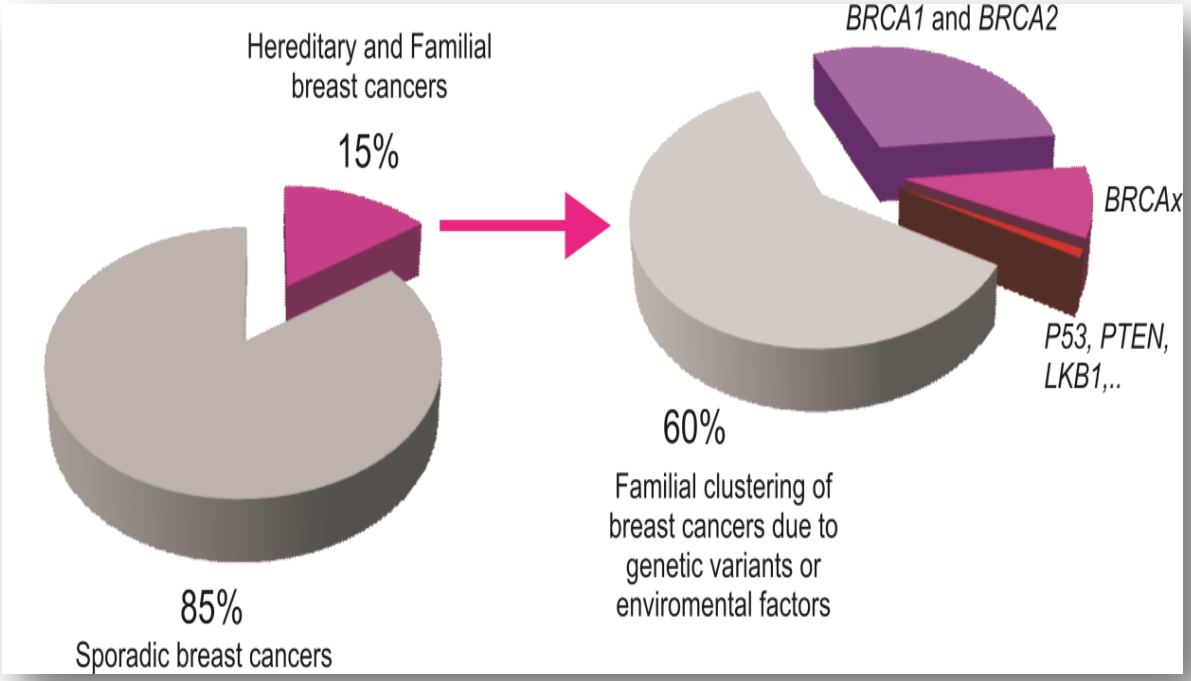

Figure 2.9: Prevalence of sporadic vs. Hereditary and Familial breast Cancer.

(About *(40%) of all inherited forms of breast cancers can be explained by mutations in single genes. (Prevalence numbers adapted from* (Ripperger *et al*, 2009)).

2.13.3. Intermediate penetrance breast cancer predisposition genes:

As numerous genetic linkage studies have failed to identify additional high risk genes, it has been hypothesized that the remaining breast cancer clustering among families might not be explained simply by inheritance of variants in additional major high risk breast cancer susceptibility genes. Chance clustering and shared environmental and lifestyle factors may account for part of the familial breast cancer cases. However, twin studies have shown that the breast cancer risk for unaffected monozygotic twins with a co-twin diagnosed with breast cancer is higher compared to the risk for dizygotic twins. This strongly suggests that a significant proportion of the remaining familial breast cancer risk is likely due to genetic factors (Ahlbom *et al*, 1997; Mack *et al*, 2002).

As *BRCA1* and *BRCA2* are involved in *DNA* repair, and heterozygous mutations in *DNA* repair genes such as *ATM* and *TP53* have been associated with an increased breast cancer risk, most candidate gene approaches now focus on genes involved in *DNA* repair, such as (*CHEK2),* (*RAD50),(BRIP1)* and (*PALB2)*. Thus far, this candidate approach has led to the identification of (5) moderate risk breast cancer genes (*CHEK2*, *ATM*, *BRIP1, PALB2* and *NBS1*). Odds ratios for heterozygous mutations in these genes lie between (2.0) and (4.3) (**Figure 2.10)**. Variants in these intermediate penetrance genes are currently estimated to account for (5%) of familial breast cancer risk and are present at (1%) allele frequency (Stratton & Rahman, 2008).

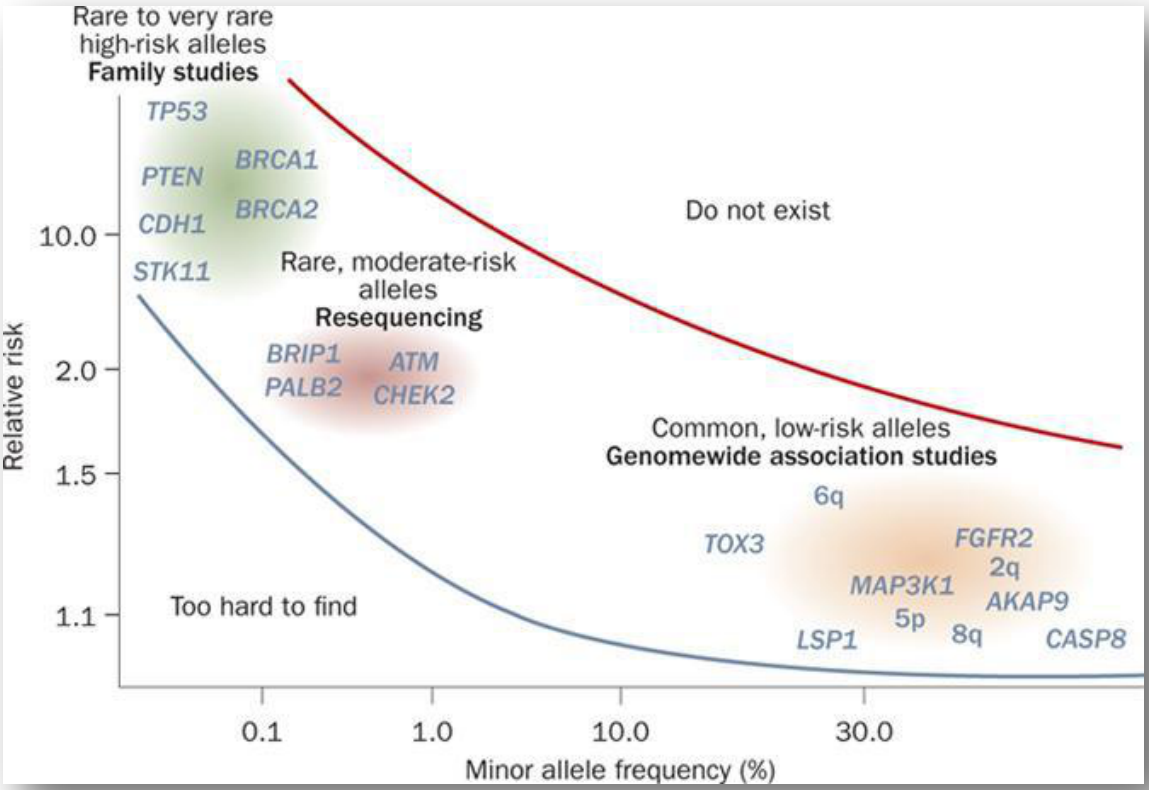

Figure 2.10: Germline mutations that confer susceptibility to breast cancer.

*Schematic representation of the relation between breast cancer risks conferred by germline variants in low risk, moderate risk and high risk breast cancer genes and their prevalence in the general population. Germline mutations in high risk breast cancer susceptibility genes are very rare in the population (<0.1%) but confer high breast cancer risk (up to 20-fold). Germline variations in moderate risk breast cancer genes are rare in the population (~1%) and confer a 2 to 4-fold risk. Low risk breast cancer alleles and SNPS are more common (up to 40%) but confer only a slight increased BC risk (on average 1.3 fold). The red line represents a natural risk limitation and the bottom line represents a virtual limitation of detection. (Adapted from (*Harris & *McCormick,* 2010*)).*

The link between *DNA* repair and breast cancer susceptibility became even more intriguing after homozygous mutations in (*BRCA)* were found to be responsible for Fanconi Anaemia (*FA*) and *BRCA2* were shown to be identical with (*FANCD1)* (Hewlett *et al*, 2002). No biallelic germline *BRCA* mutations were reported in (*FA*) patients so far, but mutations in (2) other breast cancer susceptibility genes, (*PALB2)* (*FANCN*) and (*BRIP1)* (*FANCJ*), have been identified in (*FA)* patients, giving rise to type (*NFA*) and type (*JFA*), respectively (Levy, 2010).

Recently, a (4^{th}) gene was found to be probably associated with both (*FA)* and breast cancer susceptibility. Vaz *et al* (Vaz *et al*, 2010) described an (*FA)* -like phenotype in consanguineous Pakistani family with (3) affected children, of which only one survived. These (3) patients exhibited various severe inborn anomalies, and a homozygous missense mutation in the (*RAD51C)* gene was found to be the cause of these abnormalities. Because three genes (*BRCA2*, *PALB2*, *and BRIP1*) were already known to be associated with both (*FA)* and breast cancer susceptibility, it seemed feasible to screen breast cancer families for monoallelic mutations in the (*RAD51C)* gene. Meindl *et al* (Meindl *et al*, 2010) detected (6) monoallelic pathogenic mutations in (*RAD51C)* by screening (1.100) unrelated German women with gynecologic malignancies (breast and /or ovarian tumors). Strikingly, all (6) deleterious mutations were exclusively found within (480) *BRCA1/2* negative patients from breast and ovarian cancer families. No deleterious mutations were found in breast cancer or families (Meindl *et al*, 2010). These results support (*RAD51C)* as a new breast cancer susceptibility gene and according to his function and similarities with the other breast cancer susceptibility genes its role as tumor suppressor gene and caretaker has been stated.

2.13.4. Common low penetrance breast cancer predisposition alleles:

Genome-wide association studies identified several (*SNPs)* as low-penetrance breast cancer susceptibility polymorphisms within genes as well as in chromosomal loci with no known genes. Currently, there is a growing list of reports on common (*SNPs)* in genes or chromosomal loci that have been identified in genome-wide association studies, (*FGFR2)* (Hunter *et al*, 2007; Easton *et al*, 2007), (*LSP)* (Easton *et al*, 2007), (*MAP3K1)* (Easton *et al*, 2007), (*TGFB1)* (Cox *et al*, 2007), (*TOX3)* (Easton *et al*, 2007; Stacey *et al*, 2007), as well as a locus on (*2q35)* (Stacey *et al*, 2007) and (*8q)* (Easton *et al*, 2007).The odds ratios for heterozygous and homozygous carriers range between (1.1-1.3), and (1.2-1.6) respectively. These (*SNPs)* are common in the general population up to (40%) and more studies to validate the relative risk in different large cohorts will be needed.

Under a multiplicative model of disease susceptibility, the low- penetrance variants are estimated to explain about (8.3%) of the familial clustering of the disease (Mavaddat *et al*, 2010). This suggests that many other variants remain to be identified.

2.14. Risks associated with germline mutations in *BRCA1* &*BRCA2:*

(BRCA2) mutations have been linked to a wide spectrum of cancers, including prostate cancer, pancreatic cancer, fallopian tube cancer, male breast cancer and skin cancer. In contrast, a (*BRCA1)* mutation is primarily associated with breast and ovarian cancers. Plausible explanations include the connection between (*BRCA1)*, but not (*BRCA2)*, and steroid hormone receptors (Lee, 2008) and the role of (*BRCA1)* in mammary luminal epithelial lineage determination (Visvader, 2009).

Several approaches have been used to estimate the average age-specific cumulative cancer risks associated with mutations in (*BRCA1)* and (*BRCA2)*. In

general, for (*BRCA1)* carriers, the estimated cumulative risks to the age of (70) are (65-80%) for breast cancer and (39-60%) for ovarian cancer. The corresponding risks for (*BRCA2)* carriers are (45-60%) for breast cancer and (11-30%) for ovarian cancer **(Figure 2.11)** (Antoniou *et al*, 2003; Milne & Antoniou, 2011). In comparison, the average woman in the general population has an (11%) lifetime risk of developing breast cancer and a (1.5%) risk of developing ovarian cancer. After the initial diagnosis of breast cancer in a (*BRCA1)* or (*BRCA2)* carrier, the risk of cancer in the contralateral breast (a new primary cancer) increases by approximately (3%) per year (Metcalfe *et al*, 2004; Verhoog *et al*, 2000).

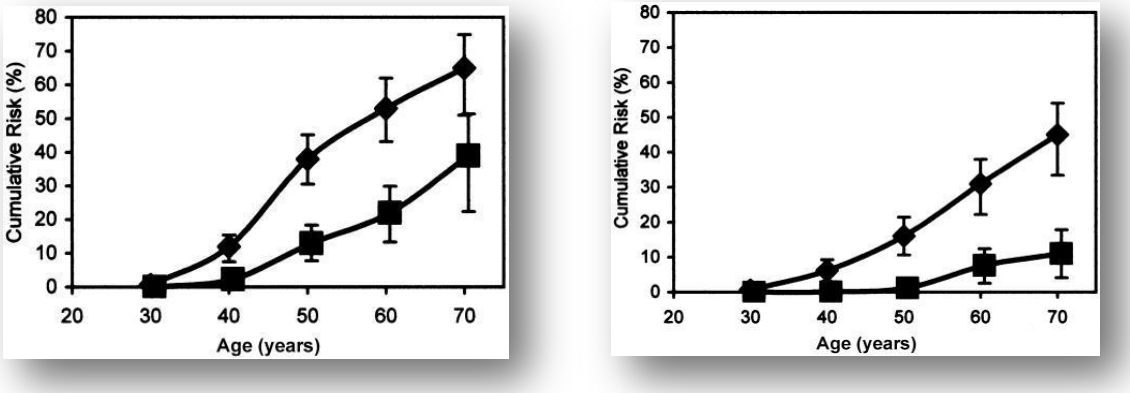

Figure 2.11: Cumulative risk

Plots *for breast and ovarian cancer associated with BRCA1 and BRCA2 mutations (right panel) mutation carriers (adapted from (Antoniou et al, 2003)).*

The broad range of associated risks reported in literature is consistent with the hypothesis that risks in (*BRCA1)* or (*BRCA2)* mutation carriers can vary substantially due to the presence of additional risk factors for breast cancer, including genetic modifiers. Common genetic modifiers of breast cancer risk for carriers of mutations in (*BRCA1)* and (*BRCA2)* have been identified in essentially three ways: studies of single nucleotide polymorphisms (*SNPs*) in candidate genes (Cox *et al*, 2007; Antoniou *et al*, 2007; Engel *et al*, 2010), studies of common (*SNPs)* (minor allele frequency > 0.13) found in genome-wide association studies (*GWAS*) to be associated with a small increased breast cancer risk (odds ratio <1.30) in the general population (reviewed in (Milne & Antoniou, 2011)) and (*GWAS)* carried out in mutation carriers (Antoniou *et al*, 2010). (*BRCA1)* and (*BRCA2)* mutation carriers could potentially be among the (1^{st}) groups of individuals for whom clinically applicable risk profiling could be developed using the common breast cancer susceptibility variants identified through (*GWAS)*.

Other studies investigated risk variation in mutation carriers based on factors such as parity, age at first live birth, breastfeeding and mammographic density (Antoniou *et al*, 2003; Andrieu *et al*, 2006; Cullinane *et al*, 2005; Antoniou *et al*, 2006). Although the results from those studies are not fully consistent, they suggest that the relative risks conferred by these factors in germline mutation carriers may be similar to the relative risks in non - carriers.

2.15. Others major genes involved in breast cancer:

2.15.1. *TP53:*

Is localized at chromosome (17), on the short arm region p. It encodes protein of (53) *KD* (nuclear phosphoprotein) that binds *DNA* sequence. The protein acts as a negative regulator of cell growth and proliferation. It usually restricts cell growth at *G1* phase until *DNA repair* mechanism proceeds before *DNA synthesis* (Harris & Hollstein, 1993) and also plays a vital role in programmed

cell death. Loss of *p53* protein will lead to persistent damage to *DNA* ultimately leading to malignancy (Malkin, 1993).

2.15.2. *CHEK2:*

It plays an important role in *DNA damage* repair response pathway (Bell *et al*., 1999). Mutations present on this gene accounts for around (4-10%) of familial breast cancer cases with an overall increase of (1.5) fold to (2) fold increased risk of female breast cancer (Offit *et al*, 2003; Neuhausen *et al*, 2004; Ohayon *et al*, 2004). Although association of *CHEK2* with breast cancer has been established by extensive research but in order to calculate the absolute risk large scale studies are still required (Oldenburg *et al*, 2003).

2.15.3. *PTEN:*

Germline mutations of this gene are responsible for Cowden's syndrome. It encodes a protein with tyrosine phosphatase with homology to tensin, located on (*10q23*). Loss of hetrozygosity at *PTEN* locus observed in high proportion suggests its putative role as a tumor suppressor gene (Lynch *et al*, 1997). It also acts as a late regulatory molecule of cytoskeleton function and responsible for maintaining normal cell physiology (Myers & Tonks, 1997).

2.15.4. *ATM:*

ATM increases risk of malignancies especially of hematological nature. Epidemiological studies also suggest a statistically increased breast cancer risk among female heterozygote carriers, with an estimated relative risk of (3.9-6.4) (Swift *et al*, 1987; Easton, 1994; Olsen *et al*, 2001). In breast cancer further research regarding relative risk estimation is required.

2.15.5. *STK1:*

Chromosome (*19p13.3*) possesses this gene termed as *STK*. Germline mutations of this gene account for (50%) of Peutz-Jeghers Syndrome (*PJS*) cases. It has been observed that patients suffering from this syndrome are more susceptible to breast, gastrointestinal and other malignancies (Giardiello *et al*, 2000).

The complex processes that characterize the development and progression of malignant tumors, the *hallmarks of cancer*, have been well described. They include self sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, unlimited explicative potential, sustained angiogenesis and tissue invasion and metastasis **(Figure 2.12)** (Hanahan & Weinberg, 2000) (plus the ability of the cancer to escape the immune response through several complex processes and events (Bhardwaj, 2007; Dunn *et al*, 2002).

Also, research on morphologic and molecular features of hereditary breast cancer, especially in patients with germline mutations in *BRCA1* (Palacios *et al*, 2008) a candidate stem cell regulator (Foulkes, 2004; Liu *et al*, 2008) has increased our understanding of breast cancer biology. Gene expression profiling studies have extended our understanding of the molecular mechanisms involved in tumorigenesis and progression of breast cancer. Basic research on genes involved in signaling pathways modulating proliferation, apoptosis, survival, angiogenesis, invasion, metastasis and drug resistance have provided more answers to the heterogeneity of breast cancer. There is increasing evidence that this heterogeneity finds its source in genetic variability (Morabito *et al*, 2003; Pavelic & Gall, 2001; Beckmann *et al*, 1997; Cuny *et al*, 2000).

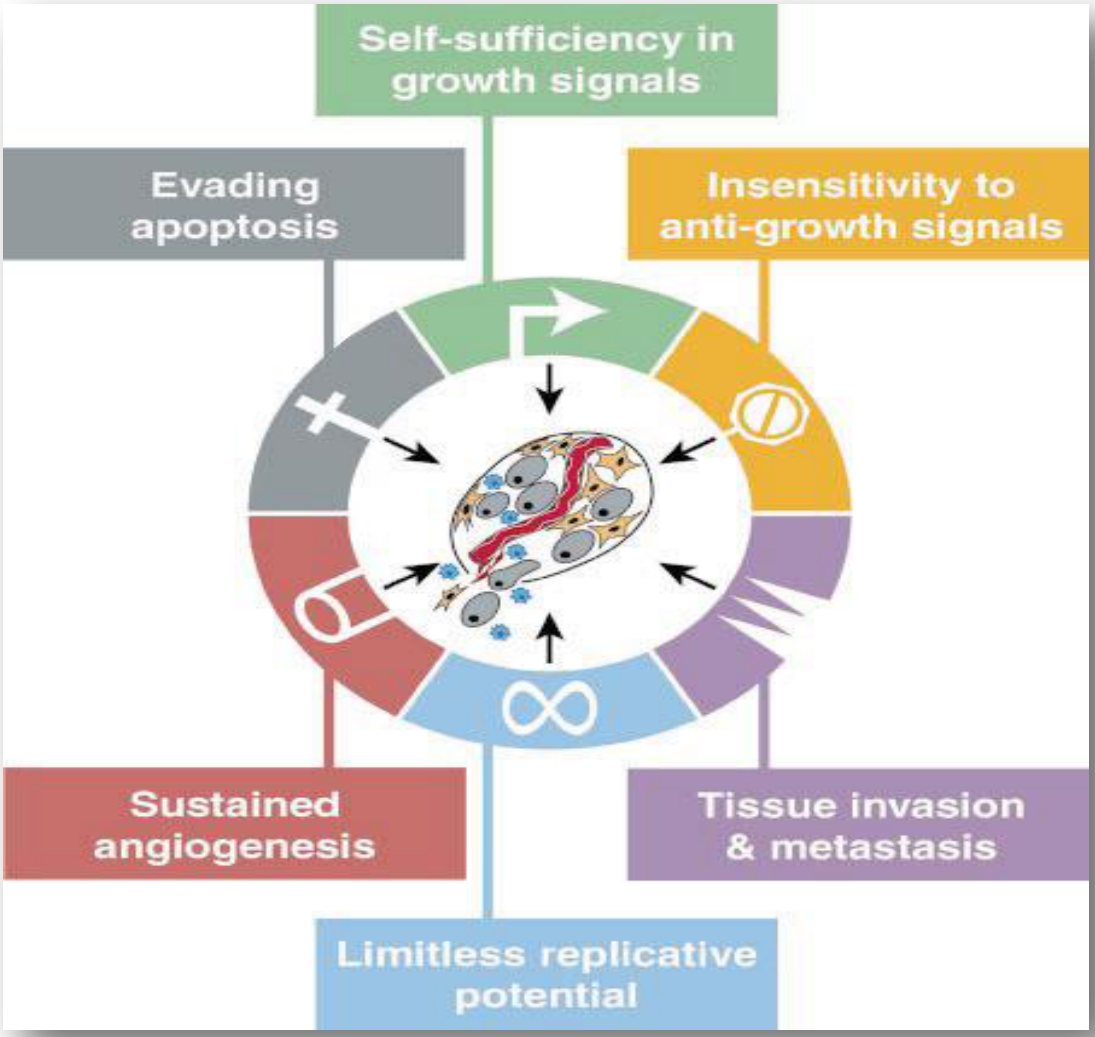

Figure 2.12: Hallmarks of cancer

(*Loss of normal growth control as a hallmark of cancer which encompasses four (self-sufficiency in growth signals, insensitive to antigrowth signals, sustained proliferation and evasion of apoptosis) of the six hallmarks of cancer as defined by Hanahan and Weinberg (2000) involve control over the cell cycle* Adapted from (Hanahan & Weinberg, 2000).

Despite the site of origin of malignancies, cells must acquire various biological traits to form a malignant tumor. Reviewed in Hanahan *et al* (2000), these biological attributes include:

i. Self-sufficiency in growth i.e. not requiring external growth signals to proliferate.
ii. Insensitivity to antigrowth signals i.e. becoming able to block the inhibition of proliferation initiated by external growth signals.
iii. The ability to evade apoptosis - the majority of cancer cells has acquired some mechanism of resistance to apoptosis.
iv. Potentially limitless replication-overcoming the intrinsic switch to senescence after numerous cell divisions.
v. Sustained angiogenesis - angiogenic properties are normally tightly regulated but essential for transition from an aberrant proliferative lesion to a larger malignant tumor entity.
vi. Ability to invade surrounding tissue and metastasize- tumors only become malignant upon invasion of surrounding tissue.

2.16. Mutation spectrum of *BRCA1/2:*

Since the identification of *BRCA1* and *BRCA2*, numerous studies have been performed worldwide and more than (1000) sequence alterations have been reported in both genes (*BIC*). Mutations are scattered throughout the large coding regions of the genes, making mutation screening challenging both technically and financially. Vast majority of reported mutations are small insertions or deletions, nonsense mutations, or alterations affecting the splice-site (*BIC*). Many missense substitutions have also been identified, but their role in carcinogenesis is much harder to establish, and therefore, most of them are classified as alterations with unknown significance (*BIC*).

The observed mutation spectrum is largely influenced by the techniques used in mutation screening. As most studies have used *PCR*-based methods, large

genomic rearrangements and regulatory mutations have been missed, and it has been estimated that only (63%) of mutations can be detected by conventional screening techniques (Ford *et al*, 1998). And indeed, several large genomic rearrangements have recently been identified particularly in *BRCA1* (Petrij-Bosch *et al*, 1997; Puget *et al*, 1997; Swensen *et al*, 1997; Puget *et al*, 1999; Rohlfs *et al*, 2000; the (*BRCA1)* exon (13) duplication screening group, 2000; Unger *et al*, 2000; Gad *et al*, 2002; Hofmann *et al*, 2002; Montagna *et al*, 2003), and to a lesser extent in (*BRCA2)* (Nordling *et al*, 1998; Wang *et al*, 2001a). High frequency of such mutations in *BRCA1* is thought to result from unusually high concentration of Alu-elements in the intronic sequences of the gene, rendering it particularly prone to Alu - mediated unequal recombination (Smith *et al*, 1996; Puget *et al*, 1997).

Most mutations are unique to the families they have been identified in, but in many populations recurrent founder mutations also exist. The founder effect is most clearly seen in Iceland, where one *BRCA2* mutation (999 del5) is estimated to account for up to (76%) of families with multiple cases of female breast cancer and/or male breast cancer (Thorlacius *et al*, 1996). Population frequency of this mutation is estimated to be about (0.5%) (Johannesdottir *et al*, 1996; Thorlacius *et al*, 1997). Strong founder effect is also seen among Ashkenazi Jews, where approximately (2.5%) of individuals carry (1) of the (3) founder mutations (*185delAG or 5382insC in BRCA1, 6174delT in BRCA2*) (Roa *et al*, 1996; Struewing *et al*, 1997; Hartge *et al*, 1999). These mutations are estimated to account for (45%) of high-risk families among Ashkenazis (Tonin *et al*, 1996). The commonness of the mutations is exemplified in one large kindred where all (3) mutations have been identified (Liede *et al*, 1998). As data on *BRCA1* and *BRCA2* mutations accumulates, recurrent founder mutations are being identified in a growing number of populations. So far these include for example French Canadians (Simard *et al*, 1994; Tonin *et al*, 1998), Austrians (Wagner *et al*, 1996), Dutch (Petrij-Bosch *et al*, 1997), Belgians (Goelen *et al*,

1999), Swedes (Hakansson *et al*, 1997), Russians (Gayther *et al*, 1997a), British (*BRCA1* exon 13 duplication screening group, 2000), Greek (Ladopoulou *et al*, 2002), African-Americans (Olopade *et al*, 2003), and Japanese, Chinese, Philippines, and Pakistanis (Liede &Narod, 2002). Founder effect is also evident in Finland, where (6) recurrent *BRCA1* and (5) *BRCA2* mutations were shown to account for the majority (84%) of *BRCA1* and *BRCA2* positive families when the whole coding regions and exon-intron boundaries were screened (Vehmanen *et al*, 1997a, b; Huusko *et al*, 1998).

2.17. Prevalence:

Early linkage studies on high-risk families indicated that up to (80 -100%) of hereditary breast cancer susceptibility was due to *BRCA1* or *BRCA2* (Easton *et al*, 1993; Narod *et al*, 1995; Wooster *et al*, 1995). A few years later in a large collaborative study where families with at least (4) cases of breast cancer diagnosed below (60) years were analyzed (52%) of the families showed linkage to or had germline mutations in *BRCA1* and (32%) in *BRCA2 (*Ford *et al*, 1998). The vast majority (81%) of families with both breast and ovarian cancer were linked to *BRCA1*, whereas families with male breast cancer cases were mainly (76%) due to *BRCA2*. Interestingly, substantially smaller frequencies were observed in families with (4) or (5) female breast cancer cases but no ovarian cancer or male breast cancer as only (32%) and (9%) of such families were linked to *BRCA1* or *BRCA2*, respectively (Ford *et al*, 1998).

The prevalence of *BRCA1* and *BRCA2* germline mutations has been extensively studied in breast and/or ovarian cancer families in different populations. Altogether, it seems that germline mutations in *BRCA1* and *BRCA2* account for about (20-50%) of hereditary breast cancer families a fraction substantially smaller than originally thought, and the proportion appears to vary a great deal between different populations.

Mutation screening of *BRCA1* and *BRCA2* is challenging due to the wide mutation spectrum and large size of the genes. It is therefore not surprising that only a few studies have reported estimates of the mutation frequencies among unselected breast cancer patients. The largest studies have been carried out in Ashkenazi Jewish and Icelandic populations where individual, highly recurrent founder mutations have been found in (7-12%) and (8-9%) of unselected breast cancer patients, respectively (Johannesdottir *et al*, 1996; Thorlacius *et al*, 1997; Fodor *et al*, 1998; Warner *et al*, 1999). The specific nature of such very recurrent founder mutations, and the presence of possible genetic or environmental modifying factors in those particular, isolated populations, mean that the results may not be used to estimate the contribution of *BRCA1* and *BRCA2* to breast cancer incidence in other populations. In some other reports patients have been unselected for family history, but the focus has been on early-onset disease (FitzGerald *et al*, 1996; Langston *et al*, 1996; Krainer *et al*, 1997; Malone *et al*, 1998; Hopper *et al*, 1999; Peto *et al*, 1999; Southey *et al*, 1999). Mutation frequencies in these studies have varied from (1.9%-13%) for *BRCA1*and (2.2-2.7%) for *BRCA2*. In a few studies where patients have been unselected both for family history and age at diagnosis the sample size has been quite small (130-211) and only *BRCA1* has been analyzed (Newman *et al*, 1998; Tang *et al*, 1999). The mutation frequencies in these studies have varied between (1.4-3.8%). Taken together, in many populations the contribution of *BRCA1* and *BRCA2* to breast cancer burden remains to be determined.

A complete list of the seven founder mutations can be found in (**Table 2.1).** These mutations are so common that often individuals of French Canadian ancestry initiate testing of the *BRCA* genes with a panel of the founder mutations and if negative then consider reflex to complete evaluation of the genes (Culver *et al*, 2006, 2007**)**.

Table (2.1): Selected examples of recurrent and founder mutations in the *BRCA* genes (Culver *et al*, 2006, 2007**).**

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

2.18. Immunohistochemical and molecular profile of *BRCA1* & *BRCA2* associated tumors:

2.18.1. Steroid hormone receptors:

Since the discovery of the (*ER*) in (1960), it has become one of the most important prognostic and predictive markers for breast cancer (Osbome, 1998). It is well known that (*ER)* expression is inversely correlated with tumor grade (Henderson & Patek, 1998). *BRCA*- associated tumors, which are more often of higher grade than that of sporadic breast cancer, would therefore be predicted to be more often (*ER) negative.* Indeed, numerous studies (Osien *et al*, 1998; Osien, 1998; Johannson *et al*, 1997; Armes *et al*, 1999) have shown low levels of (*ER*) expression in familial breast cancers. Osien *et al* (Osien *et al*, 1998; Osien, 1998) have shown that when (*ER*) was assessed in *BRCA* associated tumors in comparison with a grade-matched control group, the expression of (*ER)* in *BRCA1*-associated tumors was still significantly lower (8 versus 26%). In contrast, the expression of (*ER)* in *BRCA2*-associated tumors appears to be similar to that in sporadic breast cancers.

The detection of (*ER)* immunohistochemically (*IHC*) does not necessarily reflect its functional competence, and a certain proportion of tumors that express (*ER)* are known to be resistant to antiestrogen therapy. The function of (*ER*) is dependent on the ability to transactivate so-called (*ER)* dependent genes. Expression of progesterone receptor (*PgR*) and (*PS2*) protein is indirect evidence of retained transcriptional activation activity of (*ER),* and it has been shown that (*PgR)* and (*PS2*) expression have stronger correlation with prognosis in breast cancer than (*ER*) expression alone (Loakim *et al*, 1997).

Osien *et al* (Osien *et al*, 1998) showed that, although nine out of (40) familial breast cancer patients were (*ER*) positive, only two of these were also (*PgR*) positive. This suggests that even in cases where (*ERs*) could be identified immunohistochemically, their functional ability may be compromised. Another

intriguing question is at what stage of progression do tumors become hormone independent? It has been demonstrated that both the invasive and in-situ component in *BRCA*-associated tumors have a similar status of steroid hormone receptor expression, suggesting that loss of hormonal response is a relatively early event in progression of these tumors (Osien *et al*,1998; Osien, 1998). These data strongly suggest resistance to antiestrogen therapy of *BRCA*-associated tumors (*BAT*), and undoubtedly could have very serious practical implications in view of proposed antiestrogen prophylaxis for patients from high-risk families.

2.18.2. HER-2/neu:

A large number of studies have been performed on the functional role of *c-erbB2* oncogenes (*HER-2/neu*) in breast cancer. (*HER-2/neu*) product is a tyrosine kinase receptor that belongs to the same family as epidermal growth factor receptor. It is overexpressed in approximately (20-30%) of high-grade invasive breast cancers and has been shown to be a valuable prognostic indicator. (*HER2/neu)* status also predicts response to antiestrogen and cytotoxic chemotherapy. Antibodies directed against the (*HER2/neu)* protein have attracted a lot of attention recently because of the availability of the monoclonal antibody herceptin for treatment of breast cancer (Ross &Eletcher, 1999). Clearly, the role of (*HER2/neu*) in familial breast cancer is of interest.

Data on (*HER2/neu*) are limited and conflicting. Armes *et al* (Armes *et al*, 1999) and Robson *et al* (Robson *et al*, 1998) have not shown a difference in (*HER2/neu*) expression between sporadic and familial cancers. The study by Johannsson *et al* (Johannsson *et al*, 1997), however, demonstrated that (*c-erbB2)* expression in *BRCA1*-associated cancers is lower than would be predicted on the basis of their histological grade. Data from a large Breast Cancer Linkage Consortium (*BCLC)* study are awaited to clarify this issue.

2.18.3. *TP53:*

P53 protein is one of most important guardians of stability and integrity of the genome, and acts to prevent cell proliferation after *DNA* damage and activates apoptosis in case of unrepairable damage. Mutations in the *TP53* gene are the most common genetic alterations in human cancers and are encountered in (20-40%) of sporadic breast cancers.

The frequency of these mutations correlates with tumor grade. Detection of *p53* protein by immunohistochemistry has become a routine method in pathology practice, and the presence of detectable *p53* protein is an important prognostic marker that correlates with higher histopathological grade, increased mitotic activity, aggressive tumor behavior and therefore a worse prognosis (Rudolph *et al*, 1999; Elledge &Allred, 1998). Using immunohistochemistry, Crook *et al* (Crook *et al*, 1998) reported that *BRCA*-associated tumors were more often *p53* positive than were grade-matched sporadic breast cancers (77% *BRCA1*, 45% *BRCA2*, and 35% sporadic).

Further evidence for an important role for *p53* in familial breast cancer comes from the detection of mutations at a higher frequency than in sporadic cancers. The mutations in *BRCA*-associated cancers were often multiple and their locations unusual, which is marked contrast to sporadic cancer (Crook *et al*, 1998; Crook, 1997).

Studies of *TP53* gene function in *BRCA* tumors have been performed using *in vitro* models. These show that the identified mutants are unique not only in their number and location, but also in their function. The mutants retain some of the wild *p53*-dependent activities, such as transactivation, suppression of proliferation and apoptosis induction (in particular via *PIG3* transactivation). At the same time, these mutants fail to suppress transformation and they exhibit gain of function (Smith *et al*, 1999).

In sporadic breast cancer an inverse correlation between loss of *p53* expression and high proliferation index on one side, and low expression of the antiapoptotic gene *BCL2* on the other hand has been demonstrated. Surprisingly two studies (Armes *et al*, 1999; Robson *et al*, 1998) have shown that *BRCA1*/*BRCA2* tumors have the same level of *BCL2* expression as the control group, despite being highly proliferative and with frequent *p53* mutations. Clearly the regulation of both cell cycle and apoptosis is multifactorial and relatively high expression of antiapoptotic *BCL2* is probably one of the mechanisms of tumor survival in conditions where apoptosis- inducing genes are still transactivated by mutant *p53*.

2.18.4. Cell cycle proteins:

The cyclin- dependent kinase inhibitor *p21* blocks transition from (*G1)* to (*S)* phase and suppresses cell proliferation. The *p21* is thought to be major downstream effectors of the wild-type *p53*-mediated growth arrest pathway that is induced by *DNA* damage. In sporadic breast tumors the expression of *p21* is inversely correlated with *p53* expression and high tumor grade (Elledge & Allred, 1998; McClelland *et al*, 1999). The proposed explanation is that mutated *p53* is unable to activate *p21* transcription. Immunohistochemical studies (Crook *et al*, 1998) have failed to demonstrate a relationship between *p21* and *p53* in *BRCA1*/*BRCA2* tumors, suggesting that *p21* transactivation in this group could be mediated by a *p53*-independent mechanism. This finding could be of practical significance, because there is an increase in *p21* expression and apoptosis in cells with wild-type *p53* exposed to chemotherapy (el-Deiry *et al*, 1994).

Another cyclin-dependent kinase complex inhibitor that plays an important role in breast cancer pathogenesis is *p27*. There are reports that patients whose tumors overexpress *p27* have significantly higher survival rates. In small breast

cancers (stages T1a & b) *p27* expression was reported as the only independent prognostic factor (Tan *et al*, 1997).

Data regarding *p27* expression in familial *BRCA*-associated breast cancer are scarce and contradictory. Robson *et al* (Robson *et al*, 1998) reported that *p27* expression does not differ between sporadic and *BRCA*-associated cancers. This is contrary to our own observations, however, where we found *p27* to be over expressed in *BRCA1*/*BRCA2* breast cancers (86% in familial tumors versus (65%) in sporadic tumors; Osien PP, unpublished observations). Some studies (Mohammed *et al*, 1998) have demonstrated a better prognosis for familial breast cancer. If this is substantiated (and there is evidence that this may not be correct (Robson *et al*, 1998), the over expression of *p27* could be one possible explanation.

Cyclin *D1* is a regulator of progression from (*G1)* to (*S*) phase in cell cycle. It represents an important part of hormonal regulation of mammary epithelium growth: cyclin *D1* is known to be up regulated by estrogen and progestin and to be down regulated by antiestrogens (Gillett, 1998). The transcription of *ER*-regulated genes is also modulated by cyclin *D1* (McMahon, 1999; Neuman *et al*, 1997). Overexpression of cyclin *D1* is a common event in breast cancer and is especially frequent in early onset breast cancer, probably because of high levels of estrogens in this age group (Barnes &Gillett, 1998). *BRCA1*/*BRCA2*-associated tumors show very low expression of cyclin *D1* in both the invasive and in-situ components, however (14% in both invasive and ductal carcinoma *in situ* components in *BRCA1*/*BRCA2* tumors, versus (35-36%) in invasive ductal carcinoma *in situ* in sporadic tumors) (Osien *et al*, 1998) .

Taken together with the absence of (*ER)* and (*PgR*) in *BRCA1*/*BRCA2* cancers, the absence of cyclin *D1* in these tumors could be additional evidence of hormone independence of *BRCA*-associated familial breast cancers.

2.18.5. Miscellaneous markers:

Disruption of normal regulation of the cell cycle leading to increased mitotic activity is a manifestation of tumourigenesis. In addition to traditional counting of mitotic figures, a number of immunohistochemical methods have become a part of routine tumor investigation. A high proliferative index in *BRCA*-associated tumors compared with a grade matched control group has been demonstrated (Breast Cancer Linkage Consortium, 1997; Lakhani, 1998), and recent studies (Armes *et al*, 1999) have confirmed it by demonstrating high *Ki67* labeling index (83 versus 48%). Cathepsin *D* belongs to a family of proteases that are involved in the tissue remodeling. Overexpression of cathepsin *D* in breast cancer has been found to correlate with poor prognosis (Duffy, 1996; Rochefort & Liaudet-Coopman, 1999; Fulco, 1998) and the expression of cathepsin *D* in host stromal cells is associated with higher intratumoural microvessel density. Expression of cathepsin D in *BRCA1*/*BRCA2*-associated breast cancers was not found to be different from that in sporadic breast cancers in two studies (Armes *et al*, 1999; Robson *et al*, 1998).

E-cadherin, an epithelial adhesion molecule is mutated at high frequency in invasive lobular carcinoma. Epidemiological studies have shown a link between familiality and lobular carcinoma, although this does not appear to be a phenotype of either *BRCA1* or *BRCA2* cancer. Investigation of *E*-cadherin expression in familial breast cancer is limited, and there is no evidence at present that there are any significant differences between familial and sporadic cancers (Armes *et al*, 1999).

2.19. Cell cycle regulators and proliferation:

The cell is a highly organized and complex process comprised of a series of tightly controlled events that drives the replication of *DNA* and ensures correct cell division. Cells are normally in the resting phase (*G0*), and after appropriate

stimuli they enter the proliferative phases of the cell cycle which is made of four phases; (*G1*), (*S*), (*G2*) and (*M*) phases **(Figure 2.13).**

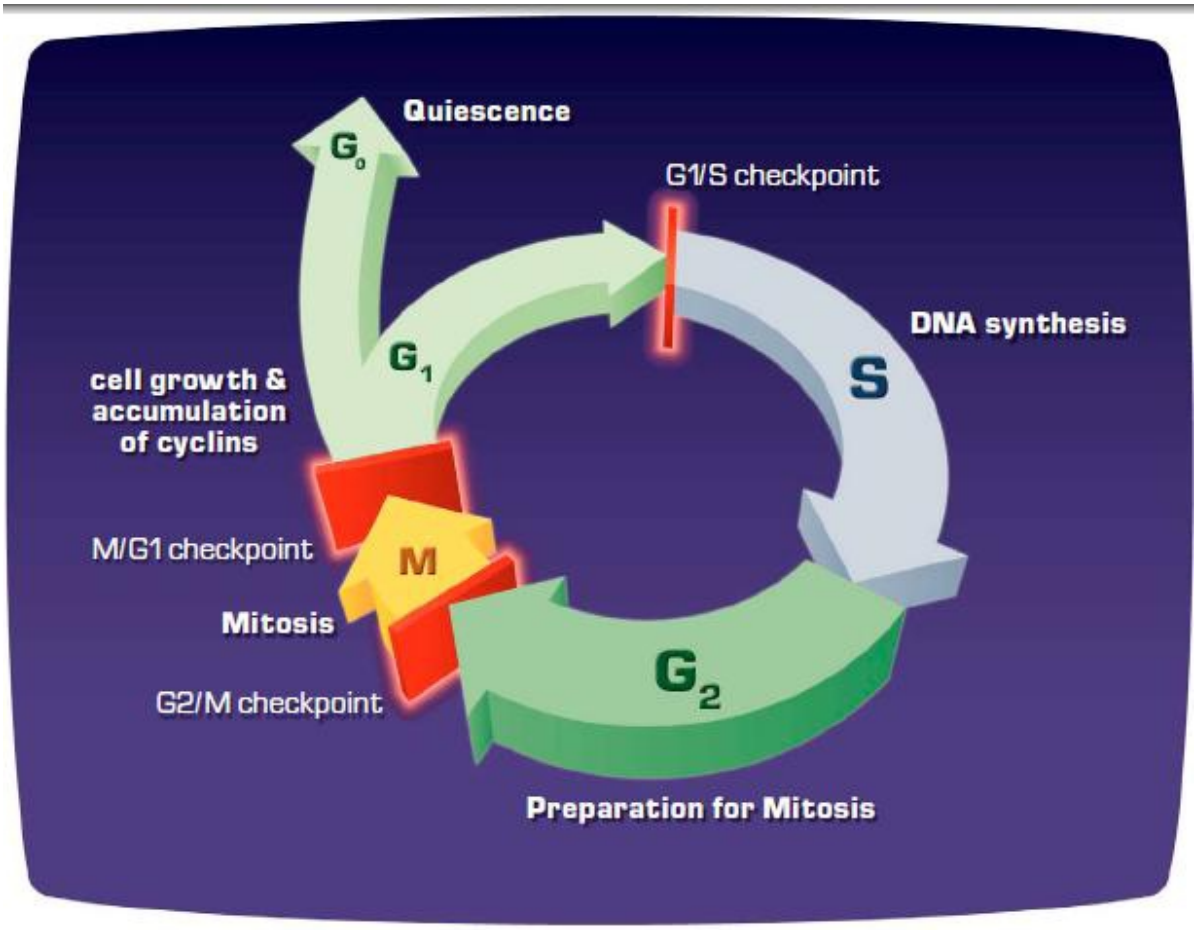

Figure 2.13: The stages of the cell cycle

The cell cycle is an ordered process of events that occurs in four stages. During the two gap phases, G1 and G2, the cell is actively metabolizing but not dividing. In S (synthesis) phase, the chromosomes duplicate as a result of DNA replication. During the M (mitosis) phase, the chromosomes separate in the nucleus and the division of the cytoplasm (cytokinesis) occurs. There are checkpoints in the cycle at the end of G1 and G2 that can prevent the cell form entering the S or M phases of the cycle. Cells that are not in the process of dividing are in the G0 stage, which includes most adult cells (Vermeulen *et al*, 2003).

In the (*G1)* phase, the cell is in a preparation for the (*S)* phase, in which *DNA* synthesis occurs followed by a second gap phase (*G2*) in preparation for the phase (*M*) in which the cell undergoes mitosis to generate (2) diploid (*G0)* cells which may reenter the cell cycle or persist in the resting phase (Fernandez *et al*, 1998; Gillett & Barnes, 1998). Cells are stimulated to divide in response to numerous external signals, including growth factors, hormones and cellular adhesion (Fernandez *et al*, 1998; Gillett & Barnes, 1998; Michalides, 1999). During the (*G1)* phase of the cell cycle, cells are responsive to the external stimuli and are dependent on them until they reach the restriction point (*R*). This is a point of no return beyond which the cell is committed to enter the cell cycle and there after the process becomes autonomous (Michalides, 1999).

The transition through the cell cycle phases is mediated by sequential assembly and activation of a family of serine/threonine proteins, the cyclin dependent kinases (*CDK*; *CDK1*, *CDK2*, *CDK4*, *CDK6* and *CDK7*) and the (*CDK)* inhibitors (*CKI*; *INK4* family: *p15*, *p16*, *p18*, *p19*; *Cip*/*Kip* family: *p21*, *p27*). The (*CKI)* are regulated by both internal and external signals such as the (*TP53)* tumor suppressor gene and Transforming Growth Factor (*TGF*).

Control of cell proliferation in the normal cell cycle has several check-points **(Figure 2.13)** to ensure an orderly sequence of events in the cell cycle as well as complete and accurate replication of the cell before division (Gillett &Barnes, 1998). Of these, the *DNA* damage check points (*G1*/*S* and *G2*/*M*) are well elucidated. Although it appears that oncogenic defects may target any major check-point, the most frequently involved is the (*G1*/*S)* transition, and it encompasses many of the important cell cycle events that may be specifically altered in breast cancer including actions of the oncogenes (such as cyclin *D1* and cyclin *E*) and tumor suppressors (such as *p27*).

Mammary gland is steroid hormone dependent, and it involves complex interactions with other hormones, growth factors and cytokines as well as proto-oncogenes (*c-myc*, cyclin *D1* and cyclin *E1*). Proliferation is essential for tissue

turn over but it exposes the cell to the occurrence of *DNA* damage (Gompel *et al*, 2004). Cell proliferation plays an important role in the clinical behavior of breast carcinoma (van *et al*, 2004) and it is a significant prognostic factor in breast cancer. Tissue hemostasis results from the balance between cell proliferation, differentiation and death in the form of apoptosis. An imbalance between cell proliferation and apoptosis contributes to tumorigenesis and tumor progression (De Jong *et al*, 1998).

2.20. Tumors arising in *BRCA1* and *BRCA2* mutation carriers:

Several distinctive histopathological features have been associated with breast tumors arising in patients with *BRCA1* mutations, suggesting that the pathogenesis of these tumors may differ from that of sporadic cases. Breast tumors in *BRCA1* - carriers are highly proliferating, frequently aneuploid, poorly differentiated, have more nuclear pleomorphism, and show higher histologic grade and more lymphocytic infiltration than sporadic tumors (Marcus *et al*, 1996; Johannsson *et al*, 1997; Breast Cancer Linkage Consortium, 1997; Armes *et al*, 1998; Noguchi *et al*, 1999). Overrepresentation of medullary or atypical medullary breast carcinomas is also frequently observed (Marcus *et al*, 1996; Breast Cancer Linkage Consortium, 1997; Armes *et al*, 1998; Eisinger *et al*, 1998; Verhoog *et al*, 1998). Somatic (*p53)* alterations and overexpression of (*p53)* protein are common in (*BRCA1)*- tumors, whereas they are more often negative for estrogen and progesterone receptor and show low expression of (*ERBB2)* (Johannsson *et al*, 1997; Armes *et al*, 1999; Noguchi *et al*, 1999 ; Lakhani *et al*, 2002). Interestingly, frequent allelic loss of *BRCA1* in *BRCA2* - associated tumors and vice versa has also been observed (Staff *et al*, 2001). In contrast to tumors in *BRCA1* - carriers, *BRCA2*- associated breast tumors appear to be more heterogeneous and show no characteristic clinicopathological features compared to sporadic tumors (the Breast Cancer Linkage Consortium, 1997; Armes *et al*, 1999; Noguchi *et al*, 1999). Both *BRCA1* and *BRCA2*

associated cancers are diagnosed at younger age and include higher incidence of bilateral tumors than sporadic cases (Easton *et al*, 1993, 1995; Ford *et al*, 1994; Miki *et al*, 1994; Wooster *et al*, 1994; Marcus *et al*, 1996; Johannsson *et al*, 1997; Verhoog *et al*, 1998; Noguchi *et al*, 1999; Hamann & Sinn, 2000).

By using comparative genomic hybridization, Tirkkonen *et al* (1997) observed that the total number of somatic genetic alterations in tumors from *BRCA1*and *BRCA2 carriers* was nearly double of that seen in control tumors. This supports the proposed role of *BRCA1* and *BRCA2* in maintaining genomic integrity. However, the changes in *BRCA1*- tumors differed from those in *BRCA2*- tumors. In (*cDNA*) microarray analysis significant differences in gene expression profiles were observed between *BRCA1*, *BRCA2* , and sporadic breast tumors, further supporting the molecular difference between cancers with underlying germline *BRCA1* and *BRCA2* mutations as well as cancers without such mutations (Hedenfalk *et al*, 2001).

2.21. Preventive measurements to reduce breast cancer risk in *BRCA1/2* mutation carriers:

Individuals carrying mutations in *BRCA1/2* have a (45-80%) chance of developing breast cancer. There is evidence that strategies to reduce the risk of cancer in populations who carry these mutations are effective (Peters *et al*, 2005), therefore it is important to identify those patients who will benefit from genetic testing. Women with a *BRCA1* or *BRCA2* mutation may consider several options for breast cancer prevention. The (3) main options are prophylactic mastectomy, prophylactic oophorectomy, and chemoprevention.

The goal of prophylactic mastectomy is to prevent breast cancer, thereby eliminating the potential for metastatic spread and death from the disease. Studies suggest that the residual breast cancer risk after mastectomy is minimal (< 5%), and much less than the risk of breast cancer in the general population (Hartmann *et al* , 2001; Meijers *et al*, 2001; Rebbeck *et al*, 2004).

It has been shown that *BRCA1*- associated triple negative breast cancers are hormonally associated (Narod, 2002). The purpose of an anti-hormonal therapy is to eliminate or block the effect of ovarian estrogen, and probably progesterone, or to prevent aromatization of androgen to estrogen. Tamoxifen and oophorectomy have been well studied in women with *BRCA1* or *BRCA2* mutations. Cohort studies estimate the reduction in hereditary breast cancer risk associated with a premenopausal oophorectomy to be about (50% ; Kauff *et al*, 2002; Rebbeck *et al*, 1999; Rebbeck *et al*, 2002).

Tamoxifen is a selective estrogen receptor modulator (*SERM*) which competes with estrogen for binding to the estrogen receptor. In humans, tamoxifen acts as an estrogen antagonist in breast tissue, inhibiting the growth of estrogen-dependent breast tumors (Pritchard, 2001). On theoretical grounds, tamoxifen should not reduce the incidence of estrogen-receptor (*ER*) negative breast cancers, and most breast cancers that occur in *BRCA1* but not *BRCA2* carriers are (*ER*) negative.

More recently, Poly (*ADP-ribose*) polymerase (*PARP*) inhibitors were found as a promising new class of targeted agents for the treatment of patients with various malignancies (breast, ovarian and prostate cancer) (Fong *et al*, 2009). The (*PARP)* enzyme plays a key role in the repair of *DNA* breaks. Evidence suggests that normal cells can tolerate (*PARP)* inhibitors by activating backup repair pathways that depend upon the activity of *BRCA1* and *BRCA2* proteins. Though there is widespread enthusiasm to move these drugs forward quickly, much remains to be understood about the optimal use of the novel agents.

2.22. *BRCA1* and *BRCA2* in sporadic breast cancer:

Soon after identification, it became evident that somatic mutations in neither *BRCA1* nor *BRCA2* play a major role in the tumorigenesis of sporadic breast cancer (Futreal *et al*, 1994; Lancaster *et al*, 1996; Miki *et al*, 1996; Teng *et al*, 1996). In subsequent years the observation has been confirmed, with reports on

such acquired inactivating mutations being extremely rare (Weber *et al*, 1999 Papa *et al*, 1998; Khoo *et al*, 1999; van der Looij *et al*, 2000). Still, *BRCA1* and *BRCA2* may affect the pathogenesis of non-familial breast carcinoma through other, more indirect mechanisms.

Both protein products may be subcellularly mislocalized into the cytoplasm, and thereby turned nonfunctional (Chen *et al*, 1995b; Spain *et al*, 1999). Marked reduction in *BRCA1* expression level has also been observed in sporadic breast tumors (Thompson *et al*, 1995; Yoshikawa *et al*, 1999), with correlation to tumor-grade (Taylor *et al*, 1998; Wilson *et al*, 1999), proliferation (Jarvis *et al*, 1998), and metastatic potential (Taylor *et al*, 1998; Seery *et al*, 1999). Possible mechanisms that may lead to low *BRCA1* expression include (*LOH*), promoter hypermethylation, regulation of protein stability; allele specific expression, (*p53*) or (*ID4)* mediated repression, activation of the negative regulatory site in the first intron, loss of activation by transcription factors, or activation of (*NBR2)* expression (reviewed in Mueller & Roskelley, 2002). In *BRCA2*, no methylation of the putative promoter region has been observed (Collins *et al*, 1997), but there are reports on frequent (*LOH*) (Cleton-Jansen *et al*, 1995; Beckmann *et al*, 1996; Hanby *et al*, 2000; Johnson *et al*, 2002).

BRCA1/*2* have been shown to serve as important central components in multiple biological pathways that regulate cell-cycle progression, centrosome duplication, *DNA* damage repair, cell growth, and apoptosis (Deng & Brodie, 2000). Evidence that the loss of *BRCA1* alleles or low expression of *BRCA1* in a large proportion of sporadic breast cancer cases supports the role of *BRCA1* in the development of sporadic breast cancer (Serry *et al*, 1999; Wilson *et al*, 1999; Yoshikawa *et al*, 1999).

BRCA1 and *BRCA2* may also themselves function normally in sporadic cancer, but mutations in other components in their pathways may lead to malignant transformation (Rahman & Stratton, 1998). So far, the extent of which *BRCA1*

and/or *BRCA2* contribute to the pathogenesis of sporadic breast cancer remains unclear.

2.23. *BRCA1* & *BRCA2* Function in the *DNA* damage response:

The ability to precisely control the order and timing of cell cycle events is essential for maintaining genomic integrity and preventing mutations able to disrupt normal growth controls. Cells exposed to *DNA* damaging agents, such as ionizing radiation, coordinately arrest the progression of the cell cycle at the *G1*/*S* phase, the *S* phase and the *G2*/*M* phase to allow adequate time for damage repair (Elledge, 1996; Kastan & Bartek, 2004). It is now widely accepted that both *BRCA1* and *BRCA2* play multiple critical roles in the maintenance of genome stability as evidenced by a profound number of chromosomal translocations, duplications, and aberrant fusion events between non-homologous chromosomes in *BRCA1* and *BRCA2* deficient cells (Venkitaraman, 2002; Moynahan *et al*, 2001; Patel *et al*, 1998).

2.23.1. *BRCA1* Function in *DNA* damage repair:

Recent studies reveal that *BRCA1* plays essential roles in *HR* repair, *NHEJ*, and nucleotide excision repair (*NER*) (Deng &Wang, 2003); *BRCA1* mediates these functions through interaction with components of the *DNA* repair machinery, and by regulating the expression of genes involved in the *DNA* damage repair pathways (Deng & Wang, 2003). Studies suggest that *BRCA1* carries out these roles in damage repair by acting as a link between the sensing and effecting components of the *DNA* damage response (Boulton, 2006; Moynahan *et al*, 2001).

BRCA1 plays a critical role in responding to *DSBs* through its function in *HR*. In ways still to be defined, *BRCA1* recruits *BRCA2*, which facilitates *Rad51* filament formation on the *ssDNA* (Zhang & Powell, 2005). *Rad51* catalyzes the invasion of the homologous sequence on the sister chromatid, which is then used

as template for accurate repair of the broken *DNA* ends (Powell *et al*, 2002; Valerie & Povirk, 2003). Other studies have shown that *BRCA1* co-localizes with *Rad50*, a member of the *MRN* complex, following the induction of *DNA* damage; *Mre11* encodes nuclease activity which resects flush ends of *DSBs* to generate *ssDNA* tracts (Haber, 1998). *BRCA1* binds *DNA* directly and inhibits this *Mre11* activity regulating the length and the persistence of *ssDNA* generation at sites of *DNA* damage (Paull *et al*, 2000). As *ssDNA* is a substrate for *DNA* repair by *HR*, it appears that *BRCA1* might play an essential role in *HR*-mediated repair of *DSBs* through its inactivation of *Mre11*; an idea confirmed by the observation the *HR* is defective in *BRCA1*-deficient cells (Moynahan *et al*, 1999).

In addition to its somewhat unclear roles in *DSB* repair, *BRCA1* has also been found to be a constituent member of a large nuclear protein complex named the *BRCA1*-associated surveillance complex (*BASC*). This complex contains the *MRN* complex, *DNA*-mismatch repair proteins *MSH2*, *MLH1* and *MSH6*, *DNA* helicase *BLM*, *ATM*, *RFC* and *PCNA* (Wang *et al*, 2000). Many of these proteins are involved in the sensing and repair of abnormal *DNA* structures, and have been linked with the repair of replication-associated *DNA* damage (Wang *et al*, 2000). Taken together this evidence suggests that *BRCA1* might function as a coordinator of multiple processes required for the maintenance of genome integrity during the process of *DNA* replication and *DNA* - replication associated repair (Gundmundsdottir & Ashworth, 2006).

2.23.2. *BRCA2* Function in *DNA* Damage Repair:

BRCA1 and *BRCA2* co-localize with *Rad51* to form *DNA* repair complexes (Scully *et al*, 1997). This co-localization of the *BRCAs* with *Rad51* at sites of recombination and *DNA* damaged-induced foci strongly suggests that the *BRCA* proteins play a role in the detection of *DSBs* (Scully *et al*, 1997). The roles played by *BRCA1* and *BRCA2* in the repair of *DSBs* by *HR* appear to differ, as

evidence indicates a more direct role for *BRCA2*. The physical interaction between *BRCA2* and *Rad51* is essential for *HR* repair of *DSBs* to take place; *BRCA2* is thought to be required for the transport of *Rad51* from its site of synthesis to the site of *DNA* damage, where *Rad51* is then released to form the nucleoprotein filament required for *HR* to take place (Zhang & Powell, 2005). Their interaction is mediated by the *BRC* repeats, and an unrelated domain located at the C- terminus of the *BRCA2* protein (Wong *et al*, 1997; Davies *et al*, 2001). In *BRCA2* defective cells, a reduction in the accurate repair of *DSBs* by *HR* is observed, along with an increase in the number of deletion events (Moynahan *et al*, 2001). These deletions are thought to arise predominantly due to the shunting of *DSB* repair down the *SSA* repair pathway (Moynahan *et al*, 2001). These cells also show an elevated sensitivity to *IR*, while the cell cycle checkpoint and apoptotic responses to *DNA* damage remain intact (Moynahan *et al*, 2001; Yu *et al*, 2000). Thus, while the function of *BRCA2* in *HR* is well characterized it remains to be seen whether *BRCA2* participates directly in cell cycle regulation via checkpoint function, or whether it simply plays an indirect role in activating cell cycle checkpoints through a deficiency in the repair of *DNA* lesions (Yoshida & Miki, 2004).

2.23.3. Functions of *BRCA1* & *BRCA2*:

Both *BRCA1* and *BRCA2* are involved in maintaining genome integrity at least in part by engaging in *DNA* repair, cell cycle checkpoint control and even the regulation of key mitotic or cell division steps (caretakers). Unsurprisingly, the complete loss of function of either protein leads to a dramatic increase in genomic instability. A summary of the involved pathways and protein - protein interactions is given in **(Figure 2.14).** The (*RING)* domain at the amino terminus of *BRCA1* mediates the interaction with (*BARD1)*. The (*BRCA1*/*BARD1)* heterodimer shows ubiquitin ligase activity (Chen *et al*, 2002; Wu *et al*, 2003). The central region interacts with the (*DNA)* repair protein complex (*MRN)*,

(*Mre11-Rad50-NBS1*) and the (*ZBRK1*) transcriptional repressor (Zheng *et al*, 2000; Zhong *et al*, 1999). The (*Mre11-Rad50-NBS1)* complex binds to and processes (*DNA)* double stranded breaks. This complex is involved in both nonhomologous end joining and homologous recombinational repair. The carboxyl terminus of *BRCA1* contains tandem (*BRCA1) C-terminal* (*BRCT*) repeats. This region binds to phospho-peptides (Manke *et al*, 2003; Yu *et al*, 2003) involved in cell-cycle checkpoints and *DNA* repair. Several proteins including (*BACH1)*, (*CtIP)*, (*Acetyl-CoA carboxylase*), (Abraxas/*CCDC98)*, and (*RAP80)* interact with the *BRCT* domain of *BRCA1* in a Phospho - dependent manner (Rodriguez & Songyang, 2008). This reveals how *BRCA1* maintains genomic stability through (*DNA)* repair and cell cycle checkpoint activation.

Three *BRCA1* protein complexes have been characterized. One complex contains (*BRCA2)* and (*PALB2)*, a (*BRCA2)* - associated protein (Sy *et al*, 2009; Zhang *et al*, 2009). The *BRC* domain in the middle third of *BRCA2* binds to the *Rad51* recombinase (Chen *et al*, 1998; Wong *et al*, 1997). (*BRCA2)* - Deficient cells show defective formation of (*IR)* - induced (*Rad51)* foci (Yuan *et al*, 1999). Both the formation of (*Rad51)* nuclear filaments and (*Rad51)* - mediated strand exchange during homologous recombination are regulated by *BRCA2* (Thorslund *et al*, 2007).

At the carboxyl terminus of (*BRCA2*) is a region with extensive secondary structure that interacts with the evolutionarily conserved protein (*DSS1)*. Based on the 3-D structure, it is predicted that the high-affinity (*ssDNA)* - binding and (*dsDNA)*- binding domains of (*BRCA2)* play critical roles in homologous recombination (Yang *et al*, 2002).

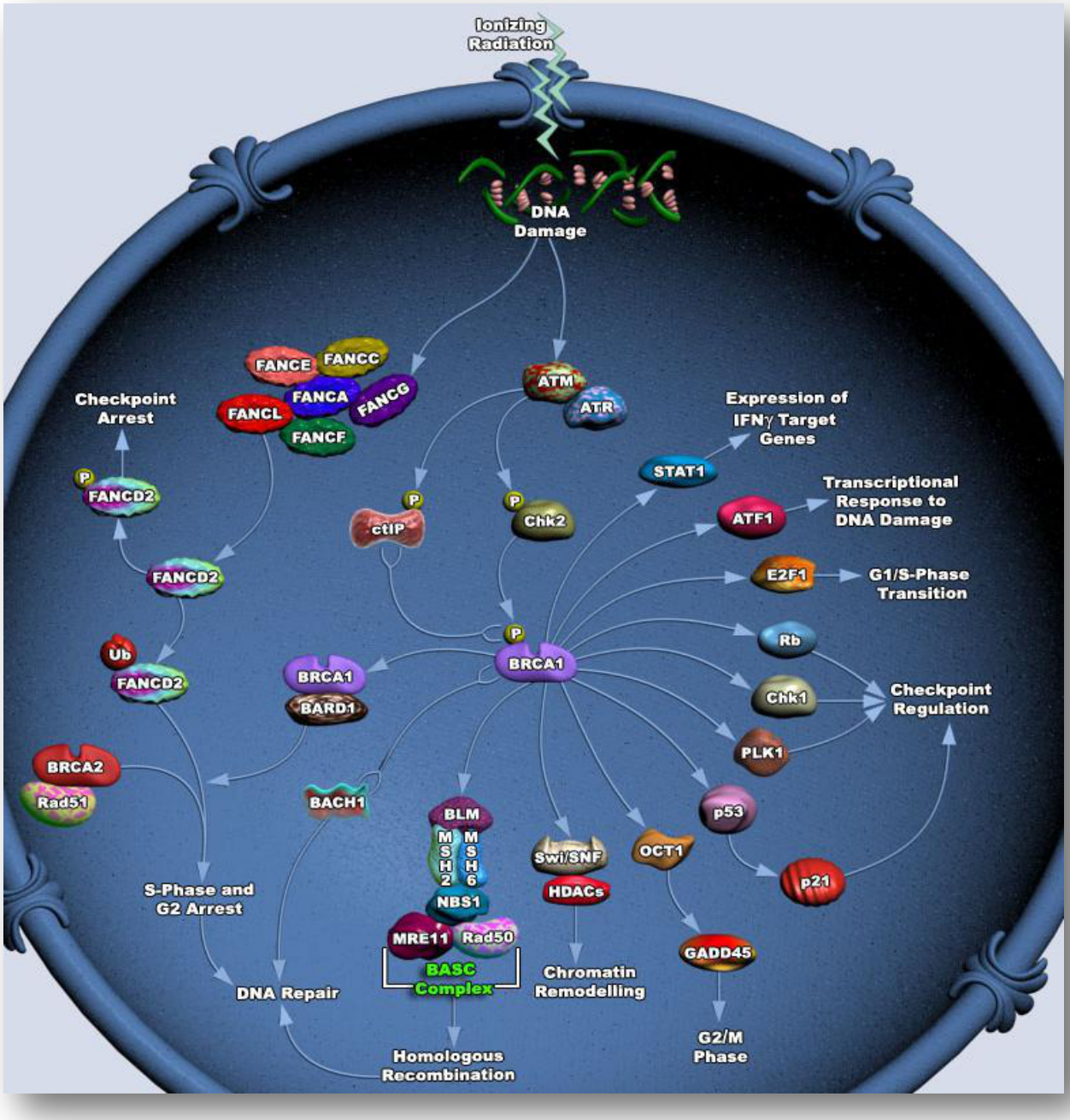

Figure 2.14: Overview of the pathways used by *BRCA1/2* to maintain cell integrity *(https://www.qiagen.com).*

2.24. Mutation detection methods:

The basic principles involved in these techniques are discussed below:

2.24.1. Allele specific oligonucleotides (*ASO*):

This method is used to detect one or more known specific mutations in population. It makes use of nucleotide probes which are short and specific for particular *DNA* sequences. This is a simpler and less expensive approach than searching for any new mutation in family members. This type of mutation detection technique is more popular in Ashkenazi Jewish (*AJ*) population and other founder mutations. The major disadvantage of this technique is that it uses radioactive material and can miss other mutations if present.

2.24.2. Protein truncation test (*PTT*):

Most *BRCA1* and *BRCA2* mutations result in short or truncated protein. *PTT* is a method that detects mutations arising from termination of *mRNA* translation process. As a result of this, the protein product is truncated. The truncated protein could have arised due to a frameshift mutation, non-sense mutation and splice site mutation. The advantage of *PTT* method is that, it can detect mutations of large kilobases. However, this method cannot detect polymorphisms, silent mutations and missense mutations.

2.24.3. Single strand conformational polymorphism (*SSCP*):

SSCP is one of the simplest screening techniques for detecting unknown mutations (microlesions) such as unknown single base substitutions, small deletions, small insertions, or microinversions. A *DNA* variation causes alterations in the conformation of denatured *DNA* fragments during migration within gel electrophoresis. The logic is comparison of the altered migration of denatured wild-type and mutant fragments during gel electrophoresis (*Kakavas et al*, 2008). In this technique, briefly, *DNA* fragments are denatured, and renatured under special conditions preventing the formation of double-stranded

DNA and allowing conformational structures to form in single-stranded fragments. The conformation is unique and resulted from the primary nucleotide sequence. Mobility of these fragments is differed through non denaturing polyacrylamide gels; detection of variations is based on these conformational structures. *PCR* is used to amplify the fragments, called *PCR-SSCP*, because the optimal fragment size can be (150 to 200) bp. About (80-90%) of potential point mutations are detected by (*SSCP*) (Kakavas *et al*, 2008; Nataraj *et al*, 1999).

2.24.4. *DNA* Sequencing (*DS*):

As a powerful technique in molecular genetics, *DNA* sequencing provides analysis of genes at the nucleotide level. The main aim of *DNA* sequencing is to determine the sequence of small regions of interest (~1 kilobase) using a *PCR* product as a template. Dideoxynucleotide sequencing or Sanger sequencing represents the most widely used technique for sequencing *DNA* (Franca *et al*, 2000). In this method, double stranded *DNA* is denatured into single stranded *DNA* with *NaOH*. A Sanger reaction consists of a single strand *DNA*, primer, a mixture of a particular *ddNTP* with normal *dNTPs* (e.g. *ddATP* with *dATP*, *dCTP*, *dGTP*, and *dTTP). A* fluorescent dye molecule is covalently attached to the dideoxynucleotide. *ddNTPs* cannot form a phosphodiester bond with the next deoxynucleotide so that they terminate *DNA* chain elongation. This step is done in four separate reactions using a different *ddNTP* for each reaction. *DNA* sequencing could be used to check all small known and unknown *DNA* variations (Franca *et al*, 2000).

2.24.5. Denaturing gradient gel electrophoresis (*DGGE*):

DGGE has been used for screening of unknown point mutations. It is based on differences in the melting behavior of small *DNA* fragments (200 -700 bp); even a single base substitution can cause such a difference. In this technique, *DNA* is first extracted and subjected to denaturing gradient gel electrophoresis. As the

denaturing condition increases, the fragment completely melts to single strands. The rate of mobility in acrylamide gels depends on the physical shape of the fragment. Detection of mutated fragments would be possible by comparing the melting behavior of *DNA* fragments on denaturing gradient gels. Approximately less than (100%) of point mutations can be detected using *DGGE*. Maximum of a nearly (1000) bp fragment can be investigated by this technique (Fodde & Losekoot, 1994).

2.24.6. Heteroduplex analysis (*HDA*):

A mixture of wild-type and mutant *DNA* molecules is denatured and renatured to produce heteroduplices. Homoduplices and heteroduplices show different electrophoretic mobilities through non denaturing polyacrylamide gels. In this technique, fragment size ranges between (200) and (600) bp. nearly (80%) of point mutations have been estimated to be detected by heteroduplex analysis (Glavac & Dean, 1995).

2.24.7. Restriction fragment length polymorphism (*RFLP*):

Point mutations can change restriction sites in *DNA* causing alteration in cleavage by restriction endonucleases which produce fragments with various sizes. *RFLP* is used to detect mutations occurring in restriction sites (Botstein *et al*, 1980).

2.24.8. Next generation sequencing (*NGS*):

In recent years, newer technologies for *DNA* sequencing in a massive scale have been emerged that are referred to as next-generation sequencing (*NGS*). High speed and throughput, both qualitative and quantitative sequence data are allowed by means of (*NGS)* technologies so that genome sequencing projects can be completed in a few days (Rabbani *et al*, 2012; Schuster, 2008). (*NGS)* systems provide several sequencing approaches including whole - genome

sequencing (*WGS*), whole exome sequencing (*WES*), transcriptome sequencing, methylome, etc. The coding sequences compromises about (1%) (30Mb) of the genome. More than (95%) of the exons are covered by (*WES)*; on the other hand, (85%) of disease-causing mutations in Mendelian disorders are located in coding regions. Sequencing of the complete coding regions (exome) (Ku *et al*, 2012), therefore, could potentially uncover the mutations causing rare, mostly monogenic, genetic disorders as well as predisposing variants in common diseases and cancer.

2.24.9. *DNA* microarray technology:

DNA "chips" or microarrays have been used as a possible testing for multiple mutations. In this technology, single *DNA* strands including sequences of different targets are fixed to a solid support in an array format. On the other hand, the sample *DNA* or *cDNA* labeled with fluorescent dyes is hybridized to the chip (Forozan *et al*, 1997). Then using a laser system, the presence of fluorescence is checked; the sequences and their quantities in the sample are determined.

2.25. Treatment:

Treatment choices are broadly classified into:

- Local therapy.
- Systemic therapy.

Methods of systemic therapy include:

a. Chemotherapy.

b. Hormonal therapy.

c. Biological therapy

Adjuvant therapy refers to additional treatment given after primary treatment of cancer to prevent its recurrence.

2.25.1. Local therapy:

Local therapy is used to control the cancer in a specific region include:

2.25.1.1. Surgical treatment of breast cancer:

Surgery is the most common form of treating breast cancer. Operations for breast cancer include Breast conserving surgery (lumpectomy), Mastectomy and axillaries lymph node dissection.

2.25.1.2. Breast conserving surgery (lumpectomy):

This surgery, a large portion of the breast where the lump is present is removed along with the surrounding margins sparing the other regions of the breast. Hence it is called as breast conserving surgery. This is often followed by radiation therapy to prevent its recurrence. This procedure is also known as segmental mastectomy.

2.25.1.3. Mastectomy:

It is a procedure where the entire breast is removed along with some lymph nodes. In modified radical mastectomy, the entire breast is removed along with axillary lymph nodes with the lining of the chest muscles. In radical mastectomy (also called as Halsted Radical Mastectomy), the breast, axillary lymph nodes, chest muscles and additional fat and skin over the chest are also removed.

2.25.1.4. Radiation therapy:

It is a method of treatment where high energy X-rays are used to treat the cancer cells. The radiation therapy can be obtained in two ways either through a machine (external radiation) or by implanting thin plastic tubes containing radioactive material (implant radiation) which requires hospital stay.

2.25.2. Systemic therapy:

Systemic therapy is used to:

- Control the spread of cancer.
- To decrease the tumor size for effective local therapy.
- To prevent recurrence.

2.25.2.1. Chemotherapy:

It is a mode of treatment where drugs are used to destroy the cancer cells. They are used either alone or in combination with one or more drugs. Some common chemotherapeutic agents are

- Anti-metabolites (e.g. methotrexate, and 5-fluoro uracil).
- Tumor antibiotics (e.g. doxorubicin and epirubicin).
- Anti-microtubule agents (e.g. paclitaxel, docetaxel, and vinorelbine).
- Alkylating agents (e.g.cyclophosphamide).

Anti-metabolites and tumor antibiotics destroy the genetic materials that are required for the survival of cancer cells. Anti-microtubule agents interfere with cell division and alkylating agents replaces certain hydrogen atoms in *DNA* with another molecule (an alkyl radical). This damages the *DNA*, and leads to cell death. They are either used to shrink the tumor before any local therapy is given (neoadjuvant therapy) or used as an additional therapy after surgery to prevent recurrence (http://www.veritas medicine.com).

2.25.2.2. Hormone therapy:

Breast tissues express some receptors for sex hormones, and cancer growth partly depends on hormonal stimulation. The aim of hormone therapy is to withdraw the stimulus either by reducing hormone production or by blocking hormone receptor binding. A well known example for hormone therapy is tamoxifen (Nolvadex) which is a serum estrogen response modulator taken in the form of oral pills for a period of five years. It competes with the receptors

for the estrogen and hence prevents it from binding. It is particularly useful for patients of estrogen receptor positive status. Other hormonal agents which are used in breast cancer are aminoglutethimide (cytadren), anastrozole (arimidex), exemestane (aromasin), fadrozole (afema), letrozole (femara), megestrol acetate (megace, pallace), methyltestosterone (android-10, testred, virilon) and testosterone (andro, androgel, testderm).

2.25.2.3. Biological therapy:

This mode of treatment employs herceptin (Trastuzumab), a monoclonal antibody which targets cell surface receptor protein, *Her-2/neu* which is essential for cell growth and division. This protein is over-expressed in (25%) of the tumors and due to this, tumors tend to grow faster and the chances of recurrence are high. The amount of *Her-2/neu* protein in tumors tissue for which herceptin has to be started is determined by a scale (0-3), where 0 means negative and (3) means strongly positive. The scaling system helps the treating physician, to decide upon the treatment options. Herceptin is given intravenously to treat the cancer.

2.25. 3. Therapy-in trail:

Scientists at University of Southern California have found a protein called contortrostatin (*CN*) from the venom of snake, southern copperhead viper which has major impact on the metastasis of breast cancer. (*CN*) prevents multiplication of cancerous cells rather than killing them. Therefore, it helps in shrinking of the tumor and it also inhibits the formation of new blood vessel. (http//news.bbc.co.uk).

Researchers have found a compound of vitamin A, fenretinide, which prevents the chances of getting a second tumor in premenopausal young women. It is also thought to be useful in high risk patients in preventing the development of breast cancer (http//news.bbc.co.uk).

Materials and Methods:

3.1. Study design:

This study is a cross sectional, hospital based study.

3.2. Study population:

The study population comprised of (52) breast cancer patients unselected for age of onset or family history from newly established Tumor Treatment & Cancer Research Center (*TTCRC*) in Shendi from the period between December (2013) to January (2015) and control sample consist of (30) healthy females with no family history of cancer.

3.3. Questionnaire:

The questionnaire (Appendix-I) has been filled by all patients. It included the demographic data and the risk factors associated with breast cancer risk such as the age of patients, height, weight, age at menarche, age at menopause, using of contraceptives pills, and family history of the study population**.**

3.4. Permission and ethical considerations:

According to research ethics, permission was obtained from Ethical committees of Almak Nimir hospital (Appendix-II) for specimen collection and performing the study. The objective of the study was explained to all participants and their consent was obtained (Appendix III).

3.5. Materials

3.5.1. Chemicals and Reagents

The chemicals and reagents used in this study include:

- Genomic *DNA* purification Kit from ***iNTRON.***
- Absolute Ethanol.
- Isopropanol Alcohol.

- Agarose.
- *Taq DNA* polymerases.

3.5.2. Disposables:

The major disposables used in this study are listed blew:

- K3-EDTA Tube.
- Sterile syringe.
- Cotton.
- Alcohol.
- Disposables powder gloves.
- Micro Tubes, (1.5) ml capacity.
- Micro Tubes, (0.2) ml capacity.
- Micropipettes tips.

3.5.3. Equipments:

All experiments of this study were done at the Alneelein University- Molecular biology Lab- Faculty of Medical Laboratory except the sequencing, which was done at ***Macrogen Incorporation-Seoul, South Korea***.

The major equipments that were used are listed below:

- Thermocycler machine.
- Microcentrifuge.
- Refregerator (-20ºC).
- Vortex Mixer.
- Micropipette Lab.
- Microwave.
- Safety Cabinet.
- UV-Transilluminator.
- Electrophoresis plate.
- Electric stabilizer.

3.6. Methods:

3.6.1. Case selection:

All patients with breast cancer diagnosed during the period of December (2013) to January (2015) were enrolled in the study except the patients who refused to be included in this study.

3.6.2. Blood sample collection:

A peripheral blood sample (3-5 ml) was collected in *EDTA* Tube. The buffy coat was separated and frozen at (- 80°C) till used.

2.6.3. Laboratory tests:

- *DNA* extraction.
- Polymerase chain reaction.
- *DNA* directs sequencing.

2.6.4. *DNA* extraction:

Genomic *DNA* was extracted from whole blood samples of cases with breast cancer as well as controls using a *DNA* extraction kit (*iNTRON* Biotechnology, South Korea) in accordance with the manufacturer's protocols as follows:

- A (300) µl whole blood was added to (1.5) ml tube containing (900) µl *RBC* lysis solutions. Mixed by vortexing and incubated for (5) min at room temperature.
- Centrifuge at (10.000) x g for (1) minute. The supernatant was removed except the white cell pellet and remained about (50-100) µl of the remnant which help cell pellet to resuspend.
- Vortex the tube to resuspend the cells.
- A (300) µl cell lysis solution was added to the resuspended cells.
- (1.5) µl RNase (optional) was added to the cell for lysate and incubated at 37°C for (15-30) min.

-The sample was chilled to room temperature, then (100) µl *PPT* buffer was added to the cell lysate and vortexed vigorously at high speed for (20) seconds.

- Centrifuge at (14.000) x g for (4) mins. The precipitated proteins will formed a tight white pellet.

- Then transferred to the (300) µl of supernatant containing the *DNA* (leaving behind the precipitated protein pellet) into a (1.5) ml tube.

- (300) µl (100%) Isopropanol was added.

- Centrifuge at (14,000) x g for (1) min. The *DNA* will be visible as small white pellet.

- Pour of the supernatant and drained the tube briefly on clean absorbant paper.

- 1ml (70%) Ethanol was added and inverted the tube several times to wash the *DNA* pellet.

- Centrifuge at (14,000) rpm x g for 1 min.

- Carefully pour off the ethanol slowly and watch the pellet.

-Inverted and drained the tube on the clean absorbent paper and allow to air dry for (10 -15) min.

- Last step (150) µl *DNA* Rehydration buffer was added and then the *DNAs* were incubated at (65 C^{o}) for (30-60) min or at (4 C^{o}) to overnight.

- For long term storage the *DNAs* were stored at (-20 C^{o}) or (-80 C^{o})

-The purity of *DNA* was measured by O.D 260:280 ratios by Nano Drop Spectrophotometer and gel electrophoresis.

3.6.4. Primers selection:

Selection of primers according to experience and previous studies in *BRCA1* were done by Elnor in Marawy (Elnor *et al*, 2010) for *BRCA1*exon (11) reigons (11-1 (C) &11-9 (D)), and the other study done by Mogtaba & Alsmawal in Khartoum state 2015 (submitted for publication) for *BRCA1* exon 20 (A), and *BRCA2* exon 11 (B) detection mutations.

The Exons fragments and its reagents are produced by Macrogen Corporation Seoul, South Korea). Exons fragments and its size range were as the following tables:

Table (3.1) Primers selected for *BRCA1* gene mutation

Primers	Exon	Annealing Temp. °C	Primer Size	Product Size
F:5'AGA GGC ATC CAG AAA AGT ATC AGG '3 **R:5' GGG AGT CCG CCT ATC ATT ACA T '3**	11 11	57 57	24 bp 22 bp	239 bp
F:5' TTG TCA ATC CTA GCC TTC CAA GAG '3 **R: 5'TTT TGC CTT CCC ATG AGT GCT AAC '3**	11 11	58 58	24 bp 24 bp	224 bp
F:5''ATA TGA CGT GTC TGC TCC AC '3 **R:5'**GGG AAT CCA AAT TAC ACA GC **'3**	20 20	55 55	20 bp 20 bp	401bp

F: forward (Sense) **R:** reverse (Antisense) **bp:** base pair

Table (3.2) Primer selected for *BRCA2* gene mutation

Primers	Exon	Annealing Temp. °C	Primer Size	Product Size
F:5'GCT CTC TGA ACA TAA CAT TAA G'3	11	50	22 bp	451bp
R:5'CAT TAT GAC ATG AAG ATC AG'3	11	50	20 bp	

F: forward (Sense) **R:** reverse (Antisense) **bp:** base pair

3.6.5. *DNA* amplification procedure:

In this step eluted DNA which has been extracted from the samples was amplified using Maxime *PCR* PreMix Kit i-Taq (20) µl (*iNTRON* Biotechnology, South Korea), which contain *Tag DAN* Polymerase, *dNTP* mixture, reaction buffer.

Then Amplification for the targeted regions was done after addition of (15-17 µl *D.W*, (1- 3) µl sample *DNA* and (1) µl of each forward and reverse primer) to the ready-to-use master mix volume. *PCR* mixture was subjected to initial denaturation step at (96)°C for (5) minutes, followed by (35) cycles of denaturation at (96) °C for (30) seconds, primer annealing at (50) °C, (55) °C, (57) °C, and (58) °C depending on the primer used for (30) seconds, followed by a step of elongation at (72) °C for (1) min, the final elongation was at (72) °C for (10) minutes according to published literature (Dufloth *et al*, 2005; Elnor *et al*, 2012).

3.6.6. Agarose Gel-electrophoresis:

The *PCR* products were visualized in (2%) Agarose gel with (0.5) µg/ml Ethidium bromide staining, the gel was prepared by dissolving (0.5) g of agarose powder in (25) ml of 1X *TBE* buffer and heated at (65)°C in microwave until the agarose completely dissolved, then left to cool at room temperature and (2) µl Ethidium bromides was added. The comb was then placed appropriately in the electrophoresis tray and then gel was slowly poured and left to set for (30) min for solidification. In a clean Eppendorf tube (10) µl of (100) bp *DNA ladder* and *PCR* product was loaded on the gel. Gel-electrophoresis was performed at (100V) and (60) Am for (30-45) minutes. Pictures were taken by gel documentation system (Gel mega, digital camera and software in a computer).

3.6.7. Interpretation of *PCR* results:

The *PCR* products length for the two overlapping fragments of *BRCA1* exon 11 (11-1(C), 11-9 (D)) *BRCA1* exon (20) and *BRCA2* exon (11) would have specific length after staining with ethidium bromide.

3.6. 8. Sequencing of *BRCA1* & *BRCA2* gene:

Sanger sequencing was performed for *PCR* products. Each sample was analyzed by 4 primers (A, B, C, and D) for the (52) patient samples and (30) negative control samples run for both forward and reverse strands targeting the four selected regions within *BRCA1&2* genes were performed by Macrogen Company (Seoul, South Korea) using the same primers.

3.7. Bioinformatics analysis:

The sequences chromatogram was viewed by (Finch TV 1.4.0 program) (http://www.geospiza.com/ Products/finchtv.shtml).

Then the nucleotides sequences of *BRCA1&2* genes were searched for sequences similarity using nucleotide *BLAST* (http://

blast.ncbi.nlm.nih.gov/Blast.cgi) (Atschul *et al*, 1997) highly similar sequences were retrieved from (*NCBI).*

According to nucleotide database reference sequences (NG_005905) on chromosome (17) which represents the whole *BRCA1* gene, and the transcript variant 1(NM_007294) *mRNA* with (7224) bp which comprises mainly the coding sequences of the gene and Protein accession numbers: (NP_009225) protein isoform 1 with (1863 a. a; the first was used to check all the three primer amplicons within gene regions nucleotides, while the second transcript variant 1 was used for assessing all the amplicons coding sequences and the comparative analysis of the patients nucleotide sequences within *BRCA1* gene, also used (NM_000059) as reference sequences for a *BRCA2* nucleotides sequences.

Any changes within the tested sequences were noticed through multiple sequences alignment using BioEdit software (Hall, 1999). All sequences were translated into amino acid sequences using online Expasy translate tool (http://web.expasy.org/translate).The resulted amino acid sequences were compared all together again using BioEdit software version 7.0.9.1 (Hall, 1999).

3.7.1. Data collection:

The *SNP* information *SNP* ID, MIM: 113705, *Ref Seq Gene* accession NO: NG_005905 on chromosome 17, *mRNA* accession NO: (NM_007294) transcript variant 1 with (7224) bp and Protein accession numbers: (NP_009225) protein isoform 1 with (1863) a. a. of the human *BRCA1* gene that was used in our computational analysis was retrieved from the National Center for Biotechnology Information (*NCBI*) database of *SNPs: dbSNP* (http://www.ncbi.nlm.nih.gov/).

3.7.2. SNP Prediction:

The SNPs were analyzed using 4 prediction online tools: SIFT (http://sift.bii.a-star.edu.sg/) (Ng & Henikoff, 2003), Polyphen-2(http:// genetics.bwh.harvard. edu/pph2/) (Ramensky, 2002), I Mutant Suite (http://gpcr2. biocomp.unibo.it/ cgi/predictors/I Mutant3.0/ IMutant3.0.cgi) and PhD-SNP (http: //snps.biofold. org/phd-snp/phd-snp.html) (Capriotti, 2006).

The tertiary model of protein and mutation analysis was done online by project Hope software (http://www.cmbi.ru.nl/hope/input) (Venselaar, 2010).

3.8. Statistical analysis:

Both qualitative and quantitative data analysis methods were used. The data analysis were made utilizing IBM *SPSS* version 20.

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

- Count, percentage and descriptive statistics.
- Chi-square test to build (2x2) tables for categorical data and cross tabulation and examine if there is statistically significance proportion differences.

Results:

The study population has been divided into two groups, breast cancer patients group which consist of (52) patients, age range (30-71), were not selected for age of onset, family history or any other criterion that would enrich for *BRCA1/2* mutation carriers, and match with age a control group of (30) healthy individual with no family history of cancer.

Demographic characteristics, family history information and other known risk factors for these patients are shown in (**Table 4.1)**

Table 4.1: Demographic characteristics and risk factors

Demographics

Age at diagnosis	**No. (%)**
30 – 40	11(21.2%)
41-50	12 (23.1%)
51- 60	24 (46.1%)
Above 60	5 (9.6%)
Resident	
Shendi	34 (65.4%)
Others	18 (34.6%)
Socioeconomic status	
house wife	44(54.6%)
Worker	2(3.9%)
Employee	6(11.5%)
Education	
Illiterate	22(42.3%)
Primary	8(15.4%)
High school	17(32.7%)
University graduate	5(9.6%)
Tribe/Ethnic	
Jaalia	40 (76.9%)
Others	12 (23.1%)
Marital status	
Single	7 (13.5%)
Married	43 (82.7%)
Divorced	2 (3.8%)

Parity	
Nulliparous	11 (21.1%)
Single parity	5(9.6%)
Multiparous	36(69.2%)
Family history	
Yes	23 (44.2%)
No	29 (55.8%)
Age at menarche	
11 year	6 (11.5%)
more than 11	46 (88.5%)
Menopause	
Postmenpause	33 (63.5%)
Premenopausal	19 (36.5%)
Physical activity	
Yes	6 (11.5%)
No	46 (88.5%)
Obesity	
Normal	14 (27%)
Less than normal	6 (11.5%)
Above normal	32 (61.5%)
Oral contraceptive	
Yes	13 (25%)
No	39 (75%)
Smoking	
Yes	5 (9.6%)
No	47 (90.4%)
Hormonal therapy	
Yes	4 (7.7%)
No	48 (92.3%)
Exposure to radiation	
Yes	17 (32.7%)
No	35 (67.3%)

Table (4. 1): Summarized that:

The average age of patients with breast cancer in the study was (2.44 ± .94), with a range of (30-71) years, most patients 24 (46.1%) were diagnosed with breast cancer at age (51-60) years.

Furthermore, the majority of patients, 34(65.4%) from Shendi, while the remaining 18(34.6%) from different regions near Shendi (Almatamma, Atbara, Aldammer, and even from Khartoum).

Regarding socioeconomic status, most patients were housewives 44 (54.6%), 2 (3.9%) were workers, and 6 (11.5%) were employees.

On the other hand 22 (42.3%) of breast cancer patients were illiterate, 8 (15.4%) were primary school pupils, 17 (32.1%) were higher secondary school students, and 5 (9.6%) were University graduates.

Concerning marital status, 43(82.7%) of patients were married, while7 (13.5%) were single, and 2(3.8%) were divorced.

Breast cancer study population was distributed as follow, 40 (76.9%) were from ethnic/tribe Jaalia, while the remaining (Shaigia, Hassania, Robatab, and Danagla) were constituted 12 (23.1%).

With regard to parity, 36 (69.2%) of patients were multiparous, 5 (9.6%) were single parous, while 11(21.1%) were nulliparous.

Out of 52 of the cases, 23 (44.2%) were found to have a family history of cancer, while 29 (55.8%) of the cases were have not a family history of cancer.

Regarding age at menarche, 46 (88.5%) of the cases have attended the menarche after (11) years, and 6 (11.5%) within (11) years old.

Moreover, 33 (63.5%) of the cases were postmenpause, while19 (36.5%) were premenopausal.

Participation to physical activities reflected that; 6 (11.5%) were *BC* patients, while 46 (88.5%) were not.

Concerning obesity, 32 (61.5%) of patients were obese, 14 (27%) with normal weight, and 6 (11.5%) were underweight.

The majority of *BC* patients, 39 (75%) were not using oral contraceptives, while 13 (25%) were using.

Regarding hormonal therapy, most of the patients 48 (92.3%) were not taking hormonal therapy and 4 (7.7%) was taking.

Furthermore, 5 (9.6%) of the patients were smokers, while 47 (90.4%) of them were not.

On the other hand, 17 (32.7%) of *BC* patients were exposed to radiation, while the remaining 35 (67.3%) were not.

Table (4.2): Breast cancer histological types versus age among case study group

Histological types of breast cancer	Age of case (yrs)				Total
	30 – 40	41-50	51-60	Above 60	
Invasive ductal carcinoma	10(21.7%)	10(21.7%)	21(45.7%)	5(10.9%)	46
Ductal carcinoma in situ	0	2 (100%)	0	0	2
Lobular carcinoma	0	0	2 (100%)	0	2
Paget disease	1(50%)	0	1(50%)	0	2
Total	11	12	24	4	52

P value 0.306

Table (4, 2): Denoted that; the invasive ductal carcinoma was found to be the most histological type of cancer diagnosed at the age of (51-60) years → 21 (45.7%); there was no significant statistical correlation observed between histological type and age; (P = 0.306).

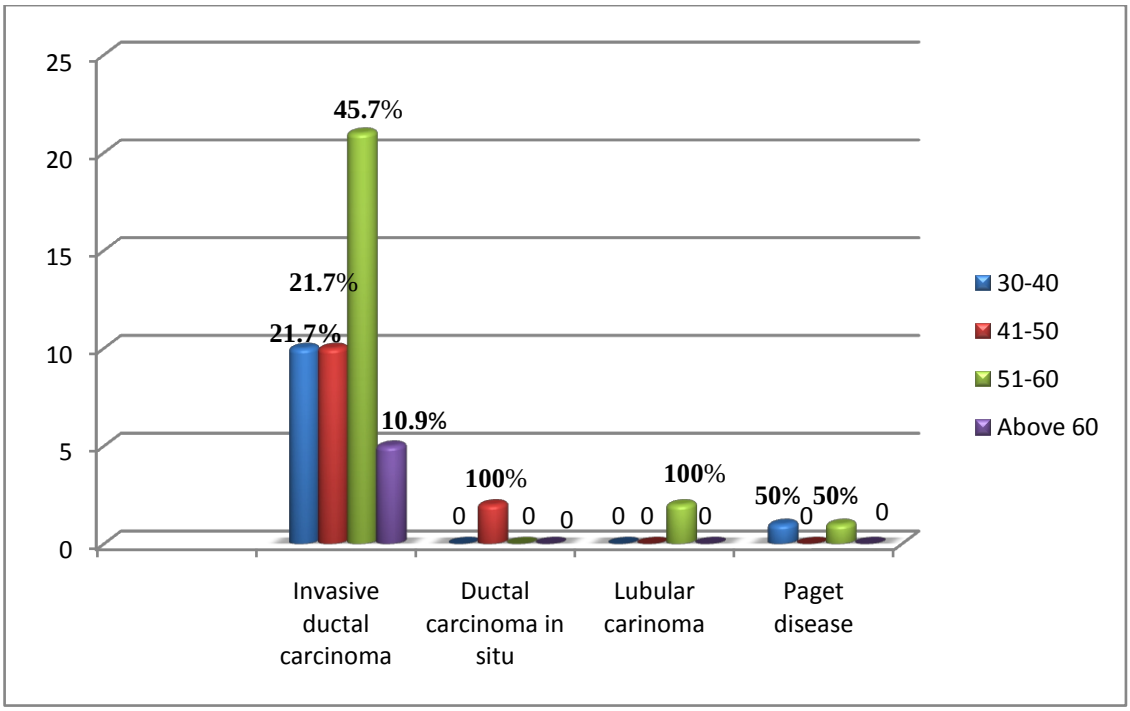

Figure (4.2): Breast cancer histological types versus age among case study group

Table (4.3): Breast cancer histological types against cancer grades

Histological types of breast cancer	grade of breast cancer			Total
	grade 1	grade II	grade III	
Invasive ductal carcinoma	1(2.1%)	31(67.4%)	14(30.5%)	46
Ductal carcinoma in situ	2 (100%)	0	0	2
Lobular carcinoma	0	0	2(100%)	2
Paget disease	0	1(50 %)	1 (50%)	2
Total	3	32	17	52

P value 0.000

Table (4, 3): Presented that; the invasive ductal carcinoma (IDC) was noted to be tightly associated with cancer grade II → 31(67.4%) compared to other types. There was a highly significant statistical correlation linkage between IDC and cancer grade II; (P = 0.000).

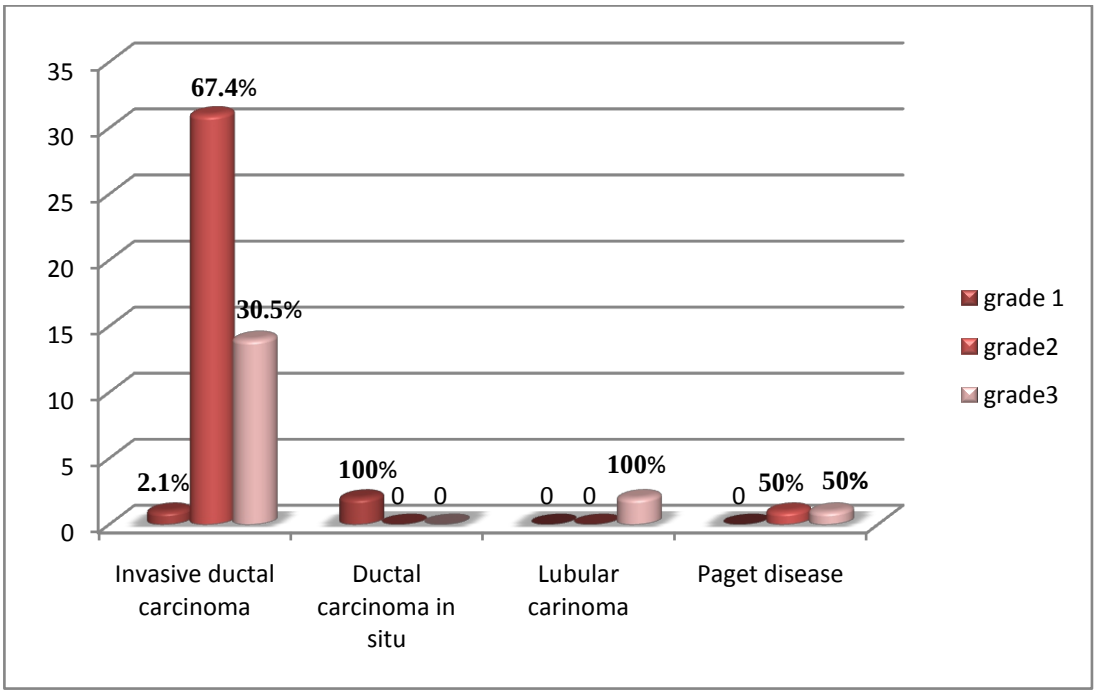

Figure (4.3): Breast cancer histological types against cancer grades

Table (4.4): Classification of breast cancer according to genetic inheritance

Classification of breast cancer	Frequency	Percent
Hereditary	23	44.2 %
Non hereditary	29	55.8 %
Total	52	100

Table (4, 4): Indicated that; the non- hereditary breast cancer (sporadic) type was revealed to be the most presented →29 (55.8%) compared to hereditary one.

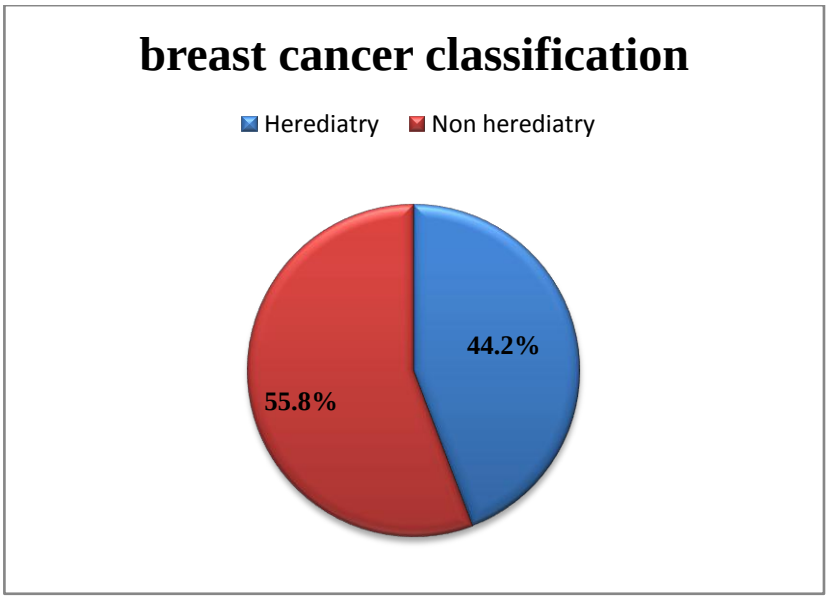

Figure (4.4): Classification of breast cancer according to genetic inheritance

Table (4.5): Family relative's degree among patients with hereditary type

	Relatives degree			Total
	First degree	Second degree	Third degree	
Hereditary type	4 (17.4%)	16(69.6%)	3(13%)	23

Table (4, 5): Revealed that: the breast cancer of familial second degree relatives was observed to be the most type of cancer occurrence among hereditary types → 16(69.6%).

Table (4.6): Cancer malignancy common types among patient's family relatives

Types of cancer in family	Frequency	Percent
Breast	17	73.9%
Other	6	26.1 %
Total	23	100

Table (4, 6): Predicted that; the breast cancer was the most common malignant cancer diagnosed among patient's family relatives → 17(73.9%), lesser other types of cancer rather than breast cancer were noted to be: cervical cancer, colorectal carcinoma, pharynx, and rectal carcinoma respectively.

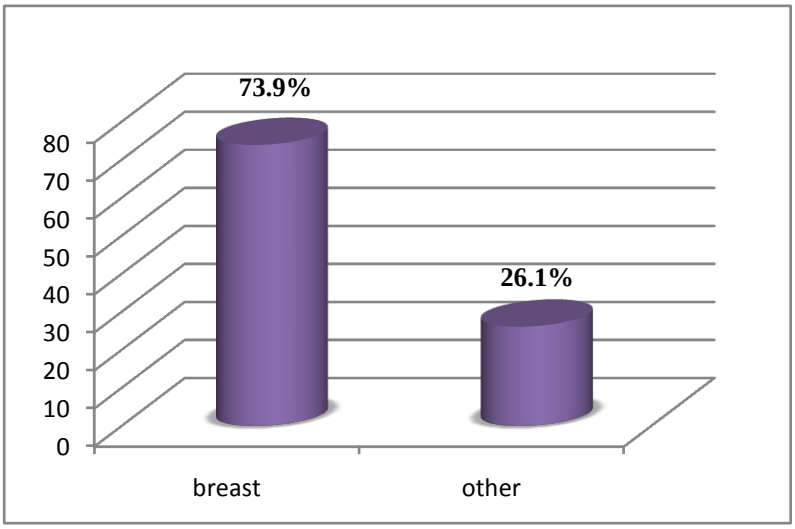

Figure (4.6): Cancer malignancy common types among patient's family relatives

Table (4.7): Frequency of *BRCA1* mutation-1 among study population

Study population	*BRCA1* Mutation-1 (Exon 20)		Total
	Positive	Negative	
Control	8 (26.7%)	22 (73.3%)	30
Case study	8(15.4%)	44 (84.6%)	52
Total	16	66	82

P value 0.214

Table (4.7): Showed that; the *BRCA1* mutation -1 gene location was detected by (PCR) and mapped on (exon 20), mutation appeared in → 8 patients with frequency (15.4%) and 8 in control with frequency → (26.7%); classified as novel point mutation; there was no significant statistical correlation calculated among study population; (P = 0.214).

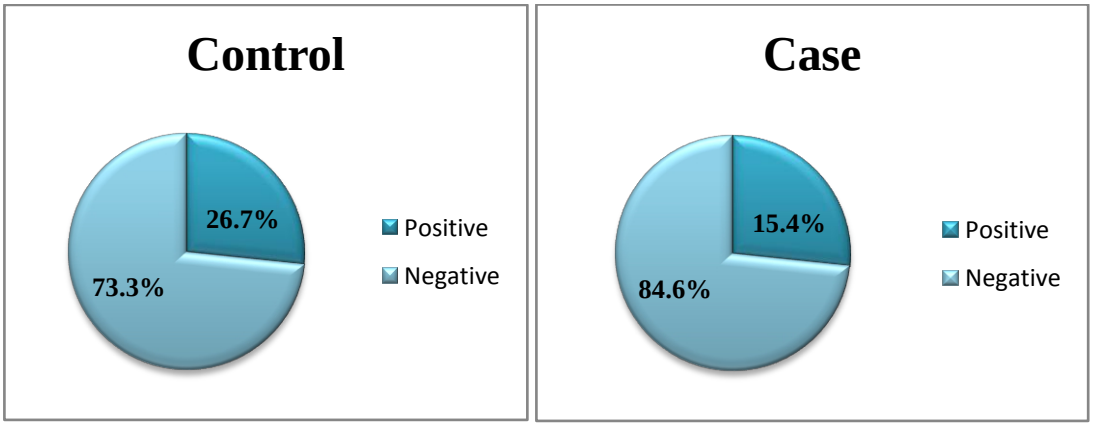

Figure (4.7): Frequency of *BRCA1* mutation-1 among study population

Table (4.8): Frequency of *BRCA1* mutation-2 among study population:

Study population	*BRCA1* Mutation-2(Exon 11)		Total
	Positive	Negative	
Control	0	30	30
Case study	3 (5.8%)	49 (94.2%)	52
Total	3	79	82

P value 0.180

Table (4, 8): Adopted that; the *BRCA1* mutation -2 gene position was detected by (PCR) and located on (exon 11); three patients were with *BRCA1*- mutation – 2 exon (11) → 3(5.8%). It was a novel mutation classified as missense mutation, there was no significant statistical relationship observed among study population; (P = 0.180).

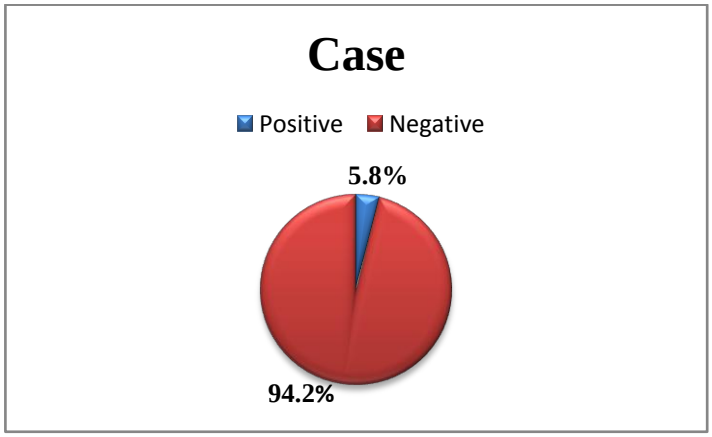

Figure (4.8): Frequency of *BRCA1* mutation -2 among study population

Table (4. 9): Age, cancer type and family history background information of the patients with *BRCA1* mutation-2

Patient index	Age /year	Cancer type	Tribe/ethnicity	Family history
18	45	IDC	Jaalia	Negative
34	56	IDC	Jaalia	Positive
37	55	IDC	Jaalia	Positive

IDC: invasive ductal carcinoma

Table (4, 9): Summarized that; the *BRCA1*mutation -2 was resulted to be affecting the three patient's indices (18, 34, and 37), with age (45, 56, and 55) years respectively. All of the patients were from Jaalia tribe and with invasive ductal carcinoma; two patients with indices (34, 37) were with a positive family history; were carrying two mutations at the same time.

Table (4.10): Frequency of *BRCA1* mutation-3 in study population:

Study population	*BRCA1* Mutation- 3		Total
	Positive	Negative	
Control	0	30	30
Case study	2 (3.8%)	50 (96.2%)	52
Total	2	80	82

P value 0.277

Table (4, 10): Prevailed that; the *BRCA1* mutation -3 gene locus was detected by (PCR) and positioned on (exon-11); two patients with *BRCA1*- mutation -3 were accounted for → 2(3.8%) classified as silent mutation. There was no significant statistical value observed among study population; (P = 0.277).

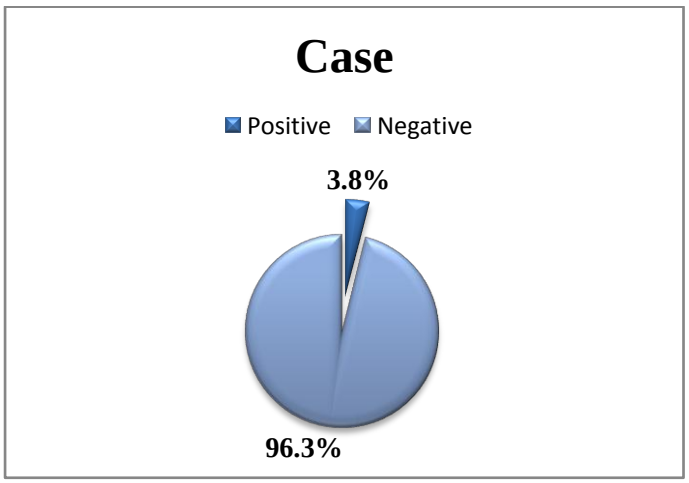

Figure (4.10): Frequency of *BRCA1* mutation-3 study population

Table (4.11): Frequency of *BRCA1* mutations - (1, 2, and 3) among study population

Study population	*BRCA1* Mutations		Total
	Positive	Negative	
Control	8 (26.7%)	22(73.3%)	30
Case study	11 (21.2%)	41(78.8%)	52
Total	19	63	82

Table (4, 11): Depicted that; the *BRCA1* mutations - (1, 2, and 3) genes loci were detected by (PCR) localized on exons (20 &11), patients with *BRCA1* – mutation (1, 2, 3) were accounted for 11 (21.2%), compared to 8 (26.7%) in control. Normal was 41(78.8%). Two patients were carried two mutations.

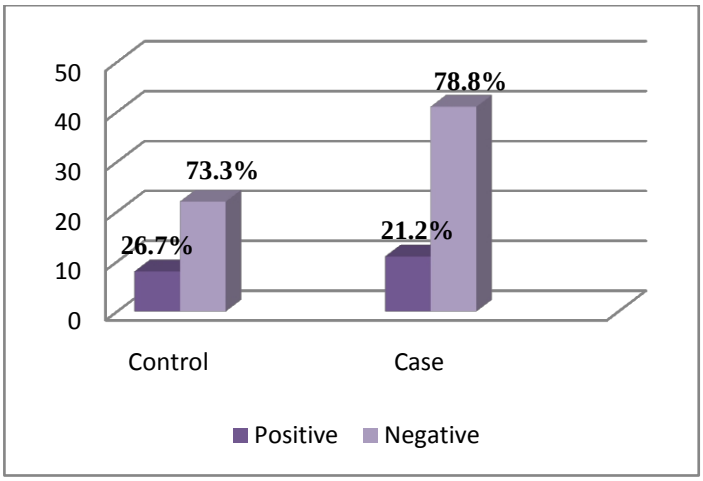

Figure (4.11): Frequency of *BRCA1* mutations (1, 2, and 3) among study population

Table (4.12): Frequency of *BRCA2* mutation among the study population

Study population	*BRCA2* mutation	Total
	Negative	
Control	30	30
Case study	52	52
Total	82	82

Table (4, 12): Demonstrated that; all population under study showed negative results for *BRCA2*- exon (11), there was no mutation occurred. *BRCA2* sequence chromatogram revealed no mutation was shown when viewed by Finch TV program 1.4.0 (http://www.geospiza.com/ Products/finchtv.shtml), and compared with *BRCA2* nucleotide reference sequences (NM_000059) from (*NCBI).*

Table (4.13): Association between breast cancer mutations and patient's Age

	Age of case (years)				Total
	30- 40	41- 50	51- 60	> 60	
Mutation	1(9.1%)	1(9.1%)	8(72.7%)	1(9.1%)	11
No mutation	10(24.4%)	11(26.8%)	16 (39%)	4(9.8%)	41
Total	11	12	24	5	52

P value 0.233

Table (4, 13): Revealed that; most breast cancer patients with *BRCA* mutation were concentrated among the age group ranged (51– 60) years, accounted for 8 (72.7%) patients. There was no significant statistical difference observed between patient's age and mutation; (P= 0.233)**.**

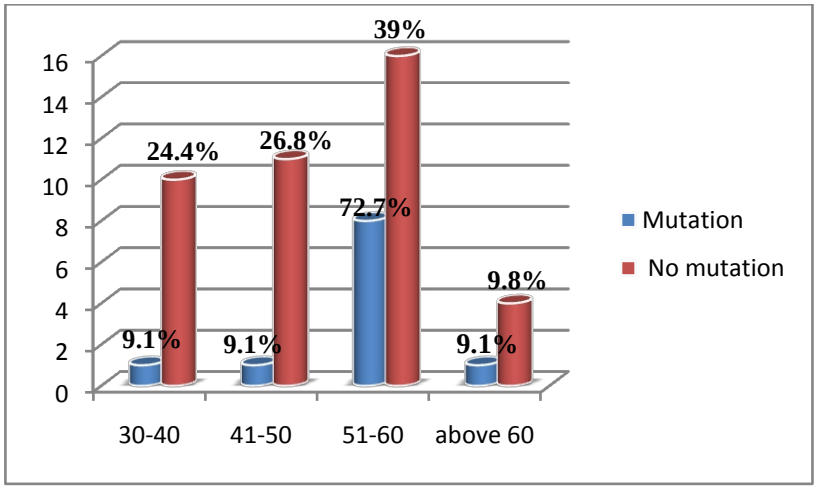

Figure (4.13): Association between breast cancer mutations and patient's age

Table (4.14): Relationship between breast cancer mutations & tribal ethnicity

	Tribe/ ethnic		Total
	Jaalia	Others	
Mutation	6 (54.5%)	5 (45.5%)	11
No mutation	34 (82.9%)	7 (17.1%)	41
Total	40	12	52

P value 0.047

Table (4, 14): clarified that; the breast cancer incidences, were observed to be more attached with Jaalia tribe compared to others → 6(54.5%) among the mutated cases; taking in consideration the ethnic point of view, there was significant statistical relationship calculated between tribal/ ethnicity and mutation; (P = 0.047).

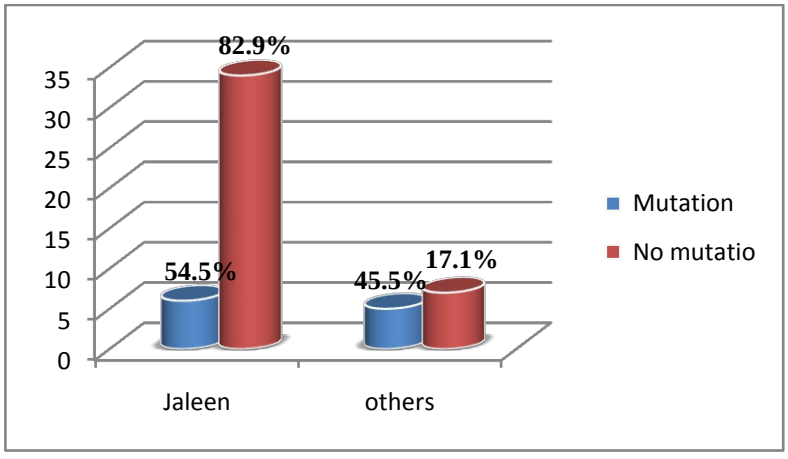

Figure (4.14): Relationship between breast cancer mutations & tribal/ ethnicity.

Table (4.15): Relationship between breast cancer mutations &parity

	Parity			Total
	Nulliparous	Single parity	Multiparous	
Mutation	3 (27.3%)	1 (9.1%)	7 (63.6%)	11
No mutation	8 (19.5%)	4 (9.8%)	29 (70.7%)	41
Total	11	5	36	52

P value 0.855

Table (4, 15): Identified that; the breast cancer occurrences were found to be more associated with multiparity accounted for → 7(63.6%) among the mutated cases, there was no statistical significant correlation observed between parity and mutation; (P = 0.855).

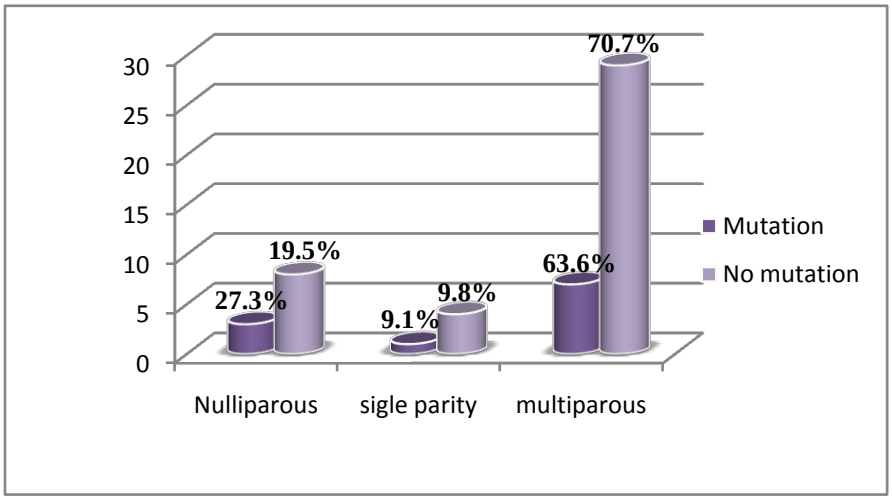

Figure (4.15): Relationship between breast cancer mutations &parity

Table (4.16): Relationship between breast cancer mutations &family history

	Family history		Total
	Yes	No	
Mutation	6 (54.5%)	5 (45.5%)	11
No mutation	17(41.5%)	24 (58.5%)	41
Total	23	29	52

P value 0.438

Regarding table **(4, 16):** the familial history of breast cancer was increasing in presence of a cancer predisposing mutation → 6(54.5%). There was no significant statistical correlation observed between the family history and mutation; (P = 0.438).

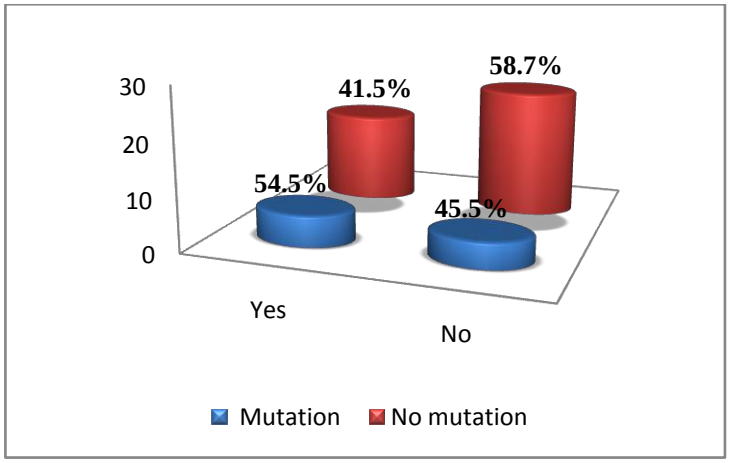

Figure (4.16): Relationship between breast cancer mutations &family history

Table (4.17): Relationship between breast cancer mutations & age at menarche

	Age at menarche		Total
	(11) years	More than (11) years	
Mutation	1 (9.1%)	10 (90.9%)	11
No mutation	5 (12.2%)	6 (87.8%)	41
Total	6	46	52

P value 0.775

Table (4, 17): indicating that, there was an increasing association between the age of more than (11) years at first menarche and breast cancer risk → 10(90.9%) among the mutated cases, there was no statistical significant relationship stated between age at menarche and mutation; (P = 0.775).

Normal universal menarche between, (12.8 -13) years.

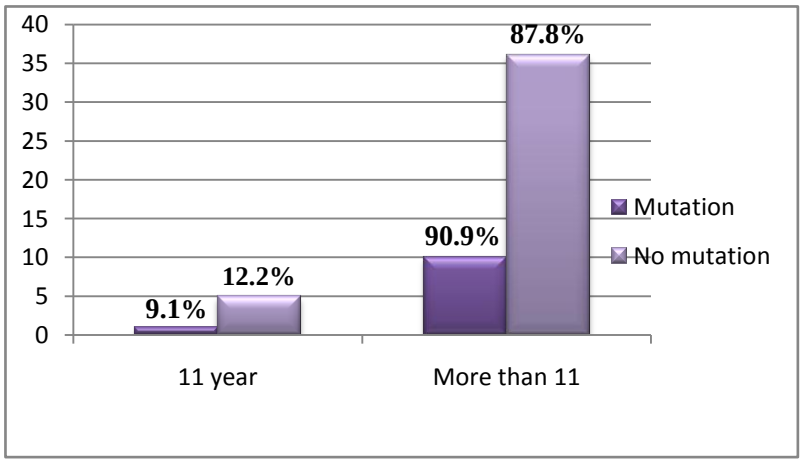

Figure (4.17): Relationship between breast cancer mutations & age at menarche

Table (4.18): Relationship between breast cancer mutations & obesity

	Obesity			Total
	Normal weight	Underweight	overweight	
Mutation	2 (18.2%)	2 (18.2%)	7 (63.6%)	11
No mutation	12 (29.2%)	4 (9.8%)	25 (61%)	41
Total	14	6	32	52

P value 0.625

Table (4, 18): Provided that obese women were more prone to breast cancer mutations, far more than others as reviewed in this study → 7(63.6%), there was no significant statistical value observed between obesity and mutation; (P = 0.625).

Underweight: (18.5)

Normal weight: (18.5 – 24.9)

Overweight: (25 – 29.9)

Obesity = 30 or more

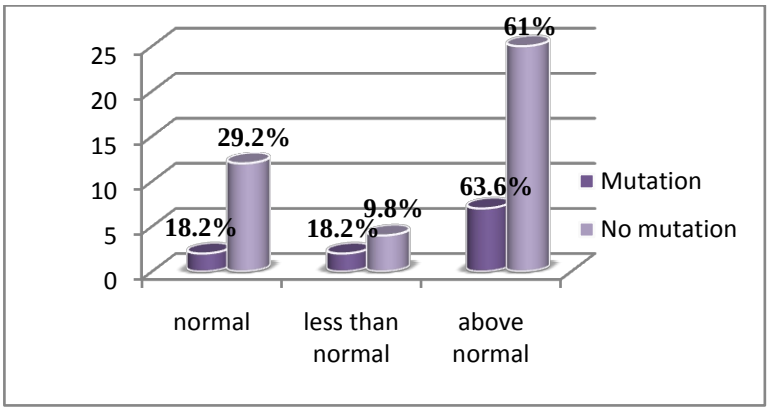

Figure (4.18): Relationship between breast cancer mutations & obesity

Table (4.19): Relationship between breast cancer mutations & using of Oral contraceptives

	Oral contraceptive		Total
	Yes	No	
Mutation	4 (36.4%)	7 (63.6%)	11
No mutation	9 (22%)	32 (78%)	41
Total	13	39	52

P value 0.327

Table (4, 19): Denoted that: there was no association between the using of oral contraceptives and the breast cancer incidences, there was no significant statistical difference observed between oral contraceptives use and mutation; (P = 0.327).

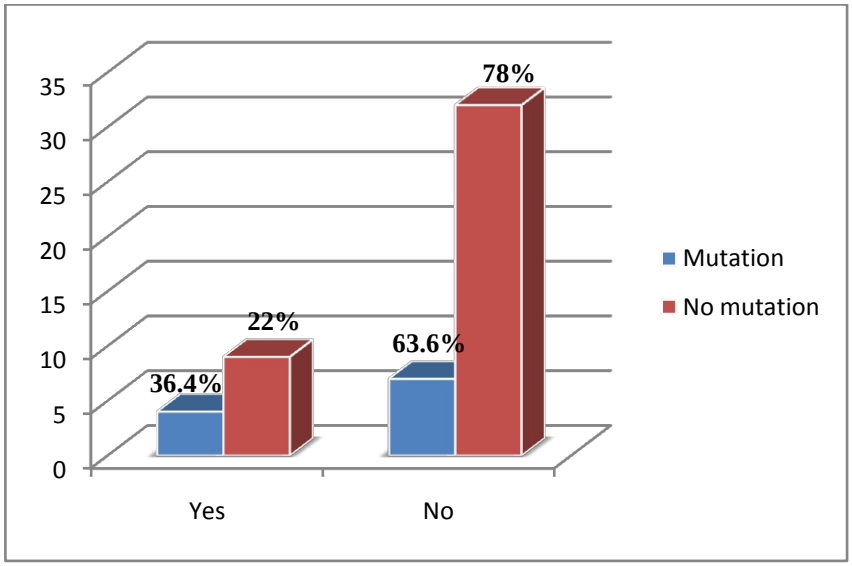

Figure (4.19): Relationship between breast cancer mutations & using of Oral contraceptives

Table (4.20): Relationship between breast cancer mutations & exposure to Ionizing radiation

	Expose to ionizing radiation		Total
	Exposed	Not exposed	
Mutation	3 (27.3%)	8 (72.7%)	11
No mutation	14 (34.1%)	27 (65.9%)	41
Total	17	35	52

P value 0.666

Table (4, 20): The findings stated that: there was no association between radiation exposure and the risk effects of breast cancer incidences. There was no significant statistical value between mutation and exposure to radiation; (P= 0.666).

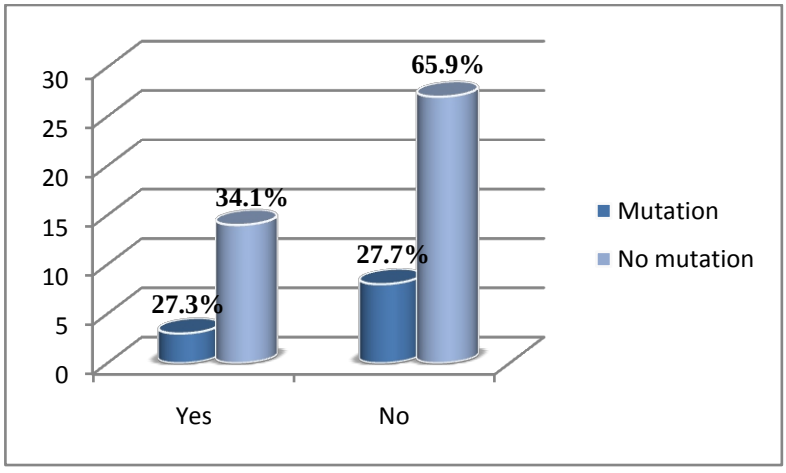

Figure (4.20): Relationship between breast cancer mutations &exposure to ionizing radiation

Table (4.21): Relationship between breast cancer mutations & menopause

	Menopause		Total
	Postmenopausal	Premenopausal	
Mutation	7 (63.6%)	4 (36.4%)	11
No mutation	26 (63.4%)	15 (36.6%)	41
Total	33	19	52

P value 0.989

Table (4, 21): Revealed that; breast cancer incidences occurring were shown to be more increasing during menopausal period → 7(63.6%) among mutated cases, there was no significant statistical relationship observed between mutation and menopause, (P= 0.989).

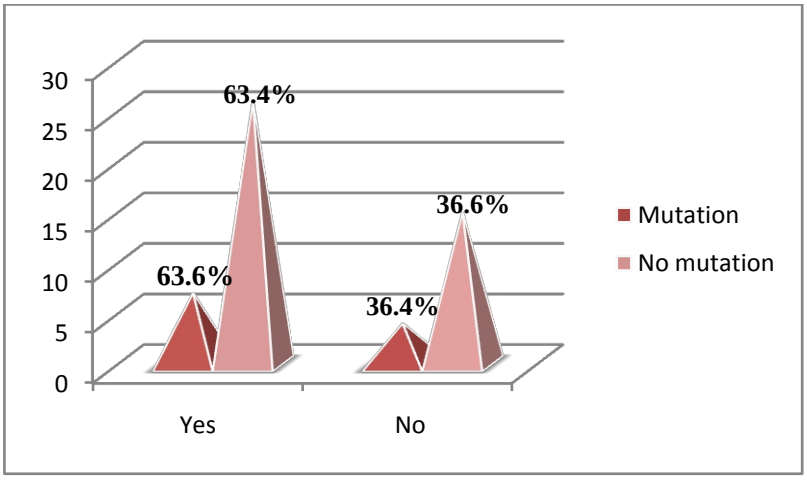

Figure (4.21): Relationship between breast cancer mutations & menopause

Table (4.22): Relationship between breast cancer mutations and histological type

	Histological types of breast cancer				Total
	Invasive ductal carcinoma	Ductal carcinoma in situ	Lobular carcinoma	Paget disease	
Mutation	11 (100%)	0	0	0	11
No mutation	35 (85.3%)	2 (4.9%)	2 (4.9%)	2 (4.9%)	41
Total	46	2	2	2	52

P value 0.611

Table (4, 22): Prevailed that; there was strong relation between the breast cancer mutation incidences and the invasive ductal carcinoma histological type compared to others → 11(100%). There was no significant statistically value observed between histological type and mutation; (P= 0.611).

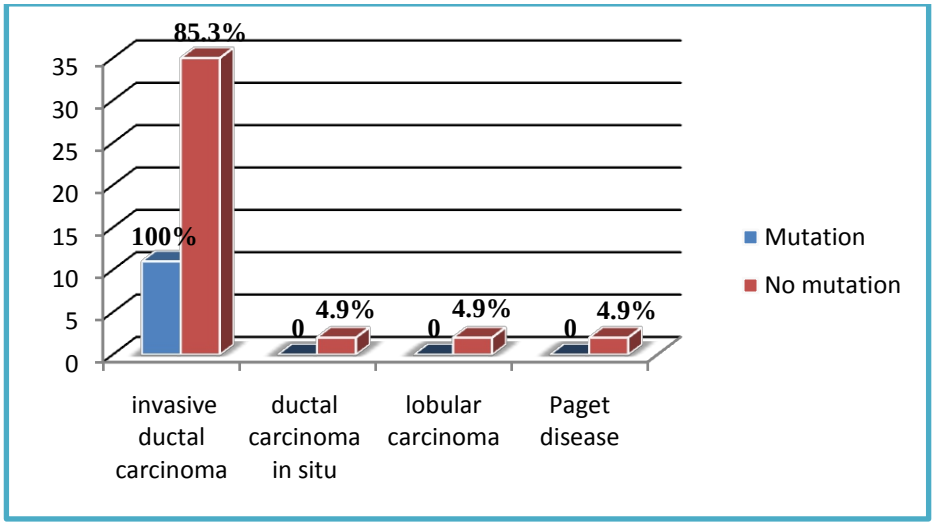

Figure (4.22): Relationship between breast cancer mutations & histological type

Table (4.23): Relationship between breast cancer mutations & cancer grades

	Grade of breast cancer			Total
	Grade I	Grade II	Grade III	
Mutation	0	6 (54.5%)	5 (45.5%)	11
No mutation	3 (7.3%)	26 (63.4%)	12 (29.3%)	41
Total	3	32	17	52

P value 0.447

Table (4, 23): Referred to that; the breast cancer mutations were shown to be more occurring with cancer grade II → 6(54.5%) among the mutated cases. There was no significant statistical difference denoted between cancer grade and mutation; (P= 0.447).

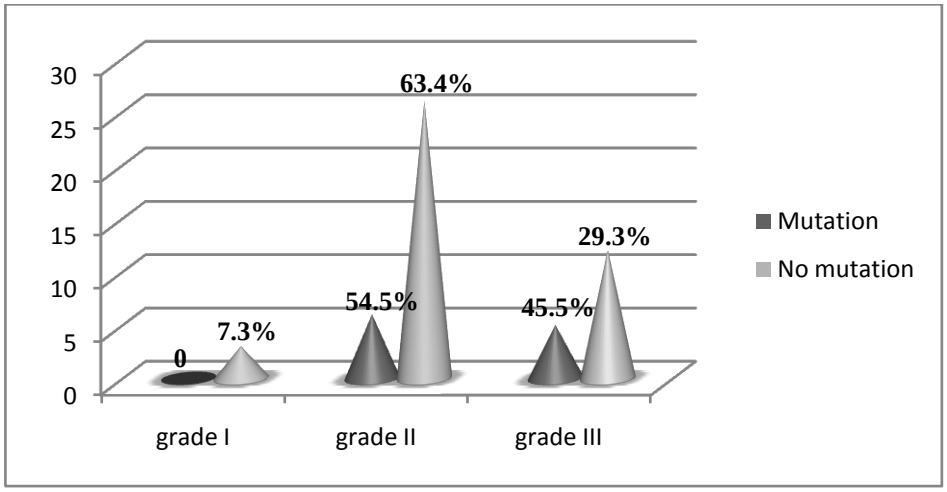

Figure (4.23): Relationship between breast cancer mutations & cancer grades

Table (4.24): Relationship between breast cancer mutations & ER (Estrogen Receptor) status

	ER status		Total
	Positive	Negative	
Mutation	5 (45.5%)	6 (54.5%)	11
No mutation	17 (41.5%)	24 (58.5%)	41
Total	22	30	52

P value 0.812

Table (4, 24): demonstrated that; breast cancer mutations were observed to be more associated with *ER* (estrogen receptor) negative → 6(54.5%). There was no significant statistical correlation between mutation and ER; (P = 0.812).

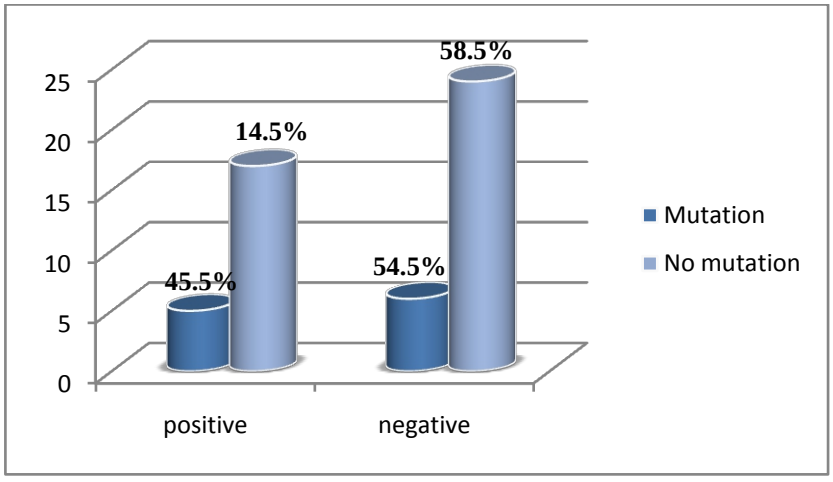

Figure (4.24): Relationship between breast cancer mutations & *ER* status

Table (4.25): Relationship between breast cancer mutations & PR (progesterone receptor) status

	PR status		Total
	Positive	Negative	
Mutation	4(36.4%)	7(63.6%)	11
No mutation	14(34.1%)	27(65.9%)	41
Total	18	34	52

P value 0.372

Table (4, 25): Summarized that; incidences of breast cancer were observed to be more associated with PR (progesterone receptor) negative → 7(63.6%) among mutated cases. There was no significant statistical correlation between mutation and PR; (P = 0.372).

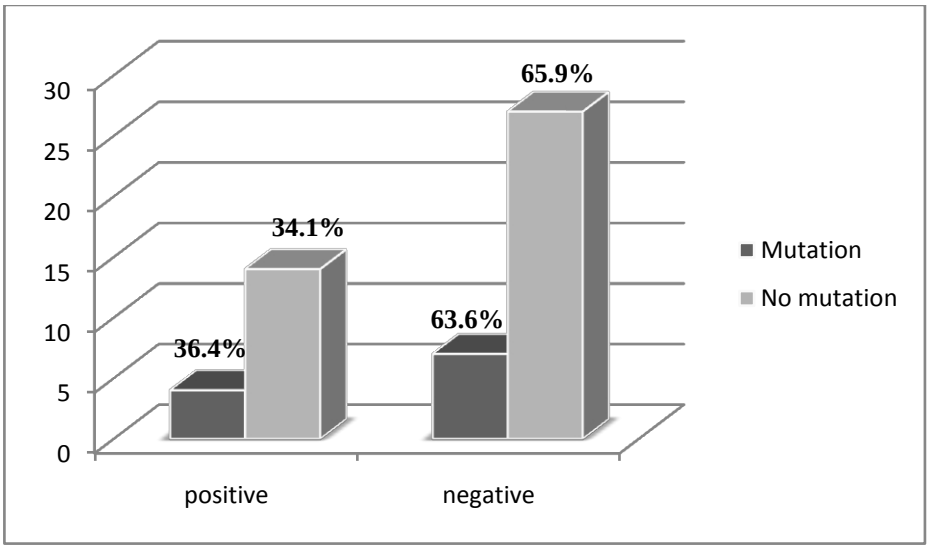

Figure (4.25): Relationship between breast cancer mutations & *PR* status

Table (4.26): Relationship between breast cancer mutations & HER-2 Status

	HER-2 status		Total
	Positive	Negative	
Mutation	1 (9.1%)	10 (90.9%)	11
No mutation	20 (48.8%)	21 (51.2%)	41
Total	21	31	52

P value 0.017

Table (4, 26): Presented that; the risk effects of breast cancer were shown to be more attached with HER-2 negative →10 (90.9%) among mutated cases, there was significant statistical relationship observed between HER-2 and mutation; (P= 0.017).

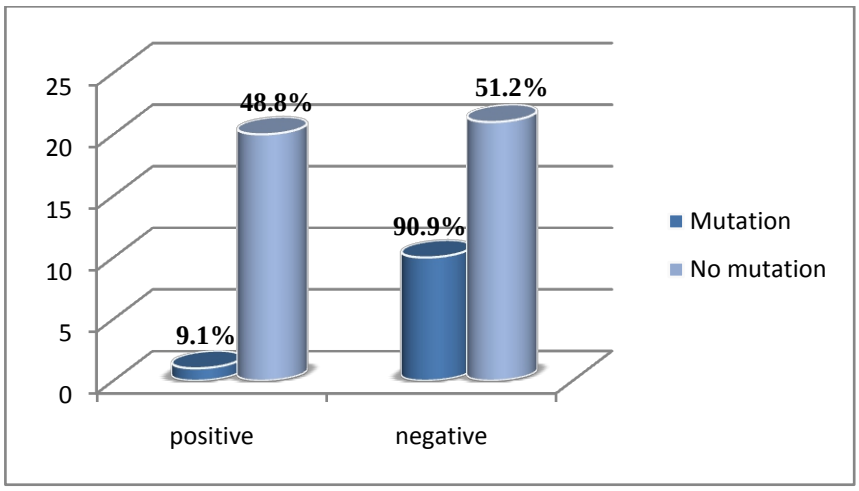

Figure (4.26): Relationship between breast cancer mutations & *HER-2* status

PCR results:

The *PCR* products length for two fragments of *BRCA1* exon 11, (C) should be (239) bp, and (D) should be (224) bp, for *BRCA1* exon 20 (A) should be (401) bp, and for *BRCA2* exon 11 (B) should be (451) bp after staining with ethidium bromide. (100) bp molecular ladder was used as illustration in below figures.

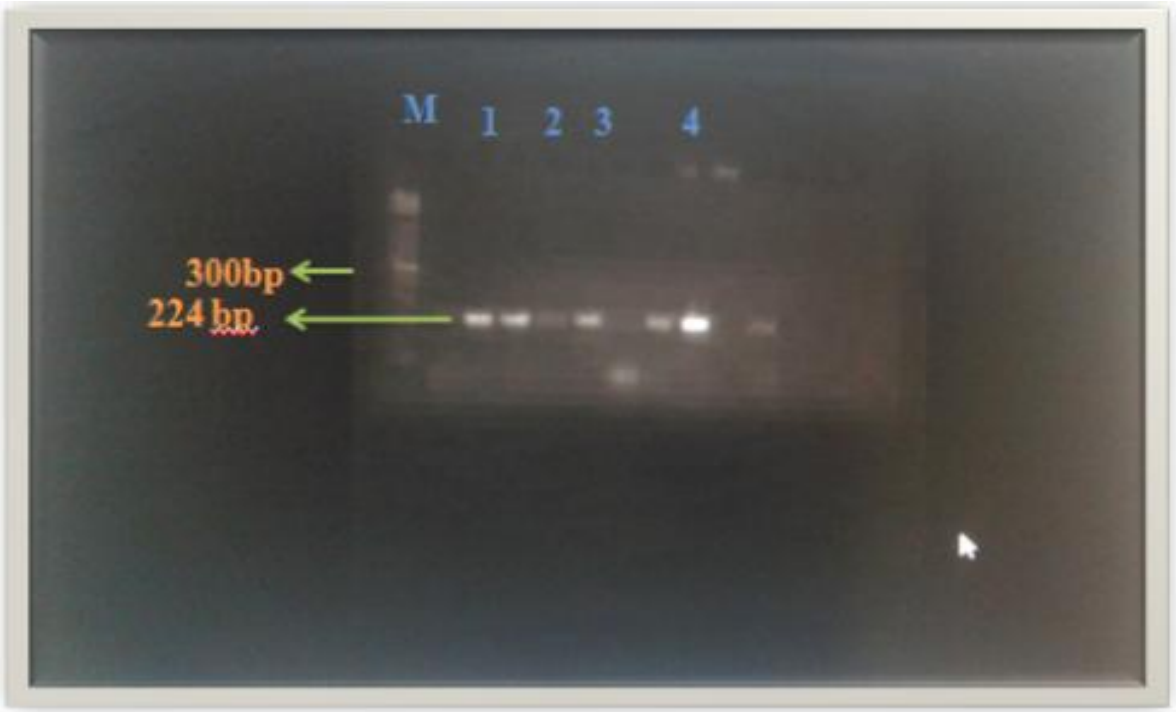

Figure (4.27): PCR for *BCRA1* gene (exon 11-D)

M: Molecular ladder 100 bp **Lane1- 4**: samples with specific band (224 bp).

Figure (4.28): PCR for *BCRA2* gene (exon 11-B)

M: Molecular ladder 100 bp **Lane 1- 5:** Samples with specific band (product with 451bp).

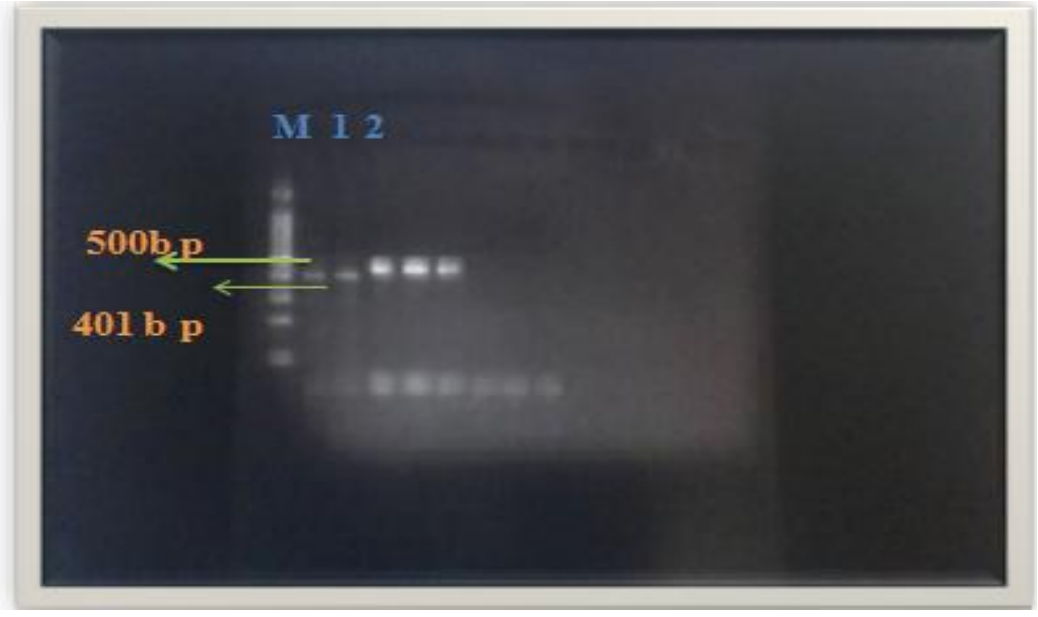

Figure (4.29): PCR for *BCRA1* (exon 20-A)

M: Molecular ladder 100 bp **Lane1&2:** Samples with specific band (product with 401bp**).**

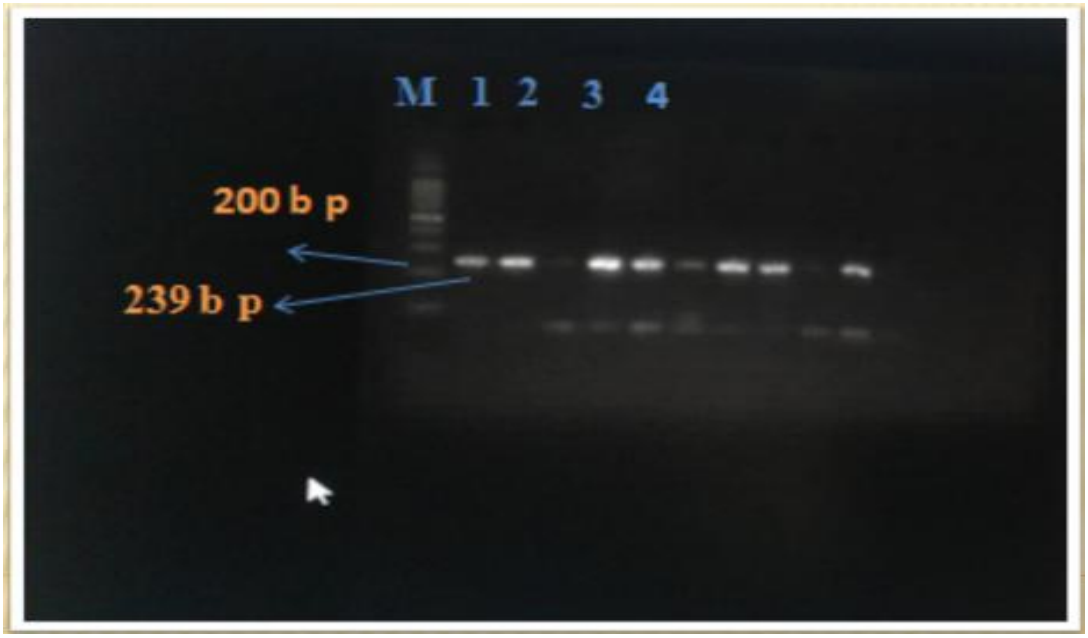

Figure (4.30): PCR for *BCRA1* gene (exon 11-C)

M: Molecular ladder 100 bp **Lane1- 4:** Samples with specific band (239 bp)

Sequencing results:

Multiple sequence alignment with reference sequence, which was obtained from National Center for Biotechnology Information (*NCBI*) (*Ncb*i. nlm. nih. gov).'Reference SNP (Refsnp) database was show three different mutations:

Mutation-1, which found in *BRCA1* exon (20), it was point mutation and was a novel (*de novo*) mutation, in which (G = guanine) was replaced by (A = adenine) (Fig 4.31) at position (160980) (Fig 4.32).

This mutation was appeared in (8) samples of *BC* patients and (8) samples of control, with frequency of (15.4%) and (26.7%) in patients and control respectively (Table 4.7).

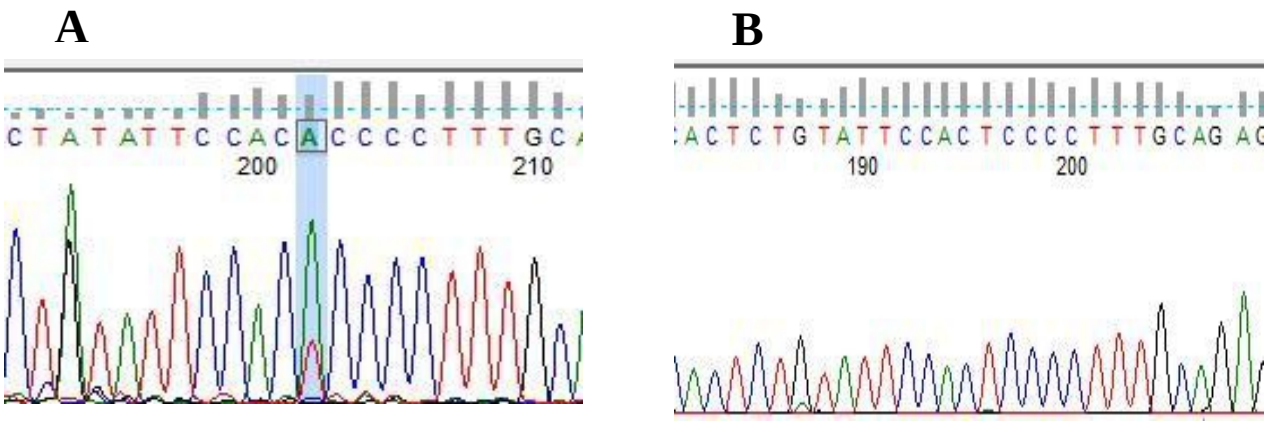

Figure (4.31): Sequence chromatogram (mutation-1)

A. substitution sequence G>A **B.** Normal sequence (Chromatogram shown by Finch TV program).

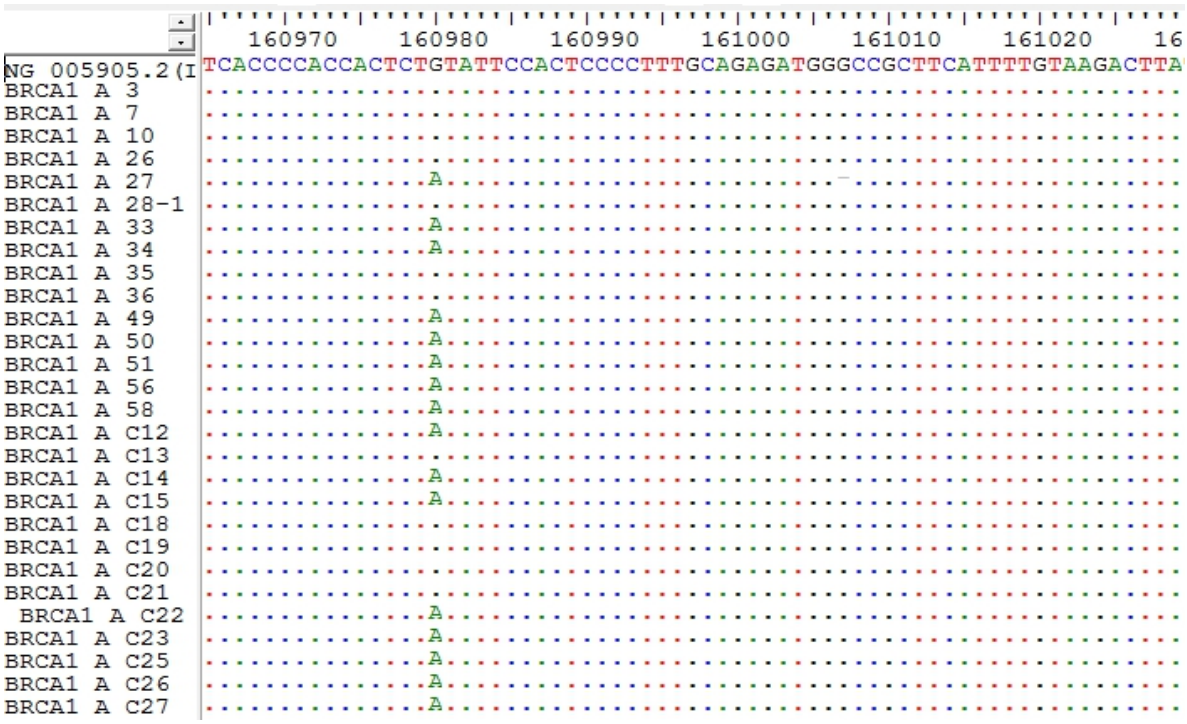

Figure (4.32): Multiple sequence alignment.

BioEdit multiple sequence alignment, point mutations (transition) in which G >A at position 160980 in (A 27, 33, 34, 49, 50, 51, 56, and 58) of *BC* patients and in (A (C12, 14, 15, 22, 23, 25, 26, 27)) of control. The alignment was performed using the Cluster W2 sequence alignment.

Mutation-2 also in *BRCA1* exon (11), it was missense mutation and also a novel one in which (G = guanine) was replaced by (C = cytosine) (**Fig 4.33)** at position (**124756**) of exon (11), after translation the sequences to amino acid sequences, the samples were aligned against *BRCA1* isoform 1 (accession number m: NP_009225), substitution of codon (**AGC**) to (**ACC**) was noted, which convert Serine (**Ser)** amino acid to Threonine (**Thr**) at position **768** (**Fig 4.34)**.

After translation to amino acid sequences, the samples were aligned against *BRCA1* protein isoform 1 (accession number: NP_009225), and then the variant was predicted with SIFT, Polyphen-2, I-Mutant-3, and PhD-SNP softwares to obtain their pathological effects, the result was seen in (**Table 4.27**). Amino acid properties for the wild and mutant residues were obtained using Project Hope software (**Fig 4.35**).

This mutation was presented in three patients (5.8%) (Table 4.8), their age were (45, 56, and 55) years, 2 patients have a positive family history; all were from Jaalia tribe, and with invasive ductal carcinoma histological types (**Table 4.9).**

A **B**

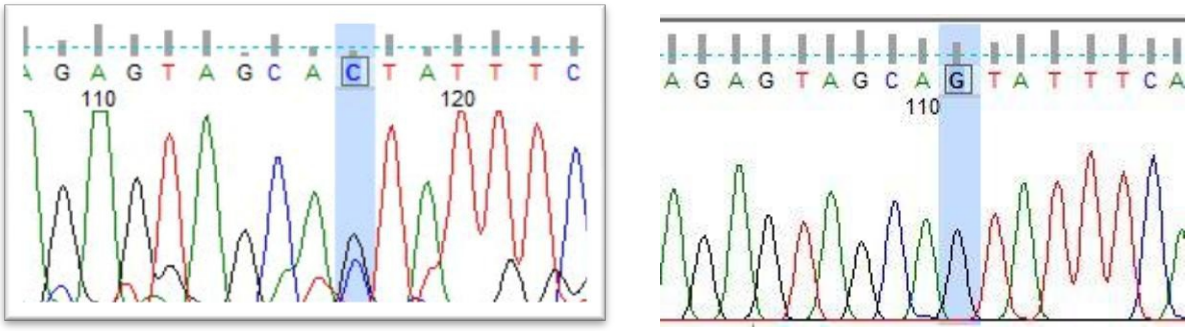

Figure (4.33): Sequence chromatogram (mutation-2)

A. Substitution sequence G > C **B.** Normal sequence (Chromatogram shown by Finch TV program).

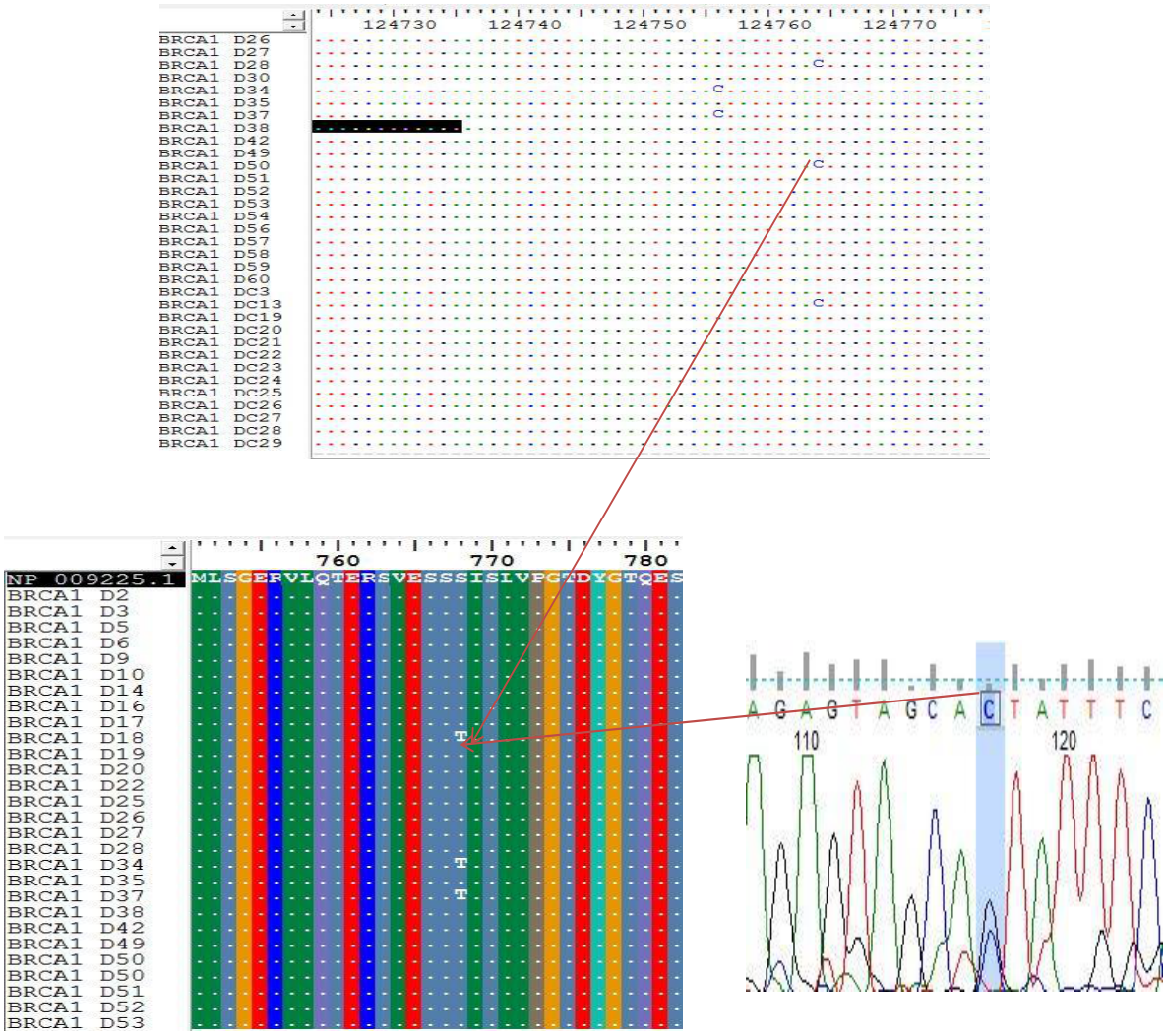

Figure 4.34: Diagram for mutation -2

substitution from a serine (S) to a threonine (Thr) at position 768 in three patients due to missense substitution mutation from guanine (G) to cytosine (C) c.124756 G>C using the transcript variant 1 NM_007294 mRNA that representing the complete *BRCA1* coding sequences used to align all patient sequences under screening, and the corresponding amino acid sequences NP_009225.

Figure 4.34: substitution from a serine (**Ser)** to a threonine (**Thr**) at position 768 in three patients due to missense substitution mutation from guanine (G) to cytosine (C) (c.124756 G>C) using the transcript variant 1 NM_007294 mRNA that representing the complete *BRCA1* coding sequences used to align all patient sequences under screening, and the corresponding amino acid sequences NP_00922.

Table (4.27): SNP prediction obtained by different software:

SNP	**SIFT**		**Polyphen-2**		**1-Mutant**			**PHD**	
	Score	prediction	Score	prediction	**SVM2** Prediction	**RI**	**DDG value Kcal/mol**	Prediction	**RI**
S768T	0.03	Protein damage	0.588	Possibly damaging	Decrease Protein Stability	4	- 0.59	Disease-Related Mutation	4

RI: reliability **DDG:** ΔΔG sign: interaction energy; **SVM:** support vector machines, **SVM2** value**:** DDG < 0: decrease stability, **DDG** >0 increase stability, **DDG** value**:** DG (New Protein)-DG (Wild Type) in Kcal/mole.

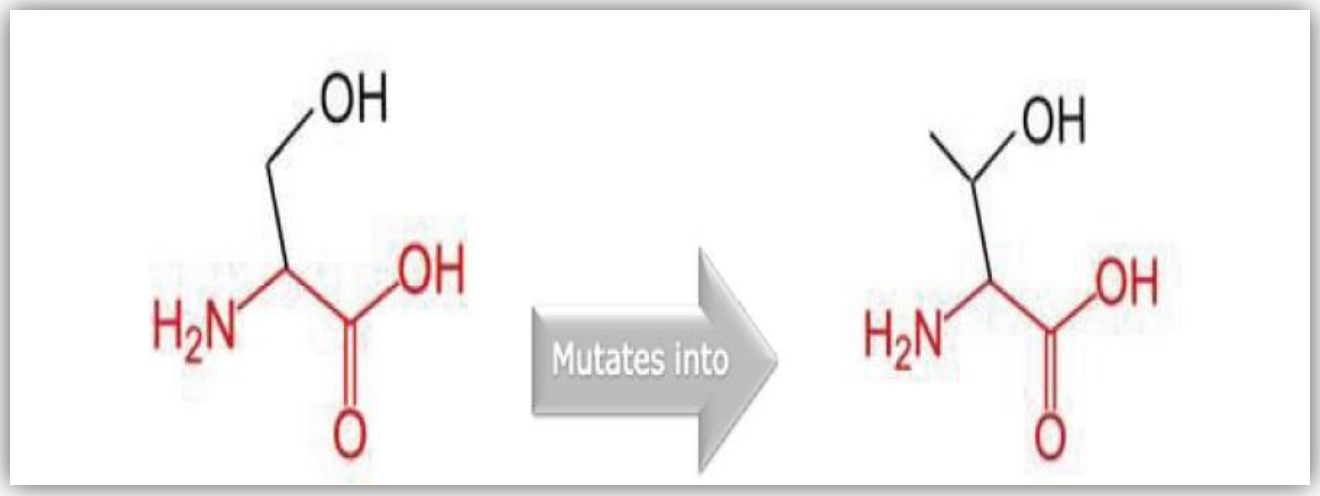

Fig (4.35): Amino acids for the wild type and mutant residues

(The mutant residue is bigger than the wild-type residue; this might lead to bumps**,** using Project Hope software).

From the above information regarding mutation-2 (**S768T**), this mutation has shown to be pathogenic, which may contribute to protein damaging.

Mutation-3 was also found in *BRCA1* - exon (11) **(Table 4.10)**, it was **silent (Synonymous mutation)** in which the third position nucleotide (T) of (**CTT**) codon was replased by (C) resulting (**CTC)** codon, which code for the same amino acid leucine at position **771**.

This is mutation was presented in 2(3.8%) patients, and it has been reported in (*NCBI*) before, referred as (**rs16940**).

The total number of patients with *BRCA1*- mutations (1, 2, and 3) were (11) patients (21.2%) and the rest (41) patients (78.8%) were normal, and 8 (26.7%) in control as shown in (**Table 4.11**).

Two patients with indices (34, 37) were carrying two mutations at the same time, age were (58 &56) respectively, and have positive family history.

BRCA2 sequence chromatogram revealed no mutation was shown when viewed by Finch TV program 1.4.0 (http://www.geospiza.com/ Products/finchtv.shtml), and compared with *BRCA2* nucleotide reference sequences (NM_000059) from (*NCBI).*

Discussion:

Breast cancer (*BC*) is the second most common cancer in the world and, by far, the most frequent malignant disease among women with an estimated (1.7) million new cases diagnosed in 2012 (25 % of all cancer cases). Despite the advancement of diagnostic techniques and treatment in the last decade, (*BC)* is still the most frequent cause of cancer deaths in women in less developed regions (324.000 deaths, 14.3 % of total) and the second cause of cancer deaths in more developed regions (198.000 deaths, 15.4%) after lung cancer (GLOBOCAN, 2012) .

The etiology of *BC* is multifactorial and includes both environmental and genetic factors, as well as genetic and epigenetic changes during progression. Up to (5-10 %) of all *BC* cases and (10-15 %) of all ovarian cancer (*OC)* cases are due to germline mutations in one of the two breast cancer susceptibility genes, *BRCA1* (MIM#113705) and *BRCA2* (MIM#600185) (Miki *et al*, 1994; Wooster *et al*, 1995; Kobayashi *et al*, 2013).

Women with either *BRCA* mutation have a cumulative lifetime risk of invasive breast cancer of about (55-85%) (Chen, 2007). Currently, the majority of clinical genetic testing for breast cancer risk is focused on identifying mutations in these genes.

In Sudan, cancer of the breast is the most commonly diagnosed type of cancer. According to a medical statistical and cancer research report from Radiation and Isotopes Center in Khartoum, is estimated as (17.2%) of all types of cancers in year 2007 and (17.9%) in year 2008 (RICK, 2009).

This is the first study conducted in Shendi to assess the role of *BRCA1&2* mutations in *BC* patients attending the newly established Center for Tumor Treatment and Cancer Research (*TTCRC*) in Shendi, and to determine the most risk factors associated with *BRCA1&2* mutations.

Analysis of *BRCA1* and *BRCA2* genes makes it possible to identify predisposing mutations in affected persons and to determine risks for family members.

A total of (82) samples (52) of unselected *BC* patients and 30 control) were analyzed for *BRCA1&BRCA2* mutation using direct sequence (Sanger sequence).

The average age of the patients with breast cancer under study was (2.44 ± .94), with a range of (30-71) years, more than half of the patients in this study, were diagnosed after (50) years of age.

Worldwide studies have shown that, breast cancer increased with age. In this recent study it seems that the study population is not different from other populations and that the incidence of breast cancer increased with age. Women after (50) years of age are more prone to develop mammary tumors. A direct correlation of age with breast cancer incidence has been observed (Feuer *et al*, 1993).

(Table 4.1): with respect to **residency**, the majority of patients in this study, were 34(65.4%) from Shendi locality, while the remaining 18 (34.6%) from different regions near Shendi, many studies have documented that; breast cancer is more common in urban areas than rural settings (Parking *et al*, 2010).

(Table 4.1): Regarding **socioeconomic status**, in this study out of (52) of cases, it was found that 44 (54.6%) were housewives, 2(3.9%) workers, and 6 (11.5%) were employees. In several studies, higher socioeconomic status (as measured by such factors as education, income and housing) has been linked with higher breast cancer risks (Okobia *et al*, 2006; Baquet & Commiskey, 2000).

(Table 4.1): In regard to **Education**, revealed that 22(42.3%) of *BC* patients were illiterate (data regarding the incidences of illiterate women in Shendi locality not available), 8(15.4%) were primary schools learners, 17(32.1%) were higher schools educated, and 5(9.6%) were university graduates. This may reflect delays seeking to medical attention until their tumors are quite advanced, this delay including a lack of knowledge concerning cancer diagnosis and

treatment, fear of surgery, non-acceptance of hospital treatment and/or preferences for alternative care (Clegg *et al*, 2009; Ibrahim & Oludara, 2012; Yarney *et al*, 2013).

(Table 4.1): Concerning **marital status**, out of (52) of cases, 43(82.7%) of patients were married, while 7 (13.5%) were single, and 2(3.8%) were divorced. In fact these women after married are more exposed to different risk factors like (parity, contraceptive used, and obesity); these are increasing the probability to get cancer of the breast.

Furthermore **(Ethnicity):** Breast cancer in this study population was more frequent in ethnic/tribe Jaalia, 40 (76.9%), while the remaining were (Shaigia, Hassania, Robatab, and Danagla) constituted 12(23.1%). This can be explained on the fact that Jaalia tribe is the most frequent tribe in Shendi, and may include either genetic or environmental factors or both.

(Table 4.1): In regard to **parity**, out of (52) of cases, 36(69.2%) of patients in this study were multiparous, 5(9.6%) were single parous, while 11(21.1%) were nulliparous. An increasing number of full-term pregnancies have been associated with a decreased risk for breast cancer in the general population. Some studies suggested that this may be due to decreased levels of estrogen and progesterone, increased levels of sex hormone-binding globulin, and pregnancy-induced differentiation of breast tissues (Chiaffarino, 2001) early study by Parkin (Parkin, 1994) suggested that multiparity was a risk factor for breast cancers diagnosed prior to (45) years of age, but an apparent protective factor for later onset cancers.

(Table 4.1): Family history, Out of (52) of cases, 23(44.2%) were found with a family history, while 29(55.8%) of patients without family history of cancer. Both males and females contribute (50%) of the inheriting predisposing genetic alterations and susceptibility may equally be contributed by mother or father sides, as incomplete dominance and gender restricted expression patterns do restrict cancer initiation. Hence this (50%) predisposition of inheritance does

not mean that everyone included in their offsprings will develop cancer (Neoman *et al*, 1998). In this study the non hereditary (sporadic) type presents more incidences than hereditary.

(Table 4.1): Regarding age at **menarche,** out of (52) of cases, 46(88.5%) of cases have attended the menarche after (11) years, while 6(11.5%) within (11) years old. Later age of menarche has been associated with a decreased risk of breast cancer in the general population. Early menarche may induce an early proliferation of mammary gland cells through early exposure to high hormonal levels causing an increased risk of breast cancer (Parsa, 2009), therefore early menarche (prior to age 12) is associated with a higher lifetime risk of breast cancer in the general population (Steiner & Knutson, 2008).

(Table 4.1): Referring to **postmenopausal,** out of (52) cases, 33(63.5%) of cases were postmenopausal, while19 (36.5%) were premenopausal. This is consistent with the two registries that have contributed to cancer in five continents over an extended period of time (Uganda and Zimbabwe); the largest increases in incidence overtime were seen among the post-menopausal age groups (Parkin *et al*, 2010; Chokunonga *et al*, 2013).

(Table 4.1): Participating with **physical activity,** was found to be out of (52) cases, 6(11.5%) of *BC* patients, while 46(88.5%) were not. Lack of exercise may increase the risk of breast cancer because exercise lowers hormone levels, alters metabolism, and boosts the immune system. Increased physical activity is associated with a decreased risk of developing breast cancer.

(Table 4.1): Concerning **obesity,** out of (52) cases, 32(61.5%) of patients were obese, 14(27%) with normal weight, and 6(11.5%) were under weight. Weight gain appeared to be predictor of postmenopausal breast cancer and has been associated with a reduced risk of premenopausal breast cancer, weight gain of more than (10) pounds between age (18 and 30) years was associated with an Increased risk of breast cancer diagnosed between age (30 and 40) years (OR =

1.44, 95% CI 1.01-2.04) and this due to the fact of decease the sex hormone during postmenopausal period (Kotsopoulos & Narod, 2005).

(Table 4.1): With respect to using **oral contraceptives** in this study, out of (52) cases, the majority of patients in this study, 39(75%) were not using oral contraceptives, while13 (25%) used to take contraceptives. Contradictory study in a large hospital- based case- control found that, significant increase in breast cancer risk were found among women who had used either oral or injectable contraceptives within the previous (10) years (Huo *et al*, 2008), this indicated that; oral contraceptives are not playing a role as risk factor for breast cancer in the study population because the most case under study are postmenopausal women.

(Table 4.1): Regarding **hormonal therapy** out of (52) cases, most of patients 48 (92.3%) were not taking hormonal therapy and 4(7.7%) were take this therapy. Hormonal replacement therapy increases a woman's risk of breast cancer. In fact, the number of breast cancers diagnosed has been dropping as fewer women have been taking hormone replacement therapy as shown in the literature.

(Table 4.1): Discussing **(smoking),** out of (52) cases, 5(9.6%) of patients were smoking, while 47 (90.4%) of them were not. Few studies considered smoking as a risk factor for breast cancer, but have been shown to impact breast cancer risk in non-African populations (Brinton *et al*, 2015).

(Table 4.1): Reflecting **(exposure to ionizing radiation**), out of (52) cases, 17(32.7%) of *BC* patients were exposed to radiations, while the remaining 35(67.3%) were not. Survivors of atomic bomb in Hiroshima and Nagasaki have shown an increased prevalence of breast cancer as a result of radiation exposure (Tokunaga *et al*, 1994; Morishita *et al*, 2005).

(Table 4.1): Paying attention to histological type versus age ranged between (51 – 60) years, out of (52) cases, results shown that: 46(88.5%) were presented with

invasive ductal carcinoma, 2(3.8%) lobular carcinoma, 2(3.8%) ductal carcinoma in situ and 2(3.8%) with Paget disease.

(Table 4.2): Concerned with the histological types against age: Invasive ductal carcinoma was found to be more presented; estimated as 21(45.7%) in the range of age (51- 60), the results concluded that, no significant statistical relationship between histological type and age was found (P 0.306), and was noted to be more.

(Table 4.3): Determined the histological type versus cancer grade**:** invasive ductal carcinoma was accounted for 31(67.4%) with grade II, showing highly significant statistical value (P 0.000) with cancer grade II.

(Table 4.4): Denoted the genetic inheritance that**,** the sporadic type: (non hereditary) was revealed to be the most presented; resulted as 29 (55.8%), while 23 (44.2%) was hereditary.

(Table 4.5): Referred to family history (family relatives degree) versus breast cancer mutation, percentages were distributed as: 16(69.6%) with second degree relatives, 4(17.4%) with first degree relatives, and 3 (13%) with the third degree relatives.

(Table 4.6): Reflected the attention towards the breast cancer mutation (patients family relatives) percentage among other cancer types, was commented on a**s:** breast cancer was accounted for 17(73.9%) and observed to be the most type of cancer occurrence within the families.

A family history of breast cancer confers a greater risk of breast cancer in an individual. In a meta-analysis of (74) studies of breast cancer risk and family history, it was found that a woman with any first-degree relative affected with breast cancer has a relative risk of (2.1) when compared to women with no family history of breast cancer. A woman with a mother diagnosed with breast cancer has a (2.0) relative risk to develop breast cancer and a woman with a sister diagnosed with breast cancer has a (2.3) relative risk, and women with a second-degree relative diagnosed with breast cancer have a relative risk of (1.5)

to develop the same type of cancer. All of these risks were increased if the relative was diagnosed at a younger age (Pharoah *et al*, 1997).

In this study, three mutations were detected among the studied population, and all are found in *BRCA1* gene.

(Table 4.7): Described the first novel mutation detected, which was point mutation, caused by *BRCA – 1* exon (20)**.** This novel mutation (***de novo***) has not been shown before, and laid in the intron part of *BRCA1* gene.

This mutation (160980 G>A), was found in (8) samples of each case and control, in a frequency of 8(15.4%) &8 (26.7%) respectively**.**

The functional and pathological consequences of this variant is difficult to assess, particularly in case of intronic mutations, because that cannot be directly associated with protein inactivation (Pagani & Baralle, 2004; Plon *et al*, 2008). So it was called **variant of unknown significant (*VUS*).**

Present of this *SNP* in patients and control can be due to one or a combination of the following reasons: possibility of residual false positives, possibility of disease risks and other common phenotypic traits (Chanock, 2001), or may be a protective *SNP*. So moving forward, cohort studies may wish to focus about the relationship of breast cancer and this variant of unknown significant.

Previous study, done by MacArthur have shown that at least (30) *SNPs* loss of function in a healthy individual (MacArthur *et al*, 2010), this is supporting our finding (present of mutation in patients and control).

In Sudan, study that was done by Mojtaba in Khartoum on a patients with positive family history of cancer at the same region (exon 20), the study revealed that; missense a novel mutations (**V1736D**), affected translation of protein (Mojtaba, data submitted for publication). This indicating that; exon 20 of *BRCA1* contributed to breast cancer in Sudan.

(Fig 4.34): Illustrated the second mutation found on *BRCA1* exon (11), was a missense mutation at position number 124756 (**G/C**) which it led to change in

the coding amino acid Serine to Threonine at position number 768. Also it was a novel one, and not described so far.

(**S768T)** SNP was found to affect the resulted translated protein**.** According to Project Hope software, it is indicated in this current study that the mutant residue Threonine (**Thr)** is bigger than the wild type Serine (**Ser**), and this might lead to bump.

(Table 4.8): Analyzed the diagnosed mutational effects caused by *BRCA-1* exon (11): this novel mutation was detected in three patients 3 (5.8%), ages were (45, 55, and 56) years, two of them with a positive family history, the third with a negative family history, all were from Jaalia tribe, and with invasive ductal carcinoma histological type.

In study performed in **northern Sudan** among secondary school girls with known breast cancer in their families, Elnour (Elnour *et al,* 2012) found that; the most mutations were concentrated at exon 11 of *BRCA1*. This has found the ground for the selection of primers to our study.

Other study, Biunno *et al* (Biunno *et al,* 2014) reported 2 novel variants (c.1088A/G: p.Asn 363 Ser and c.5090G/A: p.Cys1697Tyr) in study performed in central Sudan out of a total of 33 *BRCA1* variants were detected in (49/59) premenopausal patients. One of these was deleterious mutation and has a pathogenic impact.

(Table 4.10): Assessing the consequences of the presence of a third novel mutation in *BRCA1* exon 11, which was a (silent mutation: Synonymous) at codon 771, referred as (rs, *reference SNP* 16940) or (**p. L771L**), it was found in two patients 2(3.8%).

The (rs = reference SNP 16940) mutation was reported locally in above mentioned study in central Sudan. Biunno *et al,* (Biunno *et al,* 2014) found this mutation in (21) of patients and mostly like to be neutral mutation or not pathogenic according to the International Agency for Research on Cancer **(***IARC*) (brca.iarc.fr/LOVD).

Worldwide, it has been reported by Fackenthal *et al* in (15.2%) of unselected Nigerian woman diagnosed with invasive ductal carcinoma, Australian woman diagnosed with breast cancer before (40) years (Turkovic *et al,* 2010), Canadian woman (Ozcelik *et al,* 2012), and even found in Ashkenazi Jewish (Pereira *et al*, 2007).

(Table 4.12): Demonstrated a molecular biological linkage between *BRCA2*-exon (11) and breast cancer mutation: all population under the study showed negative results for *BRCA2* exon 11(i.e. no mutation detected in *BRCA2*). This can be explained on the basis that, there may be no *BRCA2* inherited predisposition to the disease. In addition, failure to detect a mutation does not exclude the possibility that the individual has predisposing *BRCA2* mutation as the whole gene was not completely screened; (only one region has been tested in this study). So the cancer risk equal to that of the general population, if there is no evidence for a breast cancer gene inherited. Similar finding was reported by Munsoor (Munsoor, 2013) among woman with breast cancer attending Radio &Isotope Center in Khartoum.

Also, Masri (Masri *et al,* 2002) was reported one mutation and one polymorphism in *BRCA2* of 20 patients diagnosed with breast cancer, indicated that *BRCA2* has a minor role in Sudanese patients.

Other study was done in **Central Sudan**; Awadelkarim (Awadelkarim *et al*, 2007) was found a truncation mutation in *BRCA2* in one male diagnosed with breast cancer.

The increased risk for male breast cancer has been connected with germline *BRCA2* mutations, with an estimated cumulative risk of (3- 6%) by the age (70) years (Easton *et al,* 1997; Thompson & Easton, 2001).

Other study which was done in **Khartoum** was reported another truncation mutation among premenopausal women with breast cancer (Alsmawal, data submitted for publication) at the same region of *BRCA2*.

(Table 4.11): focusing on breast cancer *BRCA1* – mutations – 1, 2, 3, exons (20, 11): In this performed study, the mutations frequencies**,** were found to be, 11(21.2%) & 8(26.7%) in case and control respectively. All were found within *BRCA1*, so that the high frequency of such mutations in *BRCA1* is thought to result from unusually high concentration of **Alu-elements** in the intronic sequences of the gene, rendering it particularly prone to Alu-mediated unequal recombination (Smith *et al*, 1996; Puget *et al*, 1997).

In some other reports patients have been unselected for family history, but the focus has been on early-onset disease (FitzGerald *et al*, 1996; Langston *et al*, 1996; Krainer *et al*, 1997; Malone *et al*, 1998; Hopper *et al*, 1999; Peto *et al*, 1999; Southey *et al*, 1999). Mutation frequencies in these studies have varied from (1.9 -13%) for *BRCA1*and (2.2 -2.7%) for *BRCA2*. In a few studies where patients have been unselected both for family history and age at diagnosis the sample size has been quite small (130-211) and only (*BRCA1)* has been analyzed (Newman *et al*, 1998; Tang *et al*, 1999). The mutation frequencies in these studies have varied between (1.4 -3.8%). This reflected that; the frequency of mutations was decreased in unselective patients.

In regard to risk factors associated with mutations in the current study population:

(Table 4.13): Revealed the presence of a junction binding breast cancer mutation with patient's age; most patients with *BRCA* mutations, 8 (72.7%) were allocated among age (51-60) years, there is no significant statistical relationship between age and *BRCA* mutations carriers (P= 0.233). This is indicating that mutations tend to be somatic rather than inherited.

Similar finding was found in a study of a meta-analysis of (22) studies unselected for family history showed that by age (50), it was estimated that approximately (40%) of *BRCA1* carriers would develop breast cancer and in *BRCA2* carriers it was estimated that approximately (15%) would develop breast cancer by age (50).

Other study of (676) Ashkenazi Jewish families and (1,272) families of other ethnicities in the (*USA*) showed that the cumulative risk to age (50) for *BRCA1* carriers was 28% (CI 24-34) for breast cancer and the cumulative risks for *BRCA2* carriers were 23% (CI 19-29) (Antoniou & Easton, 2003).

(Table 4.14): Showed a significant statistical linkage between tribe/ ethnic and breast cancer mutation: the study demonstrated that the mutated patients accounted for 6 (54.5%) were Jaalia, while 5(45.6%) from other tribes, there was significant statistical relationship between mutations and Jaalia tribal/ethnicity (P 0.047). the finding of the recent study was supported by other study, Kurian (Kurian , 2009) was found that, in individuals diagnosed with a breast cancer, *BRCA1* and *BRCA2* mutation rates across ethnic groups are fairly similar with a rate of (1-4%) for each gene. Therefore, the differences in prevalence of breast cancer among different ethnic/racial populations cannot be explained exclusively by the rate of mutations in the *BRCA1* and *BRCA2* genes in those populations. There are likely other genetic factors within racial groups that influence a woman's risk of developing breast.

Most mutations are unique to the families they have been identified in, but in many populations recurrent founder mutations also exist. The founder effect is most clearly seen in Iceland, where one *BRCA2* mutation (999del5) is estimated to account for up to (76%) of families with multiple cases of female breast cancer and/or male breast cancer (Thorlacius *et al,* 1996). Population frequency of this mutation is estimated to be about (0.5%) (Johannesdottir *et al,* 1996; Thorlacius *et al,* 1997). Strong founder effect is also seen among Ashkenazi Jewish, where approximately (2.5%) of individuals carry (1) of the (3) founder mutations (185delAG or 5382insC in *BRCA1,* 6174delT in *BRCA2*) (Roa *et al*, 1996; Struewing *et al*, 1997; Hartge *et al*, 1999). These mutations are estimated to account for (45%) of high-risk families among Ashkenazis (Tonin *et al*, 1996).

(Table 4.16): Demonstrated the presence of a genetic binding between breast cancer incidence and family history**:** the following results were observed, 6 (54.5%) have a family history, while 5 (45.5%) without family history, there was no statistically significant relationship between family history and mutations in the present study population.

Many studies have shown that familial history of either breast or ovarian cancer alone or together increase likelihood of presence of a cancer predisposing mutation (Couch *et al*, 1997; Shattuck- Eidens *et al*, 1997).

(Table 4.17): Revealed that, there was no association between breast cancer mutation risk and age at menarche: results estimated that, 10(90.9%) had attended menarche after age (11) years, while 1(9.1%) in age (11) years, there was no statistical significant relationship between age at menarche and mutation. Similar study showed no association with age at menarche and breast cancer risk in *BRCA1* or *BRCA2* mutation carriers (Chang &Easton, 2007).

One study found a later age of menarche in *BRCA1* mutation carriers was associated with a decreased breast cancer risk, however no protective effect was observed in *BRCA2* mutation carriers (Lee & Ursin, 2008).

(Table 4.18): Clarified the presence of a genetic binding between obesity and breast cancer mutation: results calculated were revealed the following, 7(63.6%) were overweight, 2(18.2%) have a normal weight, and 2(18.2%) less than normal weight; also there was no statistical significant value between obesity and mutation (P= 0.625). King and colleagues recently reported that a healthy weight defined at menarche and at age (21), as well as physical activity during adolescence, were associated with a significant delay in the age of onset of breast cancer in *BRCA1* and *BRCA2* carriers; however, such an effect could be attributable to either weight gain increasing the risk of early-onset breast cancer or to weight gain protecting against late-onset breast cancer (King *et al*, 2003). Other study of (46) *BRCA1* carriers found no significant effect of current *BM*I on the age at disease onset (Cullinane *et al*, 2005).

(Table 4.19): Denoted the presence of a correlation between oral contraceptives use against breast cancer mutation: the study indicated that; 4 (36.4%) used oral contraceptives, while the remaining were not; there was no statistically significant relationship between oral contraceptives used and mutations (P 0.327). However, using of oral contraceptives in *BRCA1* mutation carriers as prevention against ovarian cancer may increase breast cancer risk , no statistical significant value has been observed in *BRCA1* mutation carriers after using oral contraceptives for (1) year (Haile *et al*, 2006). Other recent publication has shown that; a quite opposite result which indicates that *BRCA* mutation carriers as well as women with strong familial history are more prone to exogenous hormones present in oral contraceptives (Pasanisi *et al*, 2009).

(Table 4.15): Indicated the relationship between parity and breast cancer mutations, results tabulated were showed the following: 7(63.6%) were multiparous, 1 (9.1%) was single parous, and 3 (27.3%) were nulliparous; there was no statistically significant value between parity and mutation (P = 0.855). Similar a study performed in a population of *BRCA1* and *BRCA2* mutation carriers found a decreased risk of breast cancer as the number of full-term pregnancies increased (Lee & Ursin, 2008)), however the results were not statistically significant. Another study found no statistically significant difference in the risk of breast cancer between parous and nulliparous women (Andrieu & Chang, 2006).

(Table 4.20): Showed the effects of exposure to radiation against breast cancer mutations, the study denoted that, there was no association between exposure to radiation and mutation, it was estimated for 3(27.3%), there was no statistically significant difference between exposure to radiation and mutations (P= 0.666). A similar cohort study of *BRCAs* mutation carriers treated with breast conserving therapy (*BCT*) showed no evidence of increased radiation sensitivity either in breast or any other organ of the body (Pierce *et al*, 2000).

(Table 4.21): Prevailed a molecular biological binding between menopause and breast cancer mutation: the study arrived at that, 7(53.6%) have menopause, while the other were not; there was no statistical significant relationship between menopause and mutations (P= 0.989). A study of *BRCA1* and *BRCA2* mutation carriers found no overall association with menopause and breast cancer risk (Chang & Easton, 2007). Other study found early menopause to confer an odds ratio of (0.6) when women who underwent menopause prior to age (45) were compared to those who underwent menopause after age (45) (Chiaffarino *et al*, 2001).

(Table 4.22): Identified a correlation between histological types and breast cancer mutation, the study summarized that, all mutated patients were presented with invasive ductal carcinoma, but also there was no statistically significant value between histological types and mutations (P= 0.611). This indicates that; invasive ductal carcinoma is the most histological type appears with *BRCA* mutation.

(Table 4.23): Stated an association between cancer grade and breast cancer mutation: the study demonstrated that; 6 (54.5%) have grade II, while 5 (45.5%) have grade III, there was no statistical significant difference between cancer grades and mutations (P= 0.447). Always breast cancer associated with *BRCA* mutation presents with high grade.

In respect to relation between *ER, PR,* and *HER-2* and mutations, the study found that; there was no significant statistical value binding mutations and *ER, PR* (P= 0.812, P= 0.372) respectively.

BRCA- associated tumors, which are more often of higher grade than that of sporadic breast cancer, would therefore be predicted to be more often (*ER)* negative. Numerous studies (Osien *et al*, 1998; Osien, 1998; Johansson *et al*, 1997; Armes *et al*, 1999) have shown low levels of (*ER*) expression in familial breast cancers. Osien *et al* (Osien *et al*, 1998; Osien, 1998) have shown that when (*ER*) was assessed in *BRCA* associated tumors in comparison with a grade-

matched control group, the expression of (*ER)* in *BRCA1*-associated tumors was still significantly lower (8 versus 26%). In contrast, the expression of (*ER)* in *BRCA2*-associated tumors appears to be similar to that in sporadic breast cancers.

Expression of progesterone receptor (*PgR*) is indirect evidence of retained transcriptional activation activity of (*ER)*, and it has been shown that (*PgR)* expression have stronger correlation with prognosis in breast cancer than (*ER*) expression alone (Loakim *et al*, 1997).

Osien *et al* (Osien *et al*, 1998) showed that, although (9) out of (40) familial breast cancer patients were (*ER*) positive, only two of these were also (*PgR*) positive. This suggests that even in cases where (*ERs*) could be identified immunohistochemically, their functional ability may be compromised.

In contrast, there was significant relationship between mutation and *HER-2* (P 0.017). Data on (*HER2/neu*) are limited and conflicting. Armes *et al* (Armes *et al*, 1999) and Robson *et al* (Robson *et al*, 1998) have not shown a difference in (*HER2/neu*) expression between sporadic and familial cancers. The study by Johansson *et al* (Johansson *et al*, 1997), however, demonstrated that (*c-erbB2)* expression in *BRCA1*-associated cancers is lower than would be predicted on the basis of their histological grade.

Finally a large-scale population-based study is needed to establish the clinico-pathological characteristics as well as the genetic pathology related to *BRCA* mutations in Sudanese breast cancer patients, and to identify the most risk factors contributed to breast cancer risk. However, identification of new mutations, suggesting that; genetic test plays an important role in disease progress and therapy improvement in mutation carriers.

Knowledge of the ethnic background of an individual can direct mutation testing

Conclusion:

- *BRCA1* mutations are responsible for a significant proportion of breast cancer of this study population.
- *BRCA* mutations were found in individuals with and without family history.
- *BRCA2* was not contributed to hereditary breast cancer in this study.
- Ethnicity plays a role as risk factor for mutation inheritance.

Recommendations:

- An urgent need to develop effective and affordable approaches to the early detection, diagnosis, and treatment of breast cancer among women living in less developed countries.
- Genetic counseling can help a person to understand what an ambiguous change in *BRCA1* or *BRCA2* may mean in terms of cancer risk.
- Molecular testing is the best and required way for early detection of breast cancer. It is valuable to offer genetic testing to newly diagnosed cases with breast cancer for the purpose of clinical management and as a mean to identify presymptomatic carrier relatives for prevention.
- A comprehensive understanding of *BRCA1/2* genotypes and associated tumor phenotypes is needed to enable targeted therapies.
- Furthermore, genome-wide exome sequencing and targeted high-throughput deep-sequencing will be utilized in order to obtain a more comprehensive view of the involved genetic defects in predisposition to breast cancer.
- A significant amount of additional biological and bioinformatics analyses are required to establish the clinical utility of these novel non coding intronic variants.

- Early detection of breast cancer is of high priority in medical management of the disease, and better than treating the disease in late stages.
- Mutational analysis for a limited set of founder mutations requires much less time, resources, and labor than complete sequencing. Recommendations can be made for public health action on molecular genetic testing. The increased public awareness of the nature and prevalence of breast cancer may result in an increased demand for genetic testing for breast cancer susceptibility.
- Risk factors others than tribe/ethnicity may contribute to disease, so future study with a large sample size will be recommended.

References:

Agarwal G, pradeep P, Agarwal V, Yip C and Cheung P (2007). Spectrum of Breast Cancer in Asian Woman. *World Journal of Surgery*; 31(5), 1031-1040.

Ahlbom A, *et al* (1997). Cancer in twins: genetic and non genetic familial risk factors**.** *Journal of the National Cancer Institute;* 89(4): p. 287-93.

American Cancer Society (2006). Cancer Facts and Figures. *Atlanta.*

Ames BN (1987). What are the major carcinogens in the etiology of human cancer? Environmental pollution, natural carcinogens and the causes of human cancer: six errors. *Environ Health Perspect*; 74:237-247.

Anderson DE (1980). An inherited form of large bowel cancer: Muir's syndrome. *Cancer*; 45:1103-7.

Andrieu N, *et al* (2006). Pregnancies, breast-feeding, and breast cancer risk in the International *BRCA1/2* Carrier Cohort Study (*IBCCS*). *Journal of the National Cancer Institute;* **98**(8): p. 535-44.

Antoniou AC, *et al* (2006). Parity and breast cancer risk among *BRCA1* and *BRCA2* mutation carriers**.** *Breast cancer research: BCR;* **8**(6): p. R72.

Antoniou AC, *et al* (2007). *RAD51 135G>C* modifies breast cancer risk among *BRCA2* mutation carriers: results from a combined analysis of 19 studies. *American journal of human genetics*; **81**(6): p.1186-200.

Antoniou A C, *et al* (2010). A locus on *19p13* modifies risk of breast cancer in *BRCA1* mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. *Nature genetics;* **42**(10): p. 885-92.

Antoniou A, *et al* (2003). Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *American journal of human genetics*; **72**(5): p. 1117-30.

Antoniou A, PD Pharoah, S Narod, HA Risch, and DF Easton (2003). Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2*

mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *American journal of human genetics* 72:1117-1130.

Armes JE, *et al* (1999). Distinct molecular pathogeneses of early-onset breast cancers in *BRCA1* and *BRCA2* mutation carriers: a population-based study. *Cancer Res*; **59,** 2011-7.

Armes JE, Trute L, White D, *et al* (1999). Distinct molecular pathogeneses of early- onset breast cancers in *BRCA1* and *BRCA2* mutation carriers: a population-based study**.** *Cancer Res;* **59**:2011-2017.

Atschul SF, Madden TL, Schaffer AA, *et al* (1997). Gapped BLAST and PSI-BLAST. A new generation of protein database search programmes". *Nucleic Acid Res*; 25: 3389-3402.

Avizienyte E, Roth S, Loukola A, Hemminki A, *et al* (1998). Somatic mutations in *LKB1* are rare in sporadic colorectal and testicular tumors. *Cancer Research;* 15:2087-90.

Ballard-Barbash R (1994). Anthropometry and breast cancer. Body size - a moving target**.** *Cancer;* **74 (**3 Suppl**):**1090-1100.

Bapat B, Xia L, Madlensky L, Mitri A, Tonin P, Narod SA and Gallinger S (1996). The genetic basis of Muir-Torre syndrome includes the hMLH1 locus. *Am J Hum Genet*; 59:736-739.

Baquet CR &Commiskey P (2000). Socioeconomic factors and breast carcinoma in multicultural women. *Cancer;* 88:1256-1264 [PubMed: 10705364].

Barnes DM & Gillett CE (1998): Cyclin D1 in breast cancer**.** *Breast Cancer Res Treat;* **52**:1-15.

Beckmann MW, *et al* (1996). Clinical impact of detection of loss of heterozygosity of *BRCA1* and *BRCA2* markers in sporadic breast cancer. *Br J Cancer*; **73,** 1220-6.

Beckmann MW, Niederacher D, Schnurch HG, Gusterson BA, Bender H G (1997). Multistep carcinogenesis of breast cancer and tumor heterogeneity. *J Mol Med*; **75:**429- 439.

Bell DW, *et al* (1999). Heterozygous germ line *hCHK2* mutations in Li-Fraumeni syndrome. *Science*; **286,** 2528-31.

Beukers R & Berends W (1960). Isolation and identification of the irradiation product of thymine. *Biochim Biophys Acta*; 41:550-551.

Bhardwaj N (2007). Harnessing the immune system to treat cancer. *J Clin Invest*; **117:**1130-1136.

Bignell GR, Barfoot R, Seal S, Collins N, Warren W and Stratton MR (1998). Low frequency of somatic mutations in the LKB1/Peutz-Jeghers syndrome gene in sporadic breast cancer. *Cancer Res;* 58:1384-6.

Bishop JM (1991). Molecular themes in oncogenesis. *Cell*; **64**(2): p. 235-48

Blount WP (1961). Turkey 'X" disease. *J Br Turkey Fed;* 9:522-528.

Bolton SJ (2006). Cellular function of the *BRCA* tumor-suppressor proteins**.** *Biochem Soc Trans*; 34:633-645.

Bork P, Blomberg N, Nilges M (1996). Internal repeats in the *BRCA2* protein sequence**.** *Nature genetic*; 13:22-23.

Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV (1997). A super family of conserved domains in *DNA* damage - responsive cell cycle checkpoint proteins**.** *FASEB J*; 11:68-76.

Botstein D, White RL, Skolnick M, *et al* (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet*; 32(3):314-31.

Boyd NF, *et al* (1999). Mammographic densities and risk of breast cancer among subjects with a family history of this disease. *J Natl Cancer Inst*; 91:1404-8.

Breast Cancer Linkage Consortium (1997). Pathology of familial breast cancer: differences between breast cancers in carriers of *BRCA1* or *BRCA2* mutations and sporadic cases. *Lancet;* 349:1505-1510.

Brugieres L, Gardes M, Moutou C, Chompret A, *et al* (1993). Screening for germ line p53 mutations in children with malignant tumors and a family history of cancer**.** *Cancer Res*; 53:452-5.

Buchholz TA, Wu X, Hussain A, Tucker SL, Mills GB, *et al* (2002). Evidence of haplotype insufficiency in human cells containing a germline mutation in *BRCA1* or *BRCA2***.** *Int J Cancer*; **97,** 557-61.

Callebaut I & JP Mornon (1997). From *BRCA1* to *RAP1*: a widespread *BRCT* module closely associated with *DNA* repair. *FEBS letters;* **400**(1): p. 25-30.

Cantu JM, Rivera H, Ocampo-Campos R, Bedolla N, *et al* (1980). Peutz-Jeghers syndrome with feminizing Sertoli cell tumor. *Cancer;* 46:223-228.

Capriotti E, Calabrese R & Casadio R (2006). Predicting the insurgence of human genetic disease associated to single point protein mutation with Support Vector Machines and evolutionary information. *Bioinformatics;* 22: p. 2729-2734.

Carter CL, Corle DK, Micozzi MS, Schatzkin A & Taylor PR (1988). A prospective study of the development of breast cancer in 16,692 women with benign breast disease**.** *Am J Epidemiol*; 128:467-477.

Case RA (1969). Some environmental carcinogens. *Proc R Soc Med*, 62:1061-1066.

Chang-Claude JN Andrieu, M Rookus, R Brohet, AC Antoniou, and Easton (2007). Age at menarche and menopause and breast cancer risk in the International *BRCA1*/2 Carrier Cohort Study. *Cancer Epidemiol Biomarkers Prev* 16:740-746.

Chen J & A Lindblom (2000). Germline mutation screening of the *STK11*/*LKB1* gene in familial breast cancer with *LOH* on *19p*. *Clinical genetics;* **57**(5): p. 394-7.

Chen PL, *et al* (1998). The *BRC* repeats in *BRCA2* are critical for *RAD51* binding and resistance to methyl methanesulfonate treatment**.** *Proceedings of the National Academy of Sciences of the United States of America;* **95**(9): p. 5287-92.

Chen WY, Colditz GA, Rosner B, Hankinson SE, Hunter DJ, *et al* (2002). Use of postmenopausal hormones, alcohol, and risk for invasive breast cancer. *Ann Intern Med*; 137 (10): 798-804.

Chen Y, *et al* (1995b). Aberrant subcellular localization of *BRCA1* in breast cancer. *Science*; 270**,** 789-91.

Cheney IW, Neuteboom ST, Vaillancourt MT, Ramachandra M and Bookstein R (1999). Adenovirus-mediated gene transfer of MMAC1/PTEN to glioblastoma cells inhibits S phase entry by the recruitment of p27Kip1 into cyclin E/CDK2 complexes**.** *Cancer Res;* 59:2318-23.

Cheng KC & Loeb LA (1993). Genomic instability and tumor progression: Mechanistic considerations. *Adv Cancer Res*; 60:157-180.

Chiaffarino F, *et al* (2001). Reproductive and hormonal factors and ovarian cancer. *Ann Oncol* 12:337-341.

Chlebowski RT, *et al* (2003). Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *JAMA;* 289:3243-53.

Chokunonga E, Borok MZ, Chirenje ZM, Nyakabau AM, Parkin DM (2013). Trends in the incidence of cancer in the black population of Harare, Zimbabwe 1991-2010. *Int J Cancer*; 133:721-729. [PubMed: 23364833].

Claus EB, Schildkraut JM, Thompson WD & Risch NJ (1996).The genetic attributable risk of breast and ovarian cancer**.** *Cancer*, **77,** 2318-24.

Clegg-Lamptey JN, Dakubo JC, Attobra YN (2009). Psychosocial aspects of breast cancer treatment in Accra, Ghana. *East Afr Med J*; 86:348-353. [PubMed: 20499785].

Cleton-Jansen, AM, Collins N, Lakhani SR, Weissenbach, J, *et al* (1995). Loss of heterozygosity in sporadic breast tumors at the *BRCA2* locus on chromosome 13q12- q13. *Br J Cancer*, **72,** 1241-4.

Collaborative Group on Hormonal Factors in Breast Cancer (1996). Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet* 347:1713-2.

Collins N, Wooster R & Stratton MR (1997). Absence of methylation of CpG dinucleotides within the promoter of the breast cancer susceptibility gene BRCA2 in normal tissues and in breast and ovarian cancers. *Br J Cancer*; **76,** 1150-6.

Cook JW, Hewett CL & Hieger I (1933). The isolation of a cancer producing hydrocarbon from coal tar. *J Chem Soc*; 395-405.

Core B B C *http://research.nhgri.nih.gov/bic/.* Accessed on July 2016.

Cox A, *et al* (2007). A common coding variant in *CASP8* is associated with breast cancer risk**.** *Nature genetics;* **39**(3): p. 352-8.

Crook T, Brooks LA, Crossland S, *et al* (1998). *P53* mutation with frequent novel codons but not a mutator phenotype in *BRCA-1* and *BRCA-2* associated breast tumors**.** *Oncogene;* **17**:1681-1689.

Cullinane CA, *et al* (2005). Effect of pregnancy as a risk factor for breast cancer in *BRCA1*/*BRCA2* mutation carriers**.** *International journal of cancer*; **117**(6): p. 988-91.

Culver J, Lowstuter K & Bowling L (2006, 2007). Assessing Breast Cancer Risk and *BRCA*1/2 Carrier Probability, 5-20.

Cuny M, Kramar A, Courjal F, *et al* (2000). Relating genotype and phenotype in breast cancer: an analysis of the prognostic significance of amplification at eight different genes or loci and of *p53* mutations. *Cancer Res*; **60:**1077-1083.

Davies AA, Masson JY, Mellwraith MJ, Stasiak AZ, Venkitaraman AR, West SC (2001). Role of *BRCA2* in control of the *RAD51* recombination and DNA repair protein. *Mol Cell*; 7:273 -282.

De Jong JS, van Diest PJ, van der Valk P, and Baak JP (1998). Expression of growth factors, growth-inhibiting factors, and their receptors in invasive breast cancer II: Correlations with proliferation and angiogenesis. *J Pathol*; **184:**53-57.

DeLeon ML, Liede A, Kwan E, Mapua CA, Cutiongeo EM, Tun A (2002). *BRCAl* and *BRCA2* mutations among breast cancer patients from the Philippines. *Int J Cancer;* 98:596-603.

Deng CX & Brodie SG (2000). Roles of *BRCA1* and its interacting proteins. *Bioessays*; 22:728-737.

Deng CX & Wang RH (2003). Roles of *BRCA1* in *DNA* damage repair: a link between development and cancer. *Hum Mol Genet*; Spec No 1:R113-23.

Dorum A, Hovig E, Trope C, Inganas M, Moller P (1999). Three percent of Norwegian ovarian cancers are caused by *BRCAl* 1675 del A or 1135 ins A**.** *Eur J Cancer;* 35:779 -781.

Duffy MJ (1996). Proteases as prognostic markers in cancer. *Clin Cancer Res;* **2**:613-618.

Dufloth RM, Carvalho S, Heinrich JK, Shinzato JY, dos Santos CC, Zeferino LC, *et al* (2005). Analysis of *BRCA1* and *BRCA2* mutations in Brazilian breast cancer patients with positive family history. *Sao Paulo medical journal Revistapaulista de medicina*; 123(4):192-7.

Dunn GP, Bruce AT, Ikeda H, Old LJ, and Schreiber RD (2002). Cancer immune editing: from immune surveillance to tumor escape. *Nat Immunology*; **3:**991-998.

Dupont WD, *et al* (1994). Long-term risk of breast cancer in women with fibroadenoma. *N Engl J Med*, 331:10-5).

Easton DF (1994). Cancer risks in A-T heterozygotes. *Int J Radiat Biol*; **66,** S177-82.

Easton DF, Bishop DT, Ford D & Crockford GP (1993). Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am J Hum Genet*; **52,** 678-701.

Easton DF, *et al* (2007). Genome-wide association study identifies novel breast cancer susceptibility loci**.** *Nature*; **447**(7148): p. 1087-93.

Easton DF, *et al* (1997). Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12-13. *Am J Hum Genet,* **61,** 120-8.

Eisinger F, *et al* (1998). Mutations at *BRCA1*: the medullary breast carcinoma revisited. *Cancer Res*; **58,** 1588-92.

El-Deiry WS, Harper JW, O'Connor PM, *et al* (1994). WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis**.** *Cancer Res;* **54**: 1169-1174.

Elledge RM & Allred DC (1998). Prognostic and predictive value of *p53* and *p21* in breast cancer. *Breast Cancer Res Treat;* 52:79-98.

Elledge SJ (1996). Cell cycle checkpoints: preventing an identity crisis. *Science*; 274:1664-1672.

Elnour AM Elderdery AY, Mills J, Mohammed BA, Elbietabdelaal D, Mohamed A O, *et al* (2012). *BRCA1&2* mutations in Sudanese secondary school girls with known breast cancer in their families. *Int J Health Sci*; 6(1):63-71.

Engel C, *et al* (2010). Association of the variants *CASP8 D302H* and *CASP10 V410I* with breast and ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*;* **19**(11): p. 2859-68.

Expasy translate tool. http://web. expasy. org/ translate. accessed 22 Sep 2016

Fackenthal J D, Zhang J, Zhang B *et al* (2012). High prevalence of *BRCA1* and *BRCA2* mutations in unselected Nigerian breast cancer patients**.** *Int J Cancer;* 131(5):1114-1123.

Family history and the risk of breast cancer: a systematic review and meta-analysis. *International journal of cancer* 71:800- 809.

Fasching PA, *et al* (2007). Evaluation of mathematical models for breast cancer risk assessment in routine clinical use. *European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation;* **16**(3): p. 216-24.

Fearon ER (1997). Human cancer syndromes: clues to the origin and nature of cancer. *Science*; **278,** 1043-50.

Ferlay J, Soerjomataram I, Ervik M, Dikshit R Eser S, Mathers C, *et al* (2013). Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: *International Agency for Research on Cancer*. Available from http://globocan.iarc.fr.

Fernandez PL, Jares P, Rey MJ, Campo E, Cardesa A (1998). Cell cycle regulators and their abnormalities in breast cancer. *Mol Pathol*; **51:**305-309.

Fero ML, Randel E, Gurley KE, Roberts, JM & Kemp CJ (1998). The murine gene p27Kip1 is haplo-insufficient for tumor suppression. *Nature*; **396,** 177-80.

Feuer EJ, Wun LM, Boring CC, Flanders WD, *et al* (1993)**.** The life time risk of developing breast cancer**.** *J Nat Cancer Inst*; 85:892-7.

FitzGerald MG, *et al* (1996). Germ-line *BRCA1* mutations in Jewish and non-Jewish women with early- onset breast cancer. *N Engl J Med*; **334,** 143-9.

Fodde R & Losekoot M (1994). Mutation detection by denaturing gradient gel electrophoresis (DGGE)**.** *Hum Mutat;* 3(2):83-94.

Foley TR, McGarrity TJ &Abt B (1988). Peutz-Jeghers syndrome: a clinicpathologic sur" Gastroenterology vey of the Harrisburg family with a 49 - year follow-up, 95:1535-40.

Fong PC, *et al* (2009). Inhibition of poly (*ADP-ribose*) polymerase in tumors from *BRCA* mutation carriers. *The New England journal of medicine;* **361**(2): p. 123-34.

Ford D & Easton DF (1995). The genetics of breast and ovarian cancer. *Br J Cancer;* 72 (4): 805-812.

Ford D, *et al* (1998). Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *Am J Hum Genet*, 62:676-89.

Forozan F, Karhu R, Kononen J, *et al* (1997). Genome screening by comparative genomic hybridization. *Trends Genet*; 13 (10):405-9.

Foulkes WD (2004). *BRCA1* functions as a breast stem cell regulator. *J Med Genet*; **41:**1-5.

Franca LT, Carrilho E, Kist TB (2002). A review of *DNA* sequencing techniques. *Q Rev Biophys*; 35 (2):169-200.

Frank TS, *et al* (2002). Clinical characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: analysis of 10,000 individuals. *J Clin Oncol*; **20,** 1480-90.

Freidman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, *et al* (1994). Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nat. Genet*; 8:399-404.

Fulco RA, Petix M, Salimbeni V, Torre EA (1998). Prognostic significance of the estrogen-regulated proteins, cathepsin-D and pS2, in breast cancer. *Minerva Med;* **89**:5-10.

Futreal PA, *et al* (1994). *BRCA1* mutations in primary breast and ovarian carcinomas. *Science*; **266,** 120 -2.

Gad S, *et al* (2002). Significant contribution of large *BRCA1* gene rearrangements in 120 French breast and ovarian cancer families. *Oncogene*; **21,** 6841-7.

Gayther, *et al* (1997a). Frequently occurring germline mutations of the *BRCA1* gene in ovarian cancer families from Russia. *Am J Hum Genet*; **60,** 1239- 42.

Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, *et al* (2000). Very high risk of cancer in familial Peutz-Jeghers syndrome; *Gastroenterology* 119:1447-53.

Gillett CE & Barnes DM (1998). Demystified cell cycle. *Mol Pathol*; **51:**310-316.

Gillett CE, Lee AH, Millis RR, Barnes DM (1998). Cyclin D1 and associated proteins in mammary ductal carcinoma *in situ* and atypical ductal hyperplasia. *J Pathol;* **184**:396-400.

Glavac D & Dean M (1995). Applications of heteroduplex analysis for mutation detection in disease genes. *Hum Mutat*; 6(4):281-7.

GLOBOCAN 2012: Estimated Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx. accessed 18 Sep 2016

Goelen G, Teugels E, Bonduelle M, Neyns B & De Greve J (1999). High frequency of *BRCA1/2* germline mutations in 42 Belgian families with a small number of symptomatic subjects. *J Med Genet*; **36,** 304-8.

Gompel A, Chaouat M, Hugol D, Forgez P (2004). Steroidal hormones and proliferation, differentiation and apoptosis in breast cells. *Maturitas*; **49:**16-24.

Gore L, de Gregori J & Porter C (2013). Targeting developmental pathways in children with cancer. *The Lancet Oncology;* 14(2), e70 - e78.

Gorsky RD, Koplan JP, Peterson HB & Thacker SB (1994). Relative risks and benefits of long-term estrogen replacement therapy: a decision analysis. *Obstet Gynecol*; 83:161-6.

Gundmundsdottir K & Ashworth A (2006). The roles of *BRCA1* and *BRCA2* and associated proteins in the maintenance of genomic stability. *Oncogenes*; 25:5864-5874.

Haber JE (1998). The many interfaces of *Mre11*. *Cell*; 95:583-586.

Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW & Weinberg RA (1999). Creation of human tumor cells with defined genetic elements**.** *Nature*; **400,** 464-8.

Haile RW, *et al* (2006). *BRCA1* and *BRCA2* mutation carriers, oral contraceptive use, and breast cancer before age 50. *Cancer Epidemiol Biomarkers Prev*; 15:1863-70.

Hakansson S, *et al* (1997). Moderate frequency of *BRCA1* and *BRCA2* germ-line mutations in Scandinavian familial breast cancer. *Am J Hum Genet*; **60,** 1068-78.

Hall NR, Angela M, Williams T, Murday VA, Newton JA & Bishop DT (1994). Muir-Torre syndrome: a variant of the cancer family syndrome. *J Med Genet*; 31: 627-631.

Hall TA (1999). "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT". *Nucl Acids Symp Ser*. 41:95-98.

Hamann U & Sinn HP (2000). Survival and tumor characteristics of German hereditary breast cancer patients. *Breast Cancer Res Treat*; **59,** 185- 92.

Hanahan D & Weinberg RA (2000). The hallmarks of cancer. *Cell*; **100,** 57-70.

Hanby AM *et al* (2000). Association between loss of heterozygosity of *BRCA1* and *BRCA2* and morphological attributes of sporadic breast cancer. *Int J Cancer*; **88,** 204-8.

Handerson IC, Murphy GP, Lawrece W Jr, Lenhard RE Jr (1992). Breast Cancer In: Clinical Oncology. *American Cancer Society Press;* NY, USA.

Harris CC & Hollstein M (1993). Clinical implications of the *p53* tumor-suppressor gene. *N Engl J Med*; 329:1318-1327.

Harris TJ & F McCormick (2010). The molecular pathology of cancer. *Nature reviews. Clinical oncology;* **7**(5): p. 251-65.

Hartge P, Struewing JP, Wacholder S, Brody LC & Tucker MA (1999). The prevalence of common *BRCA1* and *BRCA2* mutations among Ashkenazi Jews. *Am J Hum Genet*; **64,** 963-70.

Hartmann LC, *et al* (2001). Efficacy of bilateral prophylactic mastectomy in *BRCA1* and *BRCA2* gene mutation carriers. *Journal of the National Cancer Institute*; **93**(21): p. 1633-7.

Hayat MJ, Howlader N, Reichman ME & Edwards BK (2007). Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance**,** Epidemiology and End Results (SEER) Program. *Oncologist*; 12:20-37.

Hecker E (1967). Phorbol esters from croton oil: chemical nature and biological activities. *Natur wissenschaften*; 54:282-284.

Hedenfalk I, *et al* (2001). Gene-expression profiles in hereditary breast cancer. *N Engl J Med*; **344,** 539-48.

Heller I (1930). Occupational cancers. *J Ind Hyg*; 12:169-197.

Hemminki A, *et al* (1997). Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysi**s**. *Nature Genet*; 15:87-90.

Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, *et al* (1998). A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*; 391:184-187.

Henderson IC & Patek AJ (1998). The relationship between prognostic and predictive factors in the management of breast cancer**.** *Breast Cancer Res Treat;* **52**:261-288.

Hoffman E & Johnson K (1995). Women's health and health reform: who will deliver primary care to women? *Yale J Biol Med*; 68:201-6.

Hofmann W, Wappenschmidt B, Berhane S, Schmutzler R & Scherneck S (2002). Detection of large rearrangements of exons 13 and 22 in the *BRCA1* gene in German families. *J Med Genet*; **39,** E36.

Holt JT , Thompson ME, Szabo C, Robinson-Benion C, Arteaga CL, King MC & Jensen RA (1996). Growth retardation and tumor inhibition by *BRCA1*. *Nat Genet*; **12,** 298-302.

Hopper JL, Southey MC, Dite GS, Jolley DJ, Giles GG, McCredie MR, Easton DF& Venter DJ (1999). Population based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in *BRCA1* and *BRCA2*. Australian Breast Cancer Family Study. *Cancer Epidemiol Biomarkers Prev*; **8,** 741-7.

Howlett NG, et *al* (2002). Biallelic inactivation of *BRCA2* in Fanconi anemia. *Science*; 297(5581): p. 606-9.

Hsu IC, Metcalf RA, Sun T, Welsh J, Wang NJ & Harris CC (1991). *P53* gene mutational hotspot in human hepatocellular carcinoma from Qidong China. *Nature; 350*: 427-428.

http: //news.bbc.co.uk. Access on September 2016.

http: //www.ncbi.nlm.nih.gov/. Accessed on July 2016.

http: //www.veritas medicine.com. Accessed on Augest 2016.

Hunter DJ, *et al* (2007). A genome-wide association study identifies alleles in *FGFR2* associated with risk of sporadic postmenopausal breast cancer. *Nature genetics;* **39**(7): p. 870-4.

Huo D, Adebamowo CA, Ogundiran TO, *et al* (2008). Parity and breastfeeding are protective against breast cancer in Nigerian women. *Br J Cancer*; 98:992-996. [PubMed: 18301401].

Hussain GA, Rehab MM, Mohammed ME & Mohammed OM (2010). Role of Some Risk Factors in the Etiology of Breast Cancer in the Sudan. *The Open Breast Cancer Journal*; Volume 2.

Huusko P, Pääkkönen K, Launonen V, Pöyhönen M, Blanco G, *et al* (1998). Evidence of founder mutations in Finnish *BRCA1* and *BRCA2* families. *Am J Hum Genet*; **62,** 1544-8.

Ibrahim NA & Oludara MA (2012). Socio-demographic factors and reasons associated with delay in breast cancer presentation: a study in Nigerian women. *Breast*; 21:416-418. [PubMed: 22381153].

Ioakim-Liossi A, Karakitsos P, Markopoulos C, *et al* (1997). Expression of pS2 protein and estrogen and progesterone receptor status in breast cancer; *Acta Cytol*, **41**:713-716.

Isaacs C, Weber B, Moller P, Offit K, Kim-Sing C (2005). Effect of pregnancy as a risk factor for breast cancer in *BRCA1/BRCA2* mutation carriers**.** *Int J Cancer*.

Jancu J (1971). Peutz- Jeghers syndrome. Involvement of the gastrointestinal and upper respiratory tracts. *Am J Gastroenterol*; 56:545- 9.

Jarvis EM, Kirk JA & Clarke CL (1998). Loss of nuclear *BRCA1* expression in breast cancers is associated with a highly proliferative tumor phenotype. *Cancer Genet Cytogenet*; **101,** 109-15.

Jerina D M, Chadha A, Cheh A M, Schdruk M E, Wood AW & Sayer J M (1991). Covalent bonding of bay region diol epoxides to nuclei acids. *Adv Exp Med Biol*; 283:533-553.

Johannesdottir G, *et al* (1996). High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res*, **56,** 3663-5.

Johannsson OT, Idvall I, Anderson C, Borg A, Barkardottir RB, Egilsson V& Olsson H (1997). Tumor biological features of *BRCA1*- induced breast and ovarian cancer. *Eur J Cancer*; **33,** 362-71.

Johnson SM, Shaw JA & Walker RA (2002). Sporadic breast cancer in young women: prevalence of loss of heterozygosity at *p53*, *BRCA1* and *BRCA2*. *Int J Cancer*; **98,** 205-9.

Kadluar FF, Miller JA & Miller CE (1977). Hepatic microsomal N-glucuronidation and nuclei acid binding of N-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Res*; 37:805-814.

Kakavas VK, Plageras P, Vlachos TA, *et al* (2008). PCRSSCP: A method for the molecular analysis of genetic diseases. *Mol Biotechnol*; 38 (2):155-63.

Kastan MB & Bartek J (2004). Cell cycle checkpoints and cancer. *Nature*; 432:316-323.

Kauff ND, *et al* (2002). Risk-reducing salpingo-oophorectomy in women with a *BRCA1* or *BRCA2* mutation. *The New England journal of medicine*; **346**(21): p. 1609-15.

Kerber RA & Slattery ML (1997). Comparison of self-reported and database-linked family history of cancer data in a case-control study. *Am J Epidemiol*; 146:244-8.

Khoo US, *et al* (1999). Somatic mutations in the *BRCA1* gene in Chinese sporadic breast and ovarian cancer. *Oncogene*; **18,** 4643-6.

Kimbrough RD (1983). Determining exposure and biochemical effects on human population studies, *Environ Health Perspect*; 48:77-79.

King MC, Marks JH, Mandell JB (2003). Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2***.** *Science*; **302:**643-646.

Kinzler KW & B Vogelstein (1997). Cancer susceptibility genes. Gatekeepers and caretakers. *Nature*; **386**(6627): p. 761, 763.

Kinzler KW & Vogelstein B (1998). Landscaping the cancer terrain. *Science*; **280,** 1036-7.

Knudson A G (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*; 68**,** 820 -3.

Knudson AG, Jr H, W Hethcote, & BW Brown (1975). Mutation and childhood cancer: a probabilistic model for the incidence of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America;* **72** (12): p. 5116-20.

Kolodner RD, Hall NR, Lipford J, Kane MF, Morrison PT, Finan PJ, *et al* (1995). Structure of the human MLH1 locus and analysis of large hereditary

nonpolyposis colorectal carcinoma kindred for mlh1 mutations. *Cancer Res*; 15:242-248.

Koonin EV, Altschul SF, Bork P (1996). *BRCA1* protein products Functional motifs. *Nat Genet*; 13:266-268.

Koskenvuo M & Hemminki E (2002). Pregnancy weight gain and mother's breast cancer risk. *93rd Annual Meeting of the American Association for Cancer Research, San Francisc*.

Krainer M, *et al* (1997). Differential contributions of *BRCA1* and *BRCA2* too early- onset breast cancer. *N Engl J Med*; **336,** 1416-21.

Kreeger PK & Lauffenburger DA (2010). Cancer systems biology: *a network modeling perspective. Carcinogenesis*; 31:2-8.

Ku CS, Cooper DN, Polychronakos C, *et al* (2012). Exome sequencing: Dual role as a discovery and diagnostic tool. *Ann Neurol*; 71(1):5-14

Kurian AW (2009). *BRCA1* and *BRCA2* mutations across race and ethnicity: distribution and clinical implications. *Current opinion in obstetrics & gynecology*.

Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, Greenberg N & Ittmann M (2001). Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer **progression**. *Proc Natl Acad Sci U S A*; **98,** 11563-8.

Ladopoulou A, *et al* (2002). Germ line *BRCA1* & *BRCA2* mutations in Greek breast/ovarian cancer families: 5382insC is the most frequent mutation observed. *Cancer Lett*; **185,** 61-70.

Lakhani SR, Jacquemier J, Sloane JP, *et al* (1998). Multifactorial analysis of differences between sporadic breast cancers and cancers involving *BRCA1* and *BRCA2* mutations**.** *J Natl Cancer Inst*; **90**:1138-1145.

Lakhani SR, Van De Vijver MJ, *et al* (2002). The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone.

Lancaster JM, *et al* (1996). *BRCA2* mutations in primary breast and ovarian cancers. *Nat Genet*; **13,** 238- 40.

Langston AA, Malone KE, Thompson JD, Daling JR & Ostrander, EA (1996). *BRCA1* mutations in a population- based sample of young women with breast cancer. *N Engl J Med*, **334,** 137-42.

Law JC, Strong LC, Chidambaram A & Ferrell RE (1991). A germ line mutation in exon 5 of the *p53* gene in an extended cancer family. *Cancer Res*; 51:6385-7.

Lee EY (2008). Current opinion in obstetrics & gynecology; **20**(1): p. 68-73.

Lee E, *et al* (2008). Effect of reproductive factors and oral contraceptives on breast cancer risk in *BRCA1*/2 mutation carriers and noncarriers: results from a population-based study. *Cancer Epidemiol Biomarkers Prev;* 17:3170-3178.

Lemen RA, Dement JM & Wagoner JK (1980). Epidemiology of asbestoses related diseases. *Environ Health Perspect;* 20:1-21.

Levy-Lahad E (2010). Fanconi anemia and breast cancer susceptibility meet again. *Nature genetics;* **42**(5): p. 368-9.

Li FP & Fraumeni JF (1969). Soft tissue sarcomas, breast cancer and other neoplasms. A familial syndrome. *Ann Intern Med*; 71:747-752.

Li FP (1988). Cancer families: Human models of susceptibility to neoplasia-the Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res*; 48:5381-5386.

Liede A & Narod SA (2002). Hereditary breast and ovarian cancer in Asia: Genetic epidemiology of *BRCA1* and *BRCA2*. *Human Mutation;* 20:413- 424.

Liede A, Malik IA, Aziz Z, Rios P, Kwan E, Narod SA (2002). Contribution of *BRCA*l and *BRCA2* mutations to breast and ovarian cancer in Pakistan. *Am J Hum Genet;* 71:595-606.

Liede A, Metcalfe K, Offit K, Brown K, Miller S, Narod S A & Moslehi R (1998). A family with three germline mutations in *BRCA1* and *BRCA2*. *Clin Genet*; **54,** 215-8.

Lindahl T & Nyberg B (1972). Rate of depurination of native deoxyribonucleic acid. *Biochemistry;* 11:3610-3618.

Liu Q, *et al* (2000). *Chk1* is an essential kinase that is regulated by Atr and required for the *G 2/M DNA* damage checkpoint. *Genes Dev*; **14,** 1448-59.

Liu S, Ginestier C, Charafe-Jauffret E, *et al* (2008). *BRCA1* regulates human mammary stem/progenitor cell fate. *Proc Natl Acad Sci U S A*; **105:**1680-1685.

Loeb LA (1989). Endogenous carcinogenesis: Molecular oncology into twenty-first century. Cancer Res; 49:5489-5496.

Loeb LA (1991). Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res*; **51,** 3075-9.

London SJ, Connolly JL, Schnitt SJ & Colditz GA (1992). A prospective study of benign breast disease and the risks of breast cancer. *JAMA*; 267:941- 4.

Lorrick KL, *et al* (1999). RING fingers mediate ubiquitin-conjugating enzyme (E2) - dependent ubiquitination. *Proc Natl Acad Sci.USA*; 96:11364-11369.

Loury DL, Goldworthy TL & Butterworth BE (1987). The value of measuring cell replications a predictive index of tissue specific tumorgenic

Lynch ED, *et al* (1997). Inherited mutations in *PTEN* are associated with breast cancer, Cowden disease, and juvenile polyposis. *Am J Hum Genet*; 61:1254-60.

Mack TM *et al* (2002). Heritable breast cancer in twins. *British journal of cancer*; 87(3): p. 294-300.

Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, *et al* (1990). Germ line *p53* mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Sci;* 250:1233-1239.

Malone KE, *et al* (1998). *BRCA1* mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. *Jama*; **279,** 922-9.

Manke I A, *et al* (2003). *BRCT* repeats as phosphopeptide-binding modules involved in protein targeting. *Science*; **302**(5645): p. 636-9.

Marcus JN, *et al* (1996). Hereditary breast cancer: pathobiology, prognosis, and *BRCA1* and *BRCA2* gene linkage. *Cancer*; **77,** 697-709.

Mavaddat N, *et al* (2010). Genetic susceptibility to breast cancer. *Molecular oncology;* **4**(3): p. 174-91.

McClelland RA, Gee JM, O'Sullivan L, *et al* (1999). p21 (WAF1) expression and endocrine response in breast cancer. *J Pathol;* **188**: 126-132.

McMahon C, Suthiphongchai T, DiRenzo J, Ewen ME (1999). P/CAF associates with cyclin D1 and potentiates its activation of the estrogen receptor. *Proc Natl Acad Sci USA;* **96**:5382-5387.

Meijers-Heijboer H, *et al* (2001). Breast cancer after prophylactic bilateral mastectomy in women with a *BRCA1* or *BRCA2* mutation. *The New England journal of medicine;* **345**(3): p. 159-64.

Meindl A, *et al* (2010). Germline mutations in breast and ovarian cancer pedigrees establish *RAD51C* as a human cancer susceptibility gene. *Nature genetics;* **42**(5): p. 410-4.

Metcalfe K, *et al* (2004). **Contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers. Journal of clinical oncology:** *official journal of the American Society of Clinical Oncology;* **22**(12): p. 2328-35.

Michalides RJ (1999). Cell cycle regulators: mechanisms and their role in etiology, prognosis, and treatment of cancer. *J Clin Pathol*; **52:**555-568.

Miki Y, *et al* (1994). A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1***.** *Science*; **266**(5182): p. 66-71.

Miki Y, *et al* (1996). Mutation analysis in the *BRCA2* gene in primary breast cancers. *Nat Genet*; **13,** 245-7.

Milne RL & AC Antoniou (2011). Annals of oncology: *official journal of the European Society for Medical Oncology/ ESMO;* **22 Suppl 1**: p. 117.

Mohammed SN, Smith P, Hodgson SV, *et al* (1998). Family history and survival in premenopausal breast cancer. *Br J Cancer;* **77**: 2252-2256.

Montagna M, Palma MD, Menin C, Agata S, De Nicolo A, Chieco-Bianchi L & D'Andrea E (2003). Genomic rearrangements account for more than one-third of the *BRCA1* mutations in northern Italian breast/ovarian cancer families. *Hum Mol Genet*; **12,** 1055-61.

Moore MA & Sobue T (2010). Strategies for cancer control on an organ-site basis**.** *Asian Pac J Cancer Prev, Supp* l2:149-64.

Morabito A, Magnani E, Gion M, *et al* (2003). Prognostic and predictive indicators in operable breast cancer. *Clinical breast cancer*; **3:**381- 390.

Morishita M, Ohtsurut A, Nakashima M & Yamashita S (2005). Unilateral multiple primary tumors in an atomic-bomb survivor. *Clin Oncol*; 17:391-2.

Morrell D, Cromartie E & Swift M (1986). Mortality and cancer incidence in 263 patients with ataxia-telangiectasia**.** *J Natl Cancer Inst*; 77:89-92.

Moya M, Garbers S, Jessop DJ and Foti H (2004). A survey of breast cancer awareness and knowledge in a western population: lots of light but little illumination. *European journal of cancer*; 12(67), 123

Moynahan ME, Chiu JW, Koller BH, Jasin M (1999). *Brca1* controls homology-directed *DNA* repair. *Mol Cell*; **4**(4):511-886.

Moynahan ME, Pierce AJ, Jasin M (2001). *BRCA2* is required for homology-directed repair of chromosomal breaks. *Mol Cell*; **7:263**-272. **Mueller WH** (1982).The changes with age of the anatomical distribution of fat. *Soc Sci Med;* **16:**191-196.

Mueller CR & Roskelley CD (2002). Regulation of *BRCA1* expression and its relationship to sporadic breast cancer. *Breast Cancer Res*; **5,** 45-52.

Muir EG, Bell AJ & Barlow KA (1967). Multiple primary carcinomata of the colon, duodenum and larynx associated with kerato-acanthoma of the face. *Br J Surg*; 54:191-5

Mulligan LM, *et al* (1993). Germ-line mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. *Nature*; **363,** 458-60.

Munsoor M Munsoor, Noha Algily, Mohammed Abdulrahim (2013). Detection of *BRCA1* and 2 Genes Mutations among Women with Breast Cancer Attending Radio and Isotope Center in Khartoum, Sudan. *Journal of Science and Technology* vol.15**.**

Myers MP & Tonks NK (1997). *PTEN:* sometimes taking it off can be better than putting it on. *Am J Hum Genet*; 61:1234-8.

Nakagawa H, Koyama K, Tanaka T, Miyoshi Y, Ando H, *et al* (1998). Localization of the gene responsible for Peutz-Jeghers syndrome within a 6-cM region of chromosome 19p13.3. *Hum Genet*; 102:203-6.

Narod SA (2002). Modifiers of risk of hereditary breast and ovarian cancer. *Nature reviews. Cancer*; **2**(2): p.113-23.

Narod SA, *et al* (1995). An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. Breast Cancer Linkage Consortium. *Am J Hum Genet*; **56,** 254-64.

Nataraj AJ, Olivos-Glander I, Kusukawa N, *et al* (1999). Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis*; 20 (6):1177-85.

Neuhausen SL (1999). Ethnic differences in cancer risk resulting from genetic variation. *Cancer;* 86(Suppl 11):2575-2582.

Neuman E, Ladha MH, Lin N, *et al* (1997). Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4**.** *Mol Cell Biol***; 17**:5338-5347.

Newman B, Austin MA, Lee M & King MC (1998). Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. *Proc Natl Acad Sci*; 85:3044-8.

Newman B, Mu H, Butler LM, Millikan RC, Moorman PG & King, MC (1998). Frequency of breast cancer attributable to *BRCA1* in a population-based series of American women. *Jama*; **279,** 915-21.

Ng PC & Henikoff S (2001). Predicting deleterious amino acid substitutions. *Genome Res*; 11(5): 863-74.

Noel J C, Fayt I & Buxant F (2010). Proliferating activity in Paget disease of the nipple. *Pathol Oncol Res*; 16:7-10.

Noguchi S, Kasugai T, Miki Y, Fukutomi T, Emi M & Nomizu T (1999). Clinicopathologic analysis of *BRCA1*- or *BRCA2*- associated hereditary breast carcinoma in Japanese women. *Cancer*; **85,** 2200-5.

Nordlin M, Karlsson P, Wahlstrom J, Engwall Y, Wallgren A & Martinsson T (1998). A large deletion disrupts the exon 3 transcription activation domain of the *BRCA2* gene in a breast/ovarian cancer family. *Cancer Res*; **58,** 1372-5.

Offit K, Pierce H, Kirchhoff T, Kolachana P, Rapaport B, *et al* (2003). Frequency of *CHEK2*1100delC* in New York breast cancer cases and controls. *BMC Med Genet*; **4,** 1.

Oh H, Wang SC, Prahash A, Sano M, Moravec CS, Taffet GE, *et al* (2003). Telomere attrition and *Chk2* activation in human heart failure. *Proc Natl Acad Sci U S A*; **100,** 5378-83.

Okobia M, Bunker C, Zmuda J, *et al* (2006). Case-control study of risk factors for breast cancer in Nigerian women. *Int J Cancer*; 119:2179-2185. [PubMed: 16823837].

Oldenburg RA, Kroeze-Jansema K, Kraan J, *et al* (2003). The *CHEK2*1100delC* variant acts as a breast cancer risk modifier in non-*BRCA1/BRCA2* multiple-case families. *Cancer Res*; 63:8153-7.

Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P & Eeles RA (2003). Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and genotype. *Cancer Res;* 63:6643-50.

Olopade OI, Fackenthal JD, Dunston G, Tainsky MA, Collins F & Whitfield-Broome C (2003). Breast cancer genetics in African Americans. *Cancer*; **97,** 236-45.

Olschwang S, Boisson C & Thomas G (2001). Peutz-Jeghers families unlinked to *STK11/LKB1* gene mutations are highly predisposed to primitive biliary adenocarcinoma. *J Med Genet*; 38:356-360.

Olsen JH, Hahnemann JM, Borresen-Dale AL, Brondum-Nielsen K, *et al* (2001). Cancer in patients with ataxia-telangiectasia and in their relatives in the Nordic countries. *J Natl Cancer Inst*; **93,** 121-7.

Osborne CK (1998). Steroid hormone receptors in breast cancer management. *Breast Cancer Res Treat;* **51**:227-238.

Osin P, Crook T, Powles T, Peto J, Gusterson B (1998). Hormone status of in-situ cancer in *BRCA1* and *BRCA2* mutation carriers**.** *Lancet* 1998; **351**:1487.

Osin P, Gusterson BA, Philp E, *et al* (1998). Predicted anti-estrogen resistance in BRCA-associated familial breast cancers. *Eur J Cancer;* **34**:1683-1686.

Palacios J, Robles-Frias MJ, *et al* (2008).The molecular pathology of hereditary breast cancer. *Pathobiology*; **75:**85-94.

Pankow J S, *et al* (1997). Genetic analysis of mammographic breast density in adult women: evidence of a gene effect. *J Natl Cancer Inst;* 89:549-56.

Pant K & Dutta U (2008). Understanding and management of male breast cancer: a critical review. *Med Oncol*; 25:294-8.

Papa S, Seripa D, Merla G, Gravina C, Giai M, Sismondi P, Rinaldi M, Serra A, Saglio G & Fazio VM (1998). Identification of a possible somatic *BRCA1* mutation affecting translation efficiency in an early- onset sporadic breast cancer patient. *J Natl Cancer Inst*; **90,** 1011-2.

Parkin DM, Nambooze S, Wabwire-Mangen F, Wabinga HR (2010). Changing cancer incidence in Kampala, Uganda, 1991-2006. *Int J Cancer*; 126:1187-1195. [PubMed: 19688826].

Parkin DM, Vizcaino AP, Skinner ME, Ndhlovu A (1994). Cancer patterns and risk factors in the African population of southwestern Zimbabwe, 1963-1977. *Cancer Epidemiol Biomarkers Prev*; 3:537-547. [PubMed: 7827583].

Parsa P & B Parsa (2009). Effects of reproductive factors on risk of breast cancer: a literature review. *Asian Pac J Cancer Prev;* 10:545-550.

Pasanisi P, Hédelin G, Berrino J, Chang-Claude J, Hermann S, Steel M, *et al* (2009). Oral contraceptive use and *BRCA* penetrance: a case-only study. *Cancer Epidemiol Biomarkers Prev;* 18:2107-13.

Patel KJ, Vu VP, Lee H, Corcoran A, Thistlethwaite FC, Evans MJ, *et al* (1998). Involvement of *Brca2* in *DNA* repair. *Mol Cell*; 1: 347-357.

Paull TT, Rogakou RP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM (2001). A critical role for histone *H2AX* in recruitment of repair factors to nuclear foci after *DNA* damage. *Curr Bio*; 10:886-895.

Pavelic K, Gall-Troselj K (2001). Recent advances in molecular genetics of breast cancer. *J Mol Med*; **79:**566-573.

Peters N, *et al* (2005). Knowledge, attitudes, and utilization of *BRCA1/2* testing among women with early-onset breast cancer. *Genetic testing;* 9 (1): p. 48-53.

Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, *et al* (1999). Prevalence of *BRCA1* and *BRCA2* gene mutations in patients with early- onset breast cancer. *J Natl Cancer Inst*; **91,** 943-9.

Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drusedau, *et al* (1997). *BRCA1* genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat Genet*; **17,** 341-5.

Pharoah PD, Day NE, Duffy S, Easton DF & Ponder BA (1997). Family history and the risk of breast cancer: a systematic review and meta-analysis. *Int J Cancer*; 71:800-9.

Pharoah PD, P Guilford, & C Caldas (2001). Incidence of gastric cancer and breast cancer in *CDH1* (*E-cadherin*) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology;* **121**(6): p.1348-53.

Pierce LJ, Strawderman M, Narod SA, Oliviotto I, Eisen A, *et al* (2000). Effect of radiotherapy after breast- conserving treatment in women with breast cancer and germline *BRCA1/2* mutations. *J Clin Oncol*; 18:3360-9.

Pilarski R & Eng C (2004). Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the *PTEN* hamartoma tumors syndrome. *J Med Genet*; 41:323-6.

Ponder, B (1988). Cancer. Gene losses in human tumors. *Nature*; **335,** 400-2.

Potential in"Banbury Report 25: Non genotoxic mechanism in carcinogenesis". BE Butterworth and TJ Slaga eds. *Cold Spring Harbor Press. NY*; p.119-136.

Pott P (1775). Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures and the mortifications of the toes and feet. London: Hawkes, *Clarke and Collins*.

Powell SN, Willer H, Xia F (2002). *BRCA2* keeps *Rad51* in line. High -fidelity homologous recombination prevents breast and ovarian cancer? *Mol Cell*; 10:1262-3.

Pritchard KI (2001). Breast cancer prevention with selective estrogen receptor modulators: a perspective. *Annals of the New York Academy of Sciences*; **949**: p. 89-98.

Puget N, *et al* (2002). Distinct *BRCA1* rearrangements involving the *BRCA1* pseudo gene suggest the existence of a recombination hot spot. *American journal of human genetics;* **70**(4): p. 858-65.

Puget N, Stoppa-Lyonnet D, Sinilnikova OM, Pages S, Lynch HT, Lenoir GM & Mazoyer S (1999). Screening for germline rearrangements and regulatory mutations in *BRCA1* led to the identification of four new deletions. *Cancer Res*; **59,** 455- 61.

Puget N, Torchard D, Serova-Sinilnikova OM, Lynch HT, Feunteun J, Lenoir GM & Mazoyer S (1997). A 1-kb Alu mediated germ-line deletion removing *BRCA1* exon 17. *Cancer Res*; **57,** 828-31.

Qiagen, https://www. Qiagen. com/ gene globe/ pathway view. aspx? Pathway ID Accesses on October 2015.

Rabbani B, Mahdieh N, Nakaoka H, *et al* (2012). Next generation sequencing: Impact of exome sequencing in characterizing Mendelian disorders. *J Hum Genet*; 57(10):621-32.

Radiation and Isotopes center Khartoum Sudan department of medical statistics and cancer research (2009). Distribution of the cancer and top twenty types of cancers report in years 2007-2008.

Rahman N & Stratton MR (1998). The genetics of breast cancer susceptibility. *Annu Rev Genet*; **32,** 95-121.

Ramensky V, Bork P, Sunyaev S (2002). Human non-synonymous SNPs: server and survey. *Nucleic Acids Res*; 30: 3894-3900.

Rebbeck TR, *et al* (1999). Breast cancer risk after bilateral prophylactic oophorectomy in *BRCA1* mutation carriers. *Journal of the National Cancer Institute;* **91**(17): p. 1475-9.

Rebbeck TR, *et al* (2002). Prophylactic oophorectomy in carriers of *BRCA1* or *BRCA2* mutations. *The New England journal of medicine;* 346(21): p.1616-22.

Rebbeck TR, *et al* (2004). Bilateral prophylactic mastectomy reduces breast cancer risk in *BRCA1* and *BRCA2* mutation carriers: the PROSE Study Group. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology;* **22**(6): p.1055-62.

Rehn L (1895). Bladder tumors in Fuchsine workers. *Arch Klin Chir*; 50:588-600.

Renan MJ (1993). How many mutations are required for tumorigenesis? Implications from human cancer data. *Mol Carcinog*; **7,**139-46.

Richter C, Park JW & Ames BN (1988). Normal oxidative damage to mitochondrial and nuclear *DNA* is extensive. *Proc Natl Acad Sci USA*; 85:6465 - 6467.

Riou G, Favre M, Jeannel D, Bourhis J, Le Doussal V & Orth G (1990). Association between poor prognosis in early stage cervical carcinomas and non detection of HPV DNA. *Lancet;* 335:1171-1174.

Ripperger T *et al* (2009). Breast cancer susceptibility: current knowledge and implications for genetic counseling. *European journal of human genetics*: *EJHG*, 2009.**17**(6): p. 722-31.

Roa BB, Boyd AA, Volcik K & Richards CS (1996). Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2. Nat Genet*, **14,** 185-7.

Robson M, Gilewski T, Haas B, *et al* (1998). *BRCA*-associated breast cancer in young women. *J Clin Oncol;* **16**:1642-1649.

Robson M, Rajan P, Rosen PP, *et al* (1998). *BRCA*-associated breast cancer: absence of a characteristic immunophenotype**.** *Cancer Res;* **58**:1839-1842.

Rochefort H, Liaudet-Coopman E (1999). Cathepsin D in cancer metastasis: a protease and a ligand. *APMIS;* **107**:86-95.

Rodriguez MC & Z Songyang (2008). *BRCT* domains: phosphopeptide binding and signaling modules. Frontiers in bioscience: *a journal and virtual library;* **13**: p. 5905-15.

Rohlfs EM, Puget N, Graham ML, Weber BL, Garber JE, *et al* (2000). An Alu-mediated 7.1 kb deletion of *BRCA1* exons 8 and 9 in breast and ovarian cancer families that results in alternative splicing of exon 10. *Genes Chromosomes Cancer*; **28,** 300 -7.

Ron E, Modan B, Boice J D Jr, Alfandary E, Stovall M, Chetrit A, Katz L (1988). Tumors of the brain and nervous system after radiotherapy. *N Engl J Med*, 319:1033-1039.

Ross JS & Fletcher JA (1999). HER-2/neu (c-erb-B2) gene and protein in breast cancer. *Am J Clin Pathol;* **112(suppl1)**:S53-S67.

Rudolph P, Olsson H, Bonatz G, *et al* (1999). Correlation between *p53*, *cerbB-2*, and topoisomerase II alpha expression, *DNA* ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas: prognostic implications. *J Pathol* 1999, **187**:207-216.

Rustad CF, *et al* (2006). Germline *PTEN* mutations are rare and highly penetrant. *Hereditary cancer in clinical practice;* **4**(4): p. 177-185.

Sameshima Y, Tsunematsu Y, Watanabe S, Tsukamoto T, Kawa-ha K, Hirata Y, Mizoguchi H, Sugimura T, Terada M & Yokota J (1992). Detection of novel germ-line *p53* mutations in diverse-cancer-prone families identified by selecting patients with childhood adrenocortical carcinoma. *J Natl Cancer Ins;* 84:703-7.

Santibanez-Koref MF, Birch JM, Hartley AL, Jones PH, Craft AW, Eden T, Crowther D, Kelsey AM & Harris M (1991). *P53* germline mutations in Li-Fraumeni syndrome. *Lancet*; 338:1490-1.

Saurin AJ, *et al* (1996). Does this have a familiar RING? *Trends in biochemical sciences*; **21**(6): p. 208-14.

Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh & G (1995). The complete sequence of the coding region of the *ATM* gene reveals similarity to cell cycle regulators in different species. *Hum Mol Genet*; 4:2025-32.

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, *et al* (1997). Germline and somatic mutations in the tyrosine kinase domain of the *MET* proto-oncogene in papillary renal carcinomas. *Nat Genet*; **16,** 68-73

Schuster S C (2008). Next-generation sequencing transforms today's biology. *Nat Methods*; 5(1):16-8.

Schuurman AG, van den Brandt PA & Goldbohm RA (1995). Exogenous hormone use and the risk of postmenopausal breast cancer: results from The Netherlands Cohort Study. *Cancer Causes Control;* 6:416-24.

Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunreun J, Ashley T & Livingston DM (1997). Association of *BRCA1* strand with *Rad51* in mitotic and meiotic cells. *Cell*; 88: 265-275.

Seery LT, Knowlden JM, Gee JM, Robertson JF, Kenny FS, & Nicholson R I (1999). *BRCA1* expression levels predict distant metastasis of sporadic breast cancers. *Int J Cancer*; 84**,** 258- 62.

Seshadri R, Kutlaca RJ, Trainor K, Matthews C & Morley AA (1987). Mutation rate of normal and malignant human lymphocytes. *Cancer Res*; **47,** 407-9.

Shattuck-Eidens D, Oliphant A, McClure M, McBride C, Gupte J, Rubano T, Pruss D, Tavtigian SV, *et al* (1997). *BRCA1* sequence analysis in women at high risk for susceptibility mutations. Risk factor analysis and implications for genetic testing. *JAMA*; 278:1242-50.

Shimizu Y, Kato H & Schull WJ (1990). Studies of the mortality of a bomb survivors; mortality, 1950-1985: Part2. Cancer mortality based on the recently revised doses DS86. *Radiation Res;* 121:120-141.

Shimizu Y, Kato H, Schull WJ, Preston DL, Fujita S & Pierce DA (1989). Studies of the mortality of A-bomb survivors. Mortality, 1950-1985: Part 1. Comparison of risk coefficients for site- specific cancer mortality based on the DS86 and T65DR shielded kerma and organ doses. *Radiat Res*; 118:502-24.

Simard J, Tonin P, Durocher F, Morgan K, Rommens J & *et al* (1994). Common origins of *BRCA1* mutations in Canadian breast and ovarian cancer families. *Nat Genet*; **8,** 392-8.

Singletary SE, Ames FC& Buzdar AU (1994). Management of inflammatory breast cancer. *World J Surg*; 18:87-92.

Smith GC & Jackson SP (1999). The *DNA*- dependent protein kinase. *Genes Dev*; 13:916-934.

Smith PD, Cross land S, Parker G, *et al* (1999). Novel *p53* mutants selected in *BRCA*-associated tumors which dissociate transformation suppression from other wild-type p53 functions**.** *Oncogene;* **18**:2451-2459.

Smith TM, *et al* (1996). Complete genomic sequence and analysis of 117 kb of human *DNA* containing the gene *BRCA1*. *Genome research*; **6**(11): p.1029-49.

Sommerhaug RG & Mason T (1970). Peutz-Jeghers syndrome and ureteral polyposis. *Jama;* 211:120-2.

Southey MC, Tesoriero AA, Andersen CR, Jennings KM, Brown SM, et *al* (1999). *BRCA1* mutations and other sequence variants in a population- based sample of Australian women with breast cancer. *Br J Cancer*; **79,** 34- 9.

Spain BH, Larson CJ, Shihabuddin LS, Gage FH & Verma IM (1999). Truncated *BRCA2* is cytoplasmic: implications for cancer-linked mutations. *Proc Natl Acad Sci U S A*; **96,** 13920-5.

Srivastava S, Tong YA, Devadas K, Zou ZQ, Sykes VW, Chen Y, Blattner WA, Pirollo K & Chang EH (1992). Detection of both mutant and wild-type *p53* protein in normal skin fibroblasts and demonstration of a shared 'second hit' on *p53* in diverse tumors from a cancer-prone family with Li-Fraumeni syndrome. *Oncogene*; 7:987-91.

Stacey SN, *et al* (2007). Common variants on chromosomes *2q35* and *16q12* confer susceptibility to estrogen receptor-positive breast cancer. *Nature genetics;* 39(7): p. 865-9.

Staff S, Isola JJ, Johannsson O, Borg A & Tanner MM (2001). Frequent somatic loss of *BRCA1* in breast tumors from *BRCA2* germ-line mutation carriers and vice versa. *Br J Cancer;* **85,** 1201-5.

Stanford JL, Weiss NS, Voigt LF, Daling JR, Habel LA & Rossing MA (1995). Combined estrogen and progestin hormone replacement therapy in relation to risk of breast cancer in middle - aged women. *JAMA*; 274:137-42.

Starink TM (1984). Cowden's disease: analysis of fourteen new cases. *J Am Acad Dermatol;* 6:1127-41.

Steinberg KK, Thacker SB, Smith SJ, Stroup DF, Zack MM, Flanders WD & Berkelman RL (1991). A meta-analysis of the effect of estrogen replacement therapy on the risk of breast cancer. *JAMA*; 265:1985-90.

Steiner E, D Klubert, & D Knutson (2008). Assessing breast cancer risk in women. *American family physician;* 78:1361-1366.

Stratton MR & N Rahman (2008). The emerging landscape of breast cancer susceptibility. *Nature genetics;* **40**(1): p. 17-22.

Strong LC, Stine M & Norsted TL (1987). Cancers in survivors of childhood soft tissues sarcoma and their relatives. *J Natl Cancer Inst*; 79:1213-1220.

Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC & Tucker MA (1997). The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N Engl J Med*; **336,** 1401- 8.

Swensen J, Hoffman M, Skolnick MH & Neuhausen SL (1997). Identification of a 14 kb deletion involving the promoter region of *BRCA1* in a breast cancer family. *Hum Mol Genet*; 6**,** 1513-77.

Swift M, Reitnauer PJ, Morrell D & Chase CL (1987). Breast and other cancers in families with a taxia-telangiectasia. *N Engl J Med*; **316,** 1289-94.

Sy SM, MS Huen & J Chen (2009). *PALB2* is an integral component of the *BRCA* complex required for homologous recombination repair. *Proceedings of the National Academy of Sciences of the United States of America;* **106**(17): p. 7155- 60.

Tan P, Cady B, Wanner M, *et al* (1997). The cell cycle inhibitor *p27* is an independent prognostic marker in small (T1a, b) invasive breast carcinomas. *Cancer Res;* 57:1259-1263.

Tang B, Bottinger EP, Jakowlew SB, Bagnall, KM, Mariano J, Anvers MR, Letterio JJ & Wakefield LM (1998). Transforming growth factor-beta1 is a new form of tumor suppressor with true haploid insufficiency. *Nat Med*; 4**,** 802-7.

Tang NL, Pang CP, Yeo W, Choy KW, Lam PK, Suen M, *et al* (1999). Prevalence of mutations in the *BRCA1* gene among Chinese patients with breast cancer. *J Natl Cancer Inst*; **91,** 882-5.

Tavtigian SV, *et al* (1996). The complete *BRCA2* gene and mutations in chromosome *13q*-linked kindreds. *Nature genetics*; **12**(3): p. 333-7.

Taylor J, Lymboura M, Pace PE, A'Hern RP, Desai AJ, *et al* (1998). An important role for *BRCA1* in breast cancer progression is indicated by its loss in a large proportion of non-familial breast cancers. *Int J Cancer*; **79,** 334-42.

Teng DH, Bogden R, Mitchell J, Baumgard M, *et al* (1996). Low incidence of *BRCA2* mutations in breast carcinoma and other cancers. *Nat Genet*; **13,** 241- 4.

Thakur S, *et al* (1997). Localization of *BRCA1* and a splice variant identify the nuclear localization signal. *Molecular and cellular biology*; **17**(1): p. 444-52.

The *BRCA1* Exon 13 Duplication Screening Group (2000). The exon 13 duplication in the BRCA1 gene is a founder mutation present in geographically diverse populations. *Am J Hum Genet* **67**: 207-12

Thompson D & Easton D (2001). Variation in cancer risks, by mutation position, in *BRCA2* mutation carriers. *Am J Hum Genet*, **68,** 410-9.

Thompson ME, Jensen RA, Obermiller PS, Page DL & Holt JT (1995). Decreased expression of *BRCA1* accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet*; 9**,** 444 -50.

Thomson CA & Thompson PA (2009). Dietary patterns, risk and prognosis of breast cancer. *Future Oncol*; 5:1257-69.

Thorlacius S, Olafsdottir G, Tryggvadottir L, *et al* (1996). A single *BRCA2* mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet*; **13,** 117- 9.

Thorlacius S, Sigurdsson S, Bjarnadottir H , Olafsdottir G, Jonasson JG, *et al* (1997). Study of a single *BRCA2* mutation with high carrier frequency in a small population. *Am J Hum Genet*; 60**,** 1079-84.

Thorslund TF Esashi & SC West (2007). Interactions between human *BRCA2* protein and the meiosis-specific recombinase *DMC1*. *The EMBO journal;* 26(12): p. 2915-22.

Tirkkonen M, Johannsson O, Agnarsson BA, *et al* (1997). Distinct somatic genetic changes associated with tumor progression in carriers of *BRCA1* and *BRCA2* germ-line mutations. *Cancer Res*; 57**,** 1222-7.

Tokunaga M, Land CE, Tokuoka S, Nishimori I, Soda M & Akiba S (1994). Incidence of female breast cancer among atomic bomb survivors, 1950-1985. *Radiat Res*; 138:209-23.

Tonin PN, Mes-Masson AM, Futreal PA, Morgan K, Mahon M, Foulkes WD, Cole DE, Provencher D, Ghadirian P & Narod SA (1998). Founder *BRCA1* and *BRCA2* mutations in French Canadian breast and ovarian cancer families. *Am J Hum Genet*; **63,** 1341-51.

Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, *et al* (1996). Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. *Nat Med*; 2:1179-83.

Tsou HC, Teng DH, Ping XL, Brancolini V, Davis T, Hu R, *et al* (1997). The role of *MMAC1* mutations in early-onset breast cancer: causative in association with Cowden syndrome and excluded in *BRCA1*-negative cases. *Am J Hum Genet*; 61:1036-43.

Unger MA, Nathanson KL, Calzone K, Antin-Ozerkis D, Shih HA, Martin AM, Lenoir GM, Mazoyer S & Weber BL (2000). Screening for genomic rearrangements in families with breast and ovarian cancer identifies *BRCA1* mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. *Am J Hum Genet*, **67,** 841-50.

Urbach F (1993). Environmental risk factors for skin cancer. *Recent Res Cancer Res*; 128:243-262.

Utsunomiya J, Gocho H, Miyanaga T, Hamaguchi E Kashimure A (1975). Peutz-Jeghers syndrome: its natural course and management. *Johns Hopkins Med J*; 136:71- 82.

Vachon CM, King RA, Atwood LD, Kuni CC & Sellers TA (1999). Preliminary sibpair linkage analysis of percent mammographic density. *J Natl Cancer Inst;* 91:1778-9.

Valerie K & Povirk LF (2003). Regulation and mechanisms of mammalian double-strand break repair. *Oncogenes*; 22:5792-812.

Van der Looij M, Cleton-Jansen AM, van Eijk R, Morreau H, van Vliet M, Kuipers-Dijkshoorn N, Olah E, Cornelisse CJ & Devilee P (2000). A sporadic breast tumor with a somatically acquired complex genomic rearrangement in *BRCA1*. *Genes Chromosomes Cancer*; **27,** 295-302.

Van Diest PJ, van der Wall E, Baak JP (2004). Prognostic value of proliferation in invasive breast cancer: a review. *J Clin Pathol*; **57:675-**681.

Vaz F *et al* (2010). Mutation of the *RAD51C* gene in a Fanconi anemia-like disorder. *Nature genetics*; **42**(5): p. 406-9.

Vehmanen P, Friedman LS, Eerola H, McClure M, *et al* (1997a). Low proportion of *BRCA1* and *BRCA2* mutations in Finnish breast cancer families: evidence for additional susceptibility genes. *Hum Mol Genet*; 6**,** 2309-2315.

Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhönen S, *et al* (1997b). A low proportion of *BRCA2* mutations in Finnish breast cancer families. *Am J Hum Genet*; **60,** 1050 -1058.

Venkatachalam S, Shi YP, Jones SN, Vogel H , Bradley A, Pinkel D & Donehower LA (1998). Retention of wild-type *p53* in tumors from *p53* heterozygous mice: reduction of *p53* dosage can promote cancer formation. *Embo J*; **17,** 4657- 67.

Venkitaraman AR (2002). Cancer susceptibility and the functions of *BRCA1* and *BRCA2*. *Cell*; 108:171-182.

Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G (2010). Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics;* 11(1):548.

Verhoog LC, Brekelmans CT, Seynaeve C, van den Bosch LM, *et al* (1998). Survival and tumor characteristics of breast-cancer patients with germline mutations of *BRCA1*. *Lancet*; **351,** 316-21.

Verhoog LC, *et al*. (2000). Contralateral breast cancer risk is influenced by the age at onset in *BRCA1*- associated breast cancer. *British journal of cancer;* **83**(3): p. 384-6.

Vermeulen K, Van Bockstaele DR, Berneman ZN (2003). The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. *Cell Prolif*; **36:**131-149.

Visvader JE (2009). Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes & development*; **23**(22): p. 2563-77.

Wagner TM, Moslinger R, Zielinski C, Scheiner O & Breiteneder H (1996). New Austrian mutation in *BRCA1* gene detected in three unrelated HBOC families. *Lancet*; **347,** 1263.

Waldron HA, Waterhouse JA & Tessema N (1984). Scrotal cancers in the West Midlands 1936-1976. *Br J Ind Med*; 41: 473-474.

Wang H, Shao N, Ding QM, Cui J, Reddy ES, Rao VN (1997). *BRCA1* proteins are transported to the nucleus in the absence of serum and splice variants *BRCA1a*, *BRCA1b* are tyrosine phosphoproteins that associate with *E2F*, cyclins and cyclin dependent kinases. *Oncogene15* (2): 143-57.

Wang Q, Zhang H, Kajino K and Greene MI (1998). *BRCA1* and cell signaling. Oncogene; 17:1939-1948.

Wang T, Lerer I, Gueta Z, Sagi M, Kadouri L, Peretz T & Abeliovich D (2001a). A deletion/insertion mutation in the *BRCA2* gene in a breast cancer family: a possible role of the Alu-polyA tail in the evolution of the deletion. *Genes Chromosomes Cancer*; **31,** 91-5.

Wang Y, Cortez D, Yazdi P, Neff N, Elledge S, Qin J (2000). *BASC,* A super complex of *BRCA1*- associated proteins involved in the recognition and repair of aberrant *DNA* structures. *Genes Dev*; 14:927-939.

Warner E, Foulkes W, Goodwin P, Meschino W, *et al* (1999). Prevalence and penetrance of *BRCA1* and *BRCA2* gene mutations in unselected Ashkenazi Jewish women with breast cancer. *J Natl Cancer Inst*; **91,** 1241-7.

Weber BH, Brohm M, Stec I, Backe J & Caffier H (1996). A somatic truncating mutation in *BRCA2* in a sporadic breast tumor. *Am J Hum Genet,* **59,** 962- 4.

Weinberg Robert A (2013). The biology of cancer, second edition. *Garland Science, USA,* p: G1-G30.

Welcsh PL & MC King (2001). *BRCA1* and *BRCA2* and the genetics of breast and ovarian cancer. *Human molecular genetics*; **10**(7): p.705-13.

Wetmore C, Eberhart DE & Curran T (2000). The normal patched allele is expressed in medulloblastomas from mice with heterozygous germ-line mutation of patched. *Cancer Res*; **60,** 2239-46.

Wilson CA, Ramos L, Villasenor MR, Anders KH, Press MF, *et al* (1999). Localization of human *BRCA1* and its loss in high-grade, non- inherited breast carcinomas. *Nat Genet*; **21,** 236-40.

Wilson CA, Ramos L, Villaseñor MR, *et al* (1999). Localization of human *BRCA1* and its loss in high-grade, non-inherited breast carcinoma. *Nat Genet*; 21:236-240.

Winer EP, Morrow M, Osborne CK, Harris JR (2001). Cancer of the breast. In Cancer: Principles and Practice of Oncology**.** Eds. De Vita Jr VT, Hellman S, Rosenberg SA. Philadelphia: *Lippincott Williams & Wilkins*; 1264-1333.

Wong AK, *et al* (1997). *RAD51* interacts with the evolutionarily conserved *BRC* motifs in the human breast cancer susceptibility gene brca2. *The Journal of biological chemistry;* 272**(**51): p. 31941- 4.

Wooster R & BL Weber (2003). *Breast and ovarian cancer. The New England journal of medicine;* **348**(23): p. 2339-47.

Wu, LC, *et al* (1996). Identification of a RING protein that can interact in vivo with the *BRCA1* gene product. *Nature genetics*; **14**(4): p.430-40.

Wu-Baer F, *et al* (2003). The *BRCA1*/*BARD1* heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue *K6* of ubiquitin. *The Journal of biological chemistry*; **278**(37): p. 34743-6.

Xu X, Brodie SG, Yang X, *et al* (2000). Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. *Oncogenes*; **19,** 1868-74.

Yang H, *et al* (2002). *BRCA2* function in *DNA* binding and recombination from a *BRCA2-DSS1- ss DNA* structure. *Science*; **297**(5588): p.1837-48.

Yarney J, Donkor A, Opoku SY, *et al* (2013), Characteristics of users and implications for the use of complementary and alternative medicine in Ghanaian cancer patients undergoing radiotherapy and chemotherapy: a cross-sectional study. *BMC Complement Altern Med*; 13:16. [Pub Med: 23331546].

Yoshida K & Miki Y (2004). Role of *BRCA1* and *BRCA2* as regulators of *DNA* repair, transcription, and cell cycle in response to *DNA* damage. *Cancer Sci*; 95:866-871.

Yoshikawa K, Honda K, Inamoto T, *et al* (1999) Reduction of *BRCA1* protein expression in Japanese sporadic breast carcinomas and its frequent loss in *BRCA1*-associated cases. *Clin Cancer Res*; 5:1249-1261.

Yu VP, Koehler M, Steinlein C, Schmid M, Hanakahi LA, Van Gool AJ, *et al* (2000).Gross chromosomal rearrangements and genetic exchange between non homologous chromosomes following *BRCA2* inactivation. *Genes Dev;* 14:1400-1406.

Yu X, *et al* (2003). The *BRCT* domain is a phospho-protein binding domain. *Science*; **302**(5645): p. 639-42.

Yuan SS, *et al* (1999). *BRCA2* is required for ionizing radiation-induced assembly of *Rad51* complex in vivo. *Cancer research;* **59**(15): p. 3547-51.

Zhang F, *et al* (2009). *PALB2* links *BRCA1* and *BRCA2* in the *DNA*-damage response. *Current biology;* 19(6): p.524-9.

Zhang J & Powell SN (2005). The role of the *BRCA1* tumor suppressor in *DNA* double-strand break repair. *Mol Cancer Res*; **3**(10):531- 539.

Zheng L, *et al* (2000). Sequence-specific transcriptional co repressor function for *BRCA1* through a novel zinc finger protein, *ZBRK1*. *Molecular cell*; **6**(4): p. 757- 68.

Zhong Q, *et al* (1999). Association of *BRCA1* with the *hRad50-hMre11-p95* complex and the *DNA* damage response. *Science*; **285**(5428): p. 747-50.

Zuo L, Weger J, Yang Q, Goldstein AM *et al* (1996). Germline mutations in the p16INK4a binding domain of *CDK4* in familial melanoma. *Nat Genet*; **12,** 97-9.

Appendix I

بسم الله الرحمن الرحيم

Shendi University

Faculty of postgraduate study

Questionnaire

Identification of *BRCA1*&*BRCA2* mutation in unselected breast cancer patients in Shendi

I. Personal Profile:

1. Age:

a. 30 - 40 ☐ b.41-50 ☐ c.51-60 ☐ d. More ☐ than 60

2. Weight:

a. 40 - 60 ☐ b.61-80 ☐ c.81-100 ☐ d. More ☐ than 100

3. Height:

A.140-150 ☐ b.151-160 ☐ c.161-17 ☐

d. More than 170 ☐

4. Tribe:

a. Gallyeen ☐ b. Other ☐

5. Residents:

a. Shendi ☐ b. Others ☐

6. Level of education:

a. Illiterate ☐ b. Primary ☐ c. High School ☐

d. University graduate ☐ e. Post graduate ☐

7. Women`s job:

a. House wife ☐ b. Worker ☐. Employee ☐ d. Student ☐

e. Others

8. **Marital status:**

a. Single ☐ b. Married ☐ c. Widow ☐ d. Divorced ☐

e. Separated ☐

9. **Parity:**

a. Nulliparous ☐ b. Single parity ☐. Multiparous ☐

II. Physical Environmental Risk factors:

11. Did you ever have x-ray in the past?

1) Yes 2) No

III. Chemical Environmental Risk Factors:

Contraceptive and Hormonal Therapy:

12. Have you ever take birth control pills, shots or implants for another reason such as irregular menstrual periods, menopausal symptoms?

1) Yes 2) No

13. Have you ever take any hormone replacement therapy for menopausal symptoms:

1) Yes 2) No

Smoking:

14. Did you live with any other persons who smoked in your presence?

1) Yes 2) No

IV Family history of breast cancer:

16. Have you any types of cancer in your family?

a. Yes ☐ b. No ☐

17. If yes indicated what the degree of relationship:

a. First degree relative ☐ b. Second degree relatives ☐

b. Third degree relative ☐

18. Types of cancer in your family?

a. Breast ☐ b. others ☐

19. Age at menarche

a. before 11 years ☐ b. after 11 years ☐

20. Are you in menopausal period?

a. Yes ☐ b. No ☐

<u>Pathological report:</u>

ER:

PR:

HER2:

Grade:

Types of breast cancer:

بسم الله الرحمن الرحيم

اِستبانة

جامعة شندى

كلية الدراسات العليا

بحث مقدم لنيل درجة الدكتوراه في الاحياء الجزيئية

العنوان :- التعرف على الطفرات الوراثية لجينى البركا1 والبركا2 لعينة غير مختارة من مرضى سرطان الثدى بمركز معالجة الاورام وابحاث السرطان - شندى

أ. البيانات الشخصية : -

1. العمر بالسنوات :- 30-40 ☐ 41- 50 ☐ 51- 60 ☐ اكبر من 60 ☐

2. الوزن :- 40- 60 ☐ 61 - 80 ☐ 81- 100 ☐ اكثر من 100 ☐

3. الطول :- 140-150 ☐ 151 -160 ☐ 161- 170 ☐ اكثر من 170 ☐

4. القبيلة :- جعلية ☐ اخرى ☐

5. السكن :- شندى ☐ اخرى ☐

6. المستوى التعليمى :- امى ☐ ابتدائى ☐ ثانوى ☐ جامعى ☐ فوق جامعى ☐

7. الوظيفة :- ربة منزل ☐ عاملة ☐ موظفة ☐ طالبة ☐ اخرى ☐

8. **الحالة الاجتماعيه** :- عازب ☐ متزوجه ☐ مطلقه ☐

9.عدد الولادات :- واحدة ☐ متعددة ☐ لا يوجد ☐

ب/ عوامل الخطر الفيزيائية:

هل تعرضت لاشعة قبل ذلك:

1/ نعم ☐ 2/ لا ☐

.ح /عوامل الخطر الكيميائية:

1/ هل تتعاطين حبوب منع الحمل:

1 / نعم ☐ 2/ لا ☐

هل تتعاطين حبوب بدايل الهرمونات:

1/ نعم ☐ 2/ لا ☐

د/ التدخين :

هل تدخنيين او تتعرضين للتدخين:

1/ نعم ☐ **2/لا** ☐

ها/ التاريخ العائلى للمرض:

1/ هل لديك اقرباء مصابين بالسرطان فى العائلة:

1/ نعم ☐ **2 /لا** ☐

2 / ما هى صلة القرابة اذا كانت الاجابة بنعم:

1/ اقرباء من الدرجة الاولى ☐ 2/ الدرجة الثانية ☐ 3/ الدرجة الثالثة ☐

3/ نوع السرطان فى العائلة:

1/ سرطان ثدى ☐ 2/ اخرى ☐

كم كان عمرك عند باية اول دورة شهرية

1/ قبل 11 سنة 2/ يعد 11 سنة

هل اتقطعت الدورة الشهرية:

1/ نعم 2 / لا

<u>Pathological report:</u>

ER: **PR:**

HER2:

Grade: **Types of breast cancer:**

Appendix II

E.N.U.H

Shendi University
El-mek Nimir University Hospital

جامعة شندي
مستشفى الملك نمر الجامعي

التاريخ: ١/١١/٢٠١٣م

النمرة: ج ش/م م ن/٤٧/أ/١

موافقة المؤسسة الصحية

بهذا تشهد مستشفى الملك نمر الجامعي بأنها قد منحت التصديق والموافقة والسماح **لطالبة الدراسات العليا / هادية عباس الطيب** لعمل البحث لنيل درجة الدكتوراة بأخذ العينات من المرضى الذين يترددون على قسم الأورام بالعيادات المحولة وأقسام التنويم بالمستشفى.

والله الموفق ،،،

د.حمدان صديق سراج أحمد

مدير عام مستشفى الملك نمر الجامعي

السودان . شندي . ص.ب:١٤٢ . ١٤٣ تلفون:٠١٥٤٨٨٢٩٩٩ فاكس: ٨٧٦٩٤٨(٢٦١)(٠٠٢٤٩)

Email: shendioncology10@hotmail.com

Appendix III

بسم الله الرحمن الرحيم

جامعه شندي

كليه الدراسات العليا

استمارات الموافقة الأخلاقية

استمارة موافقة المريض على المشاركة بالبحث

استمارة مشاركه في بحث مقدم لنيل درجه الدكتوراه في الأحياء الجزيئية بعنوان:- التعرف على الطفرات الوراثية لجيينى البركا1و2 لعينة غير مختارة من مرضى سرطان الثدى بمركز معالجة الاورام وابحاث السرطان - شندى

السيدة.....

أنا هادية عباس الطيب استاذة بجامعة شندى / كلية علوم المختبرات الطبية أقوم بدراسة للتعرف على دور الطفرات الوراثية فى حدوث مرض سرطان الثدي وعوامل الخطر الاخرى والتى يمكن ان تلعب دور ايضا فى حدوث وتطور المرض وهي دراسة تفيد في الكشف المبكرللمرض وتوقع حدوثه عند اقرباء المرضى فى المستقبل حتى يتمكنوا من المتابعة الدورية قبل حدوث المرض اوالتعرف عليه مبكرا لسهولة علاجه فى مراحله الاولية ولتفادى بعض العوامل اوالممارسات و تغيير نمط الحياة والتى ربما تكون ذات علاقة وطيدة بحدوث المرض. لذا آمل منكم المشاركة معي في هذا البحث وذلك بالسماح لي بأخذ عينات دم لتحديد الطفرات و التعرف على بعض العوامل والعادات المتعلقة باسلوب حياتكم على ان تكون هذه البيانات لغرض البحث فقط وسوف أقوم باتخاذ جميع الإجراءات التي تضمن سريه وخصوصية المعلومات الشخصية المتعلقة بكم ولكم مني كل الشكر والتقدير واسأل الله أن يتم عليكم نعمه الشفاء العاجل.

موافق ( ) غير موافق ( )

Appendix VI

مركز معالجة الأورام وأبحاث السرطان- شندي

Table 2-1 The standard genetic code

First position (5′ end)	Second position				Third position (3′ end)
	U	C	A	G	
U	UUU Phe } F UUC Phe } F UUA Leu } L UUG Leu } L	UCU Ser } S UCC Ser } S UCA Ser } S UCG Ser } S	UAU Tyr } Y UAC Tyr } Y UAA Stop UAG Stop	UGU Cys } C UGC Cys } C UGA Stop UGG Trp W	U C A G
C	CCU Leu } L CUC Leu } L CUA Leu } L CUG Leu } L	CCU Pro } P CCC Pro } P CCA Pro } P CCG Pro } P	CAU His } H CAC His } H CAA Gln } Q CAG Gln } Q	CGU Arg } R CGC Arg } R CGA Arg } R CGG Arg } R	U C A G
A	AUU Ile } I AUC Ile } I AUA Ile } I AUG Met M	ACU Thr } T ACC Thr } T ACA Thr } T ACG Thr } T	AAU Asn } N AAC Asn } N AAA Lys } K AAG Lys } K	AGU Ser } S AGC Ser } S AGA Arg } R AGG Arg } R	U C A G
G	GUU Val } V GUC Val } V GUA Val } V GUG Val } V	GCU Ala } A GCC Ala } A GCA Ala } A GCG Ala } A	GAU Asp } D GAC Asp } D GAA Glu } E GAG Glu } E	GGU Gly } G GGC Gly } G GGA Gly } G GGG Gly } G	U C A G