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Molecular Diagnosis of Human Papilloma Virus *(HPV)* Isolated from Breast Cancer Patients in Radiation and Isotopes Center Khartoum *(RICK)* – Sudan

Submitted By: Osman Abdalla Eltayeb Elnoubi

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Supervisor: Prof. Rashid Eltayeb Abdalla

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Author: **Osman Abdalla Eltayeb Elnoubi**

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Declaration

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Osman Abdalla Eltayeb Elnoubi **Date: March 2016**

Examination Committee Members

Supervisor: - Prof. Rashid Eltayeb Abdalla

Signature **date**

External Examiner: - Prof. Bakri Mohammed Ahamed Nuri

Signature **date**

Internal Examiner: - Pro. Ahmed Mohamed Ahamed Ibrahim

Signature **date**

Dedication

I dedicate this work,

To my father,

Who didn't spare any effort to put me on the right path of knowledge.

To my late mother,

Who overwhelmed me with her true love, care and kindness.

To my wife,

Who lived every moment of my work to reach this achievement (Um-Meral)**.**

To my daughter,

Meral which is endowed me on the most beautiful of life.

To my faithful brothers and sisters,

Who have always helped me and believed that I could do it.

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Abstract

Background:

Breast cancer is the most common among female comprises about (18%) of all female cancers, with (1.7) million new cases in the world each year. Recently some studies reported that approximately (18%) of cancer cases can be linked to infectious agents including viruses particularly Human Papilloma Virus (*HPV)*.

Objectives:

The objectives of this study were to investigate whether *human papilloma virus* is among the associated agents causing of human breast cancer (*BC)* in Sudan and which genotype of *HPV* is implicated as well as the risk factors associated with *HPV* in breast cancer by using PCR as diagnostic tools for HPV in breast tissues.

Materials and methods:

One hundred forty three females diagnosed with histopathological examinations to have breast cancer (n=100 patients) or breast inflammatory conditions (n=43patients) were employed in this study. Tissue sample of (10) μm was taken from the pathological tissues of each patient. The samples were subjected to a *PCR protocol* to determine whether the patient is infected with *HPV* or not. *HPV infected* samples were further subjected to anther *PCR protocol* to identify the genotypes of *HPV*. The risk factors associated with breast cancer were taken from patient's records. The data were subjected to *analytical and descriptive statistical analysis*.

Results & Discussion:

Out of the cases screened for breast pathology, 41(29%) patients were found to have, invasive ductal carcinoma, 21(15%) invasive lobular carcinoma, 12(8%) invasive micropapillary carcinoma, 26(18%) medullary carcinoma and 43(30%) have inflammatory breast conditions.

In this study, the presence of *HPV* in biopsies from the breast (malignant and breast inflammatory conditions) was investigated. *HPV* was detected from (4) different types of breast cancer in those biopsies. These were, invasive ductal carcinoma, 23(16.1); invasive lobular carcinoma, 14(9.8%); invasive micropapillary 9(6.3%) and medullary carcinoma, 11(7.7%). Control breast inflammatory conditions were positive only for 10 (7%) *HPV*.

A strong relationship was established between *HPV infection* and breast cancer types as using breast inflammatory conditions as control group (**P = 0.00022**).

As regards the association of *HPV* with the different types of breast cancer, there was a significant relationship between *HPV* and invasive ductal carcinoma when compared with breast inflammatory conditions **(P = 0.00022**).

Another significant relationship was found between *HPV* and invasive lobular carcinoma with respect to breast inflammatory conditions (**P = 0.000016**). A third significant association between *HPV* was also found between invasive micropapillary carcinoma and breast inflammatory conditions (**P = 0.000095**). No significant relationship was seen between *HPV* in medullary carcinoma and breast inflammatory conditions (**P = 0.098**).

Genotyping of HPV infected breast cancer specimen was performed. Out of the (143) specimens (breast cancer and breast inflammatory conditions) the following genotypes were determined and mentioned in a descending order. 22(33%) of the

HPVs were of the genotype 16, 21(31%) genotype 18=14(21%) genotype 33=14(21%) and 10(15%) genotype (31).

Significant relationship between breast cancer types and other risk factor such as, age **(P=0.0012**), breast feeding (**P= 0.0430**), stage of BC and practicing of exercises (**P = 0.0024**), duration of illness (**P=0.028**5), family history (**P=0.0432**) finally strong significant relation between breast inflammatory conditions and any type of breast cancer was founded (**P =0.0000**).

Conclusion:

In conclusion, the findings of this study provide strong association between *HPV* and *BC* in Sudan.

ملخص الدراسة :-

يعتبر سرطان الثدي من أكثر السرطانات شيوعا عند الإناث وتبلغ نسبته حوالي 18% عالميا. هنالك 1.7 مليون حالة إصابة بسرطان الثدي عند الإناث سنويا . حديثا ذكرت بعض الدراسات أن 18% من حالات الإصابة بسرطان الثدي سببها فيروسي وتحديدا فيروس الورم الحليمي البشري .

أهداف الدراسة :-

الهدف الأساسي من هذه الدراسة هو معرفه ما إن كان لفيروس الورم الحليمي البشري دور بالإصابة بسرطان الثدي في السودان . وكذلك معرفه أكثر الأنماط الجينية لفيروس الورم الحليمي البشري شيوعا في حالات الإصابة بسرطان الثدي في السودان . أيضا هدفت الدراسة إلى معرفة عوامل الخطورة الأخرى وعلاقتها بفيروس الورم الحليمي البشري . كذلك هدفت الدراسة إلى معرفة اكثر عوامل الخطورة للإصابة بسرطان الثدي شيوعا . أخيرا هدفت الدراسة لمعرفة حساسية اختبار تفاعل اختبار البلمرة المتسلسل كطريقة لتشخيص فيروس الورم الحليمي البشري .

المواد والطرق :-

تم اخذ عدد 143 عينة نسيج ثدي بعد التشخيص النسيجي (100 عينة مصابه بأنواع مختلفة من سرطان الثدي و43 عينه مصابه بالتهابات تم أخذها كعينات مرجعية). من معامل مستشفى الطب النووي والعلاج بالأشعة في الخرطوم . تم اخذ 10 ميكرون من كل عينة وتم إجراء اختبار البلمرة التسلسلي لمعرفه وجود فيروس الورم الحليمي البشري آو عدمه في كل عينة . تم إجراء اختبار بلمرة تسلسلي آخر للعينات الموجبة لمعرفه أكثر الأنماط الجينية (تركزت الدراسة علي 4 أنماط جينية وهي (16,18,31,33). عوامل الخطورة الأخرى لسرطان الثدي تمت دراستها من خلال سجلات المرضي بالمستشفى. تم إجراء اختبار الثقة للنتائج عن طريق اختبار مربع كأي وتم تثبيت قيمه الاحتمالية(ب) عند 0.05

النتائج والمناقشة:

أظهرت الدراسة النتائج التالية :- وجود 41 (29%) مصابه بسرطان الأغنية الغازي ، 21(15%) حالة سرطان الفصوص الغازي، 12(8%) حالة سرطان الحليم الصغير الغازي ،26(18%) حالة سرطان النخاع و 43 حالة مصابه بالالتهاب الثدي .

أظهرت الدراسة وجود فيروس الورم الحليمي البشري حسب نوع سرطان الثدي علي النحو التالي: 23(16.1) عينات موجبه للفيروس في سرطان الأغنية الغازي ، 14(9.8%) ، سرطان الفصوص الغازي ، 9(6.3%) ، سرطان الحليم الصغير ، 11(7.7%) ،سرطان النخاع و 10(7%) فقط في العينات المصابة بالتهاب الثدي .

وجدت الدراسة أن هنالك علاقة ذات دلاله إحصائية بين عينات سرطان الثدي المصابة بفيروس الورم الحليمي البشري عند مقارنتها مع العينات المصابة بالتهاب الثدي عند فحصها باختبار البلمرة التسلسلي (P=0.00022) ، أيضا وجدت الدراسة أن هنالك علاقة ذات دلاله إحصائية بين الإصابة بفيروس الورم الحليمي البشري في سرطان الأغنية الغازي عند مقارنتها مع عينات التهاب الثدي .
(P=0.0002) كذلك وجدت الدراسة أن هنالك علاقة ذات دلالة إحصائية بين الإصابة بفيروس الورم الحليمي البشري وسطان الثدي نوع الفصوص الغازي(0.00016) =P عند مقارنتها مع عينات التهاب الثدي، كذلك وجدت الدراسة أن هنالك علاقة ذات دلاله إحصائية في العينات المصابة بفيروس الورم الحليمي البشري في سرطان الحليم الصغير الغازي عند مقارنتها مع عينات التهاب الثدي P=0.000095

وجدت الدارسة إن أكثر الأنماط الجينية لفيروسات الورم الحليمي البشري شيوعا هي النمط الجيني 31=10(15),33=14(21),18=14(21%), 16=21(31%) .

وجدت الدراسة علاقة ذات اثر معنوي بين العمر كأحد عوامل الخطورة وسرطان الثدي P=0.0012 أيضا وجدت الدراسة علاقة ذات دلاله إحصائية بين مدة الرضاعة والإصابة بسرطان الثدي P=0.043 كذلك وجدت الدراسة علاقة ذات دلالة إحصائية بين ممارسة الرياضة وسرطان الثدي P=0.0024، أيضا وجدت الدراسة علاقة ذات دلالة إحصائية بين مده الإصابة وسرطان الثدي P=0.0285، أيضا وجدت الدراسة علاقة ذات دلالة إحصائية بين التاريخ العائلة وسرطان الثدي (P=0.0432)، أخيرا وجدت الدراسة علاقة ذات دلالة إحصائية كبيرة بين انتشار المرض وسرطان الثدي (P=0.0000).

الملخص :-

خلصت الدراسة إلى أن فيروس الورم الحليمي البشري من أهم مسببات سرطان الثدي عن الإناث في السودان، كذلك خلصت الدراسة إلى أن اختبار البلمرة التسلسلي يمكن أن يستخدم لتشخيص فيروس الورم الحليمي البشري من عينات الثدي كذلك خلصت الدراسة إلى أن هنالك عدد من عوامل الخطورة مثل العمر والوراثة ، نوع نسيج الثدي يمكن أن تزيد من احتماليه الإصابة بسرطان الثدي

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List of Abbreviation:

ABC	Advanced Breast Cancer.
ACS	American Cancer Society.
AIB1	Amplified in Breast Cancer -1.
AIs	Aromatase Inhibitors.
AMF	Autocrine Motility Factor.
Amp	Ampere.
AP	Associated Protein.
AP – 1	Activator Protein – 1.
AR	Androgen Receptor.
ASCO	American Society of Clinical Oncology.
AT	Adriamycin and Taxotere.
ATAC	Adjuvant Tamoxifen Versus Arimidex Combined Study.
ATPase	Adenosin-Tri-Phosphatase.
BAX	Bcl-2- Associated X Protein.
BC	Breast Cancer.
Bcl-2	β- Cell lymphoma -2.
Bp	Base pair.
BRCA1/2	Breast Cancer Gene 1/2.
BSE	Breast Self-Examination.

CAP	College of American Pathologists.
CBE	Clinical Breast Examination.
CCL21	Chemokine –C- ligand 21.
CCR	Chemokine – C – Receptor (gene).
CDC-2	Cell Division Cycle – 2.
CDH1	Cadherin 1.
CDK	Cyclin-Dependent kinases.
CDKN1A	Cyclin Dependant kinase Inhibitor Gene 1A (P21).
CDKN1B	Cyclin Dependant kinase Inhibitor Gene 1B (P27).
CDKN2A	Cyclin Dependant kinase Inhibitor Gene 2A (P16).
CDKN2B	Cyclin Dependant kinase Inhibitor Gene 2B (P15).
CHEK2	Check Point kinase 2.
CHEK-2	Cell Cycle Checkpoint kinase Gene-2.
CIP	Cancerous Inhibition Protein.
CISH	Chromatid Insitu Hyperdization
CR 1,2,3	Conservative Region1, 2, 3.
CRC	Colorectal Cancer.
CT	Computerized Tomography.
CXCL12	Chemokine –X- (C motif) ligand 12.
Cyto –P	Cytochrome – P.

DAP	Di-Amino Para- benzidine.
DCIS	Ductal carcinoma in situ.
DES	Di Ethyl Stilbestrol.
DLg	Disc large (suppressor gene protein).
DNA	Deoxyribonucleic Acid.
DNTP	Deoxynucleotide Triphosphate.
E	Early.
E2B	E2 Binding protein.
E2BSs	E2 Binds Sequences (palindromic).
E2F	E2 Factor of Transcription.
E6 , E7	Early protein 6, 7.
E6 AP	Early 6 Associated proteins.
EBP	E Binding Protein.
EBV	Epstein- Barr Virus.
ECM	Extracellular Matrix.
EDTA	Ethylene Diamine Tetra Acetic Acid.
EGFR	Epidermal Growth Factor Receptor.
ER	Estrogen Receptor.
ERDr	Estrogen Receptor Down Regulation.
ERDs	Estrogen Receptor Downstream.

ESR	Erythrocyte Sedimentation Rate.
EU	European Union.
FISH	Fluorescence In Situ Hyperdization.
FNA	Fine Needle Aspiration.
G phase	Gap (phase).
G phase	Growth Phase.
G_1 phase	Gap 1 (phase).
G_2 phase	Gap 2(phase).
GAPDH	Glyceraldehydes 3-phosphate dehydrogenase
GSPT	G1- S- Phase Transition.
GST	G1 – S transcription.
GST	Glutathione –S- Transferase.
GSTM-1	Glutathione -S - Transferase Mu -1.
GSTP	G1 – S transcription phase.
GSTP-1	Glutathione –S - Transferase Pi -1.
HEC	Higher Education Commission.
Her2/neu	Human Epidermal receptor 2 Neural Tumor.
HMSH-2	Human Mutator - S- homology-2inhibitors.
HNPCC	Hereditary Non Polyposis Colorectal Cancer.
HNSCC	Head and Neck Squamous Cell Carcinoma.

HPRBP	Hypophosphorylated Retinoblastoma Protein.
HPV	Human papilloma Virus.
HRAS 1	Harvey Rat Sarcoma - 1.
HR-HPVs	High-risk human papilloma Viruses.
HRT	Hormone Replacement Therapy.
HSV	Herpes Simplex Virus.
HTERT	Human Telomerase Reverse Transcriptase.
IARC	International Agency for Research on Cancer.
ICC	Immunocytochemistry.
ICTV	International Committee on Taxonomy of Viruses.
IGF-I	Insulin-like Growth Factor-1.
IHC	Immunohistochemistry.
ISH	In situ- Hyperdization.
kD	Kilo-Dalton.
Ki 67	Kiel University 67.
KIP	Kinase Inhibition Protein.
K-ras	Kirsten rat sarcoma.
KSHV	Kaposi Sarcoma Herpes Virus.
L	Late.
LCIS	Lobular Carcinoma In Situ.

LCR	Long Control Region.
LN	Lymph Node.
µl	Micrometers'.
M (phase)	Mitosis (phase).
MAD4	Mothers Against Decapentaplegic Homolog - 4.
MHC	Major Histocompatibility.
Min	Minutes.
Ml	Micrometers'.
MLH1	Mutator- L- Homolog 1.
MLH-1	(Human) Mutator L homolog-1.
MMPs	Matrix Metalloproteinases.
MMR	Mismatch Repair.
MMTV	Mouse Mammary Tumor Virus.
MMTV-LS	Mouse Mammary Tumor Virus-like Sequences.
MRI	Magnetic Resonance Imaging.
mRNAs	Messenger ribonucleic Acid.
MSH2	Mismatch 2.
MYC	Myelocytoma.
MYH	Mutator Y- Homolog (E. coli).
NCI	National Cancer Institute.

NCR	Non-Coding Region.
ORFs	Open Reading Frames.
P(110)Rb	Protein (110) Retinoblastoma.
P53	Protein 53.
p97	Protein 97.
PBS	Primer Binding Site.
PCR	Polymerase Chain Reaction.
p-CR	Pathological complete Response
PDZ	PSD95, DLg1, ZO-1.
PET	Positron Emission Tomography.
PET	Paraffin Embedded Tissue.
PgR	Progesterone Receptor.
PI3K	Phosphotidylinositol -3 kinase.
PI3K	Protein-13- kinase.
PMS2	Post Meiotic Segregation 2.
PTENH	Phosphatase and Tensin Homolog.
PV	Papilloma Virus.
Ras	Rat Sarcoma Virus.
Rb	Retinoblastoma.
RICK	Radiation and Isotopes Center Khartoum.

Rpm	Round Pear Minutiae
S phase	Synthetic Phase.
SCC	Squamous Cell Carcinoma.
SCR	Sudan Cancer Registry.
SP -1	Specificity Protein – 1.
Src	Sarcoma.
STAT	Signal Transducer & Activator Transcription.
STAT3	Signal Transducer and Activator of Transcription - 3.
STK	Serine /Threonine Kinase.
STK11	Serine/Threonine Kinase 11.
Taq	Thermus aquaticus (DNA polymerase).
TBE	Tris Borate EDTA.
TDLU	Terminal Duct lobular Unit.
TFIID	Transcription Factor II D.
TNM	(Tumor – Nodes – Metastasis) Staging System.
TP53	Tumor Protein 53.
UK	United Kingdom.
URR	Upstream Regulatory Region.
US	Ultra Sound.
USA	United State of America.

UTRs	Un Translated Regions.
UV	Ultra Violet.
V	Volte.
VDR	Vitamin D Receptor.
VEGFR	Vascular Endothelial Growth Factor Receptor.
Wg	Wingless.
WHO	World Health Organization.
Wnt	Wingless Type.
XRCC	X-ray Crosses Complementing.
ZO-1	Zona Occludens.

Glossary:

Adenovirus	A medium-sized DNA virus that does not integrate into the host genome. Used commonly as a gene transfer vector.
Aneuploidy	Any deviation from the normal (euploid) number of chromosomes
Apoptosis regulator	Is a protein that in humans is encoded by the *BAX* gene.
Assortative mating	Nonrandom mate selection. Autosome Any chromosome other than the sex chromosomes.
Base pair	One subunit of a double-stranded DNA sequence; either (A-T) or (G-C).
BAX	Is a family members form hetero- or homo dimmers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities.
BRCAI & BRCAII	Are human genes that produce tumor suppressor proteins.
Breast	Is a soft protruding organ on the upper front of a woman's body which secretes milk after child birth.
Breast cancer	Is cancer that develops from breast tissue.
Cell Cycle	Is the series of events that take place in a cell leading to its division and duplication (replication) that produces two daughter cells.
Cancer	An abnormal growth, capable of invading adjacent tissue and establishing colonies (metastases) in other parts of the body.
Chromatid	One half of a chromosome after the DN4 has been duplicated.
Cip/Kip proteins	Members of the Cip/Kip family of cyclin-dependent kinases inhibitors (CKIs) are well characterized for their role as negative regulators of G_1-phase cell-cycle progression

Codon	A set of three adjacent base pairs in DNA or RNA that specifies an amino acid
Diploid	Two sets of chromosomes; the normal condition in somatic cells.
DNA	DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms.
DNA replication	Is the process of producing two identical replicas from one original DNA molecule.
E6 , E7	Transforming Protein of the HPV
E6AP	Is a protein encoded by the oncogenic human papilloma viruses (HPVs) targets p53 for ubiquitin-dependent proteolysis.
Exon	A contiguous segment of DNA that is retained in mRNA after introns have been removed. Most, but not all, exons encode a series of amino acids.
Frame shift	A type of mutation that changes the amino acids specified by the entire region of DNA downstream of the mutation.
Gene	Is a locus (or region) of DNA that encodes a functional RNA or protein product, and is the molecular unit of heredity.
Genome	The total DNA of a species including genes and nongenic sequences.
Haploid	The number of chromosomes found in gametes; half the number in somatic cells. Also used to refer to DNA amount.
HeLa	Is a cell type in an immortal cell line used in scientific research.
Helicase	A protein that breaks hydrogen bonds between base pairs and separates the DNA strands locally.

HER2	Is a type of neural tumor ErbB-2 was named for its similarity to ErbB (avian erythroblastsis oncogene B), the oncogene later found to code for EGFR.
Histone	A very basic (positively charged) protein that associates with DNA.
Hybrid cell	A cell with chromosomes derived from two types of parental cells, usually made by cell fusion techniques.
Inflammation	A localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection.
Intron	A segment of DNA or RNA that lies between exons and does not appear in mRNA.
Inversion	A 180-degree realignment of a portion of a chromosome.
Karyotype	The chromosome complement of an organism or cell.
Kilobase	One thousand base pairs.
L1 sequence	The most abundant member of the LINE class of dispersed repetitive sequences.
Ligase	An enzyme that splices two single
Messenger RNA	The type of RNA molecule that is translated into polypeptides on ribosome's
Missense mutation	A change in one base pair resulting in a codon that specifies a different amino acid from the original one.
MLH1	It is a gene commonly associated with hereditary non polyposis colorectal cancer.
MSH2	Are a tumor suppressor gene and more specifically acaretaker gene that codes for a DNA mismatch repair (MMR).

Mutation	Is a permanent change of the nucleotide sequence of the genome of an organism or extra-chromosomal DNA or other genetic elements.
NIH 3T3	Refers to the cell transfer and inoculation protocol for the line.
Non disjunction	Failure of a pair of chromatids to be separated at mitosis, resulting in one daughter
Nonsense mutation	A change in base sequence that creates a stop codon in a translated sequence.
Okazaki fragments	Short pieces of newly synthesized DNA on the 3’
Oncogene	A gene that can initiate a cancer, acting in a dominant manner.
P53	Tumor suppressor gene
Phenotype	The observable properties of a cell or an organism, which result from expression of the, genotype and environmental influences.
Polymerase	An enzyme that makes long polymers of nucleotides.
Polyploidy	Presence of more than two complete sets of chromosomes in an embryo or cell.
Promoter	A region of DNA near the transcription start site of a gene, to which RNA polymerase and associated factors bind in order to initiate transcription.
Radiations	Is the emission or transmission of energy in the form of waves or particles through space or through a material medium.
Retrovirus	An RNA virus capable of being converted to DNA within a host cell and inserted into the host genome.

Reverse transcriptase	A type of polymerase that makes a DNA copy (cDNA) of an RNA molecule.
Ribosome	The complex assemblage of RNA and protein to which mRNA binds and on which protein synthesis is carried out.
Risk Factor	A risk factor is any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury.
Robertsonian translocation	An end-to-end fusion of the long arms of two acrocentric chromosomes.
Short Interspersed Element (SINE)	One of the major types of dispersed repetitive sequences.
Single nucleotide polymorphism (SNP)	A location in a DNA sequence where different individuals have a different base pair.
Sister chromatid exchange	Recombination between the two DNA strands of a single chromatid.
Stop codon	One of three trinucleotides (UAG, UAA, UGA) at which polypeptide synthesis will be terminated.
SYBR Green I	Is an asymmetrical cyanine dye used as a nucleic acid stain in molecular biology.
Telomere	The structure at the ends of chromosomes, consisting of tandem repeats of a hexanucleotide sequence with associated proteins.
TNM System	Is staging system for all solid tumors.
Topoisomerase	An enzyme required for DNA replication; it makes single
TP53	Is a gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor.

Trans gene	A gene transferred into a host organism by, molecular methods.
Transcription	Synthesis of RNA from a DNA template.
Translation	Is the final step on the way from DNA to protein, It is the synthesis of proteins directed by an mRNA template.
Translocase	A protein that transports a specific class of small molecules from one side of a membrane to the other.
Trisomy	Presence of three copies of one type of chromosome in otherwise diploid cells.
Tumor	A swelling of a part of the body.
Tumor suppressor gene	A gene whose normal function is to prevent proliferation of cells. When both copies are inactivated or lost, uncontrolled cell division ensues.

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Chapter One

(Introduction, Justification, Objectives)

1.1 Human Papilloma Virus:

HPV is a member of the *papillomaviridae* family and have double stranded circular *DNA genome* icosahedral nucleocapsid. [2] These viruses are small in size with *8kbp-long DNA genome*, no enveloped virus. [2] *HPV genome* contains early *(E)* and the late genes *(L)* which codes for early proteins *(E1-E7)* late proteins *(L1 and L2*) and a non-coding *long control region (LCR*). [2] There are more than one hundred different *HPV types* that have been discovered and these were divided into high risk and low risk types. [2] *HPV* (16, 18, 31, 33, 35 45) was classified as high risk *HPV types* associated with many of the cancer while *HPV (6*, 11) are low risks non oncogenic *HPV types.* [2, 3]

1.2 Classification of *Human Papilloma Virus:*

Table (1.1) Shows some high risk, low risk and potentially risks *HPVs*. [2, 3]

Classification	**HPV types**
High-risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59.
Low-risk	6, 11, 40, 42, 43, 44, 54, 61, 70.
Potentially high –risk	26, 53.

1.3 Breast cancer:

BC is a malignant tumor that originates in the breast tissue mainly from the inner lining of milk ducts or the lobules that supply milk to the ducts, cancers that initiates from ducts was called ductal carcinomas and those originating from lobules were called as lobular carcinomas. [19] Cancer occurs due to abnormal changes or mutations in the genes responsible for regulating the growth of cells the change in the genetic information causes a cell to no longer carry out its function properly. [20] *BC* was the most common cancer among female comprises about (18%) of all female cancers, with (1.7) million new cases in the world each year. Based on the most global recent data approximately (12.3%) of women will be diagnosed with *BC* during their lifetime. [10] *BC* is the second most common cancer after lung cancer in both sexes. *BC* is the most frequent neoplasm and cause of death in women between (35 and 55) years of age (522, 000) deaths in 2012 According to the mortality data from 2008 to 2012 *BC* incidence has been increased by more than (20%) while mortality has increased by (14%) however, there are huge inequalities between rich and poor countries. Incidence rates remain highest in more developed regions, but mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities. [12, 15]

In 2013, an estimated (296,980) new cases of *BC* was diagnosed in women in the United States of America. About (39,620) women in the *U.S.A* were died in 2013 from *BC*. [13] There were (49,961) new cases of *BC* in the *UK* in 2011. The crude incidence rate shows that there are (157) new *BC* cases for every (100,000) females in the *UK* and (1 for every 100,000) males. [13, 14, 15] Whereas in India about; (115,000) new cases were diagnosed in 2011. The incidence rate in Nigeria was (52 /100 000) at the Ibadan Population Based Cancer Registry (*IBPCR*), covering a two year period 2009-2010 In Sudan, according to Radiation & Isotopes Center in

Khartoum(*RICK)* records from 2000 to 2011 there are (9534) cases of *BC*, of whom (231) were male(2.3%) and (9303) were female (97.6%).[16, 17]

Environmental and genetic factors they play role at different points in the process of neoplastic progression. [17] Age, family history, inflammation, obesity, smoking, and alcohol are also common risk factors for *BC*. [18] Several abnormalities at the molecular level have been reported in breast carcinomas. These include mutations of oncogenes such as *k-ras*, *DNA mismatch repair genes* such as (*MLH1 and MSH2*) inactivation of tumor suppressor genes (such as *APC*, *p53*) and disturbances of *DNA methylation* and microsatellite instability. [4, 8] Recent studies reported that approximately (18%) of cancer cases can be linked to infectious agents including viruses, bacteria and parasites, examples include *HBV, EBV* and *KSHV*. Finally, an association between *HPV infection* and *BC* was recently shown.[5, 9] The *HPV onco-protein (E7)* binds and inactivates *HPRBP* this leads to up-regulation of *P16* ^{INK4A}, the product of which is tumor suppressor protein that inhibits cyclin-dependant kinases *(CDK)-4 or -6* Confirming this is the strong and consistent overexpression of *p16* ^{INK4A} in *HPV-induced cancers*. [15] Furthermore *HPV onco-proteins (E6, E7)* activate the ras family including *K-ras*, which encodes a small *21-KD protein (P21 ras)* that is involved in the transduction of signals across plasma membranes. [18]

1.4 Justification:

- To the best of our knowledge, this is the first study that attempts to determine the possible role of *HPV* in the pathogenesis of *BC* in Sudan.

- *BC* represent the eighth cancers worldwide. The majority of *BCs* are attributed to life style, type of breast tissues, alcoholic consumption and other environmental carcinogens. Recent studies have shown a strong association between *BC* and *HPV*. In Sudan, there are considerable numbers of *BC*, but there is no evidence highlighted the relation between these cancers and *HPV*. Therefore this study will investigate the *HPV* in the etiology of *BC* applying *PCR techniques*.

1.5 Objectives:

1.5.1 General objective:

The study is to evaluate the possible role of *HPV* in the pathogenesis of *BC* in Sudan.

1.5.2 Specific objectives:

- To find out the incidence of *HPV* infection in BC patients.
- To determine the association between human papilloma virus and *BC*.
- To determine the genotyping of *HPV* in *BC.*
- To find out the relation between *HPV* infections with risk factors such as age, socioeconomic status, family planning, type of tissue, obesity, martial statues in *BC* patient.
- To find out the relation between *BC* with risk factors such as age, socioeconomic status, family planning, type of tissue, weight, martial status in non infected HPV breast cancer patients.

Chapter Two

Literature Review

2.1 *HPV* and *Cancers*:

HPV genome normally was founded in the cytoplasm of infected tissues however the *DNA* of *HPV types* that associated with cancer is integrated into the host genome. [6] *HPV was* caused disruption and loss of some of the viral genes *(L1 and L2 genes)* and also increases the expression of the early genes. [7] *Onco-proteins (E5)* interacts with *MHC class I* and prevents its transport to the cell surface therefore infected cells escapes from the immune system consequently allowing the virus to establish persistent infections and thus progressing to cancer. [8] *E6 targets p53* for degradation and therefore prevents apoptosis of abnormal cells, whereas *E7* inactivates *Rb* function, which results in abnormal cell proliferation and disturbs the normal cell cycle regulation. [8, 9] *P53* and *Rb* are tumor suppressor genes which stop tumors from developing. [10] Incorporation of virus into host cell increases and sustains the growth of both virus and the host cell, thus resulting in the alteration of infected host cells into malignant cells and ultimately invasive cancer. [11]

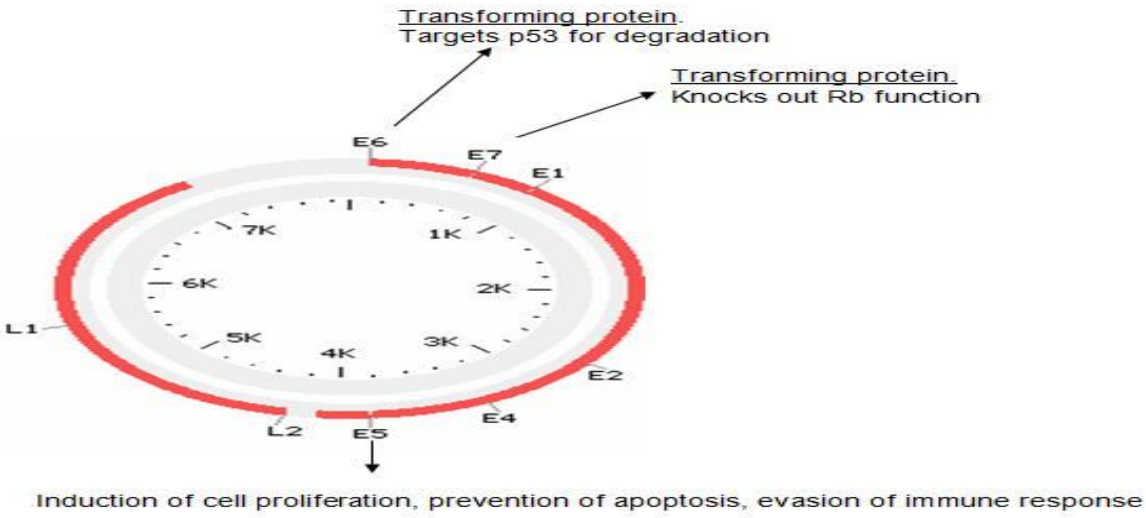

Figure (1.2) Shows different genes of HPV (Image originally from the Springer Website).

2.2 Life Cycle of *HPV*

HPVs are non-enveloped viruses with icosahedral capsids that replicate their genomes within the nuclei of infected host cells. The *double-stranded, circular DNA genomes* of all *HPV*s are approximately *8 kb* in size. *DNA* was found to be associated with cellular histones to form chromatin-like complexes. [63] The viral genomes carry on average eight major open reading frame sequences (*ORFs)*, and these are expressed from poly-cistronic *m-RNAs* transcribed from a single *DNA strand*. In the *high-risk HPV types*, transcripts are initiated at two major viral promoters, one of which initiates upstream of the (*E6) ORFs*, encodes early viral proteins, and is expressed prior to productive replication. In *HPV-16 and HPV-31* this promoter is referred to as *p97*, while in *HPV-18* it is referred to as *p105*. Coincident with the induction of productive replication, the late promoter is activated, which directs expression from a series of heterogeneous start sites clustered around nucleotide in *HPV-31.* [68] Similar promoters have been identified in *HPV-16 and HPV*-18. [46, 53] Several additional minor promoters have been identified that also play important roles during the viral life cycle.

The life cycle of *HPV* is linked to the differentiation program of the infected *host cell*, the keratinocyte, with production of mature virion particles restricted to differentiated suprabasal cells. Infection by *HPV* is thought to occur through micro wounds of the epithelium that expose cells in the basal layer to viral entry the receptor for entry of the virus into cells is currently unknown; however heparin sulfate mediates the initial attachment of virions to cells. [76] Cells in the basal layer consist of stem cells and transit amplifying cells that are continuously dividing and provide a reservoir of cells for the suprabasal regions. *HPV infection* of these cells leads to the activation of a cascade of viral gene expression those results in the production of approximately (20: 100) extra chromosomal copies of *viral DNA* per cell. This average copy number is stably maintained in undifferentiated basal cells

throughout the course of the infection among the first viral proteins to be expressed are the replication factors, *(E1 and E2*) these proteins form a complex that binds to sequences at the viral origin of replication and acts to recruit cellular polymerases and accessory proteins to mediate replication. [18, 44, 47] The *E1 protein* also exhibits helicase activity allowing for the separation of *viral DNA strands* ahead of the replication complex *E2* is a site-specific *DNA binding protein* that helps to recruit *E1* to the origin but also plays a role in regulating viral transcription from the early promoter binding sites for *E2* that located adjacent to sites for cellular transcription factors that activate the early promoter. [49] At low levels, *E2* binds its recognition sequences and activates the early promoter while at high concentrations, it represses by blocking the binding of cellular transcription factors. [39] Since the *(E1 and E2) replication factors* are also expressed from the early promoter, the ability of *E2* to activate and repress expression contributes to the control of viral copy number in undifferentiated cells.

The (*E6 and E7) proteins* of the *high-risk HPV types* act as viral onco-proteins but no such functions are associated with the corresponding proteins from the low-risk types. *High-risk E6* binds the *p53 tumor suppressor protein* as part of a trimeric complex with the cellular ubiquitin ligase, *E6AP*, leading to the rapid turnover of *p53.* [131, 155] *E7* binds to the *(Rb*) family of tumor suppressors, as well as other proteins involved in *cell cycle regulation.* [37, 110] As *HPV* infected basal cells divide, viral genomes are partitioned into daughter cells, one of which detaches from the basal layer, migrates toward the stratum granulosum and undergoes differentiation, in normal uninfected epithelia, cells exit the *cell cycle* as they leave the basal layer, and this often results in the loss of nuclei in suprabasal cells as infected cells leave the basal layer, they remain active in the *cell cycle* due to action of the (*E7) protein*. Cells reenter the *S phase* in highly differentiated cells and activate the expression of cellular replication factors required for viral replication.

The presence of (E7*)* leads to a characteristic retention of nuclei throughout all layers of infected epithelia. Not only are the viral onco-proteins necessary for cell immortalization and retention of *cell cycle* capability on differentiation, but *(E6 and E7)* have also been shown to be necessary for the maintenance of extra-chromosomal forms of *HPV* in undifferentiated basal cells.[145] The mechanism by which this occurs is not clear although it probably likely involves abrogation of checkpoints that block the long-term retention of the *extra-chromosomal DNAs*.

The functions of the *(E4 and E5*) *proteins* are not yet fully understood however they both have been proposed to be involved in regulation of late viral functions. [140] The *(L1 and L2) proteins* are assembled late and spontaneously form icosahedral capsids. Following virion assembly, mature viruses are released from the uppermost layers of the epithelium.[68] It is still not clear how the differentiation program of the host cell is able to activate the productive life cycle of *HPV*, the most likely mechanism centers on the activation of expression of the late viral promoter, resulting in high-level expression of transcripts encoding the viral replication proteins, *(E1 and E2)* along with the late genes. Unlike the early promoter, the late promoter is not negatively regulated by *E2 protein*, and high levels of expression occur upon differentiation, leading to amplification of *viral DNA*. [139] this increase in template numbers results in a further increase in expression of the replication proteins. It is possible those cellular or other viral factors are up regulated on differentiation and that these factors contribute to activation of late functions, but the identification of these proteins is only beginning. In low-grade infections, the *high-risk HPV genomes* are present as episomes, while during progression to high-grade lesions or carcinomas, the genome often is found integrated into host sequences. This integration usually occurs within the *E2 ORF* and results in loss of *E2* repressive action leading to higher levels of *(E6 and E7)* expression.[133, 134] In the following sections, it is

described in detail the present state of knowledge about how the viral factors act during the productive *life cycle* as well as during progression to malignancy.

2.3 Function of Different Genes of *HPV*

Table (1.2) Show the function of different genes of *HPV*. [133,134]

Gene/Region	Function
E1/E2	Code for proteins which control the function of *E6 and E7 genes*
E4	Function largely unknown but may control virus release from cell
E5	Codes for a hydrophobic protein which enhances immortalization of the cell
E6	Codes for proteins which inhibit negative regulators of the cell cycle .E6 products inhibit p53 which is a transcription factor for apoptosis
E7	Codes for products which bind to the retinoblastoma tumor suppressor proteins there by permitting the cell to progress through the cell cycle in the absence of normal mitogenic signals
L1/L2	Code for structural proteins and formation of complete virus particles
LCR	Necessary for normal virus replication and control of gene expression

2.4 Association of *HPV* with the Breast Cancer:

The association of *HPV* with cervical cancer and head and neck cancers is well established, the involvement of the virus in *BC* is more controversial. [11] Some studies have demonstrated the presence of *HPV high-risk types (16, 18 and 33)* in breast cancer specimens from diverse populations around the world.[12] Women that have both breast and cervical cancers were found to be infected with the same *HPV* types. [16] The controversy surrounding the role of *HPV* in *BC* may be because of the difficulty that has been encountered in detecting the virus in breast specimens in

contrast to the relative ease of detection in cervical cancers.[17] Indeed in a previous study from our group, we demonstrated that it was necessary to use *SYBR Green PCR* methods for detection of the virus in *BC* tissues.[17] Because there is a considerable proportion of *BC* specimens are non-cancerous and that the levels of virus are low in *BC*. One solution to the detection of such low levels of *HPV* is the use of *In situ PCR*. The oncogenic mechanisms by which *HPV* induces *BC* have been intensively studied. [18] In this study *HPV* used *was associated with BC* as a model. *HR-HPV* encodes a series of proteins, designated as early *(E1–E7)* or late *(L1 and L2*). Although all of the viral proteins have a role in viral replication, only a small number of the viral early proteins have a role in cellular transformation. Key to transformation are the *(E6 and E7) onco-proteins*, which work in concert to disrupt *cell-cycle regulation* by inhibiting apoptosis and stimulating *cell- cycle* progression by binding/inhibiting the *p53* and *p10 Rb tumor suppressor genes*, respectively. In addition to that the *HPV (E5 and E6)* acts early in transformation (before integration) and are known to disrupt cytokeratin causing perinuclear cytoplasmic clearing and nuclear enlargement which leads to the appearance of a koilocyte. [19]

2.5 Normal Anatomy and Histology of the Breast:

The breast consists of mammary glands, associated with a skin and connective tissues. It lies in the superficial fascia anterior to the pectoral muscle and the anterior thoracic wall. The base of each breast extends vertically from ribs (2 to 5) and transversely from the sternum to the mid axillary line. A layer of loose connective tissue (retro mammary space), separates the breast from the deep fascia and provides some degree of movement over underlying structures. [45, 46]

The mammary glands are modified as apocrine like sweat glands, classified as compound tubule-alveolar glands. It consists of (15 to 25) lobes radiating out from

the nipple embedded in a mass of adipose tissue which is subdivided by collagenous septa. Each lobe is drained by its own lactiferous duct leading directly to the nipple. Before the opening on the surface, each duct is dilated to form lactiferous sinuses for milk storage. The nipple contains smooth muscle oriented in parallel to the lactiferous ducts, as well as covered by keratinized stratified squamous epithelium. The areola in skin around the nipple contains sweat and sebaceous glands. [34]

The mammary gland composed of lobes, within the lobe, the main duct branched into numbers of terminal ducts, which leads to lobule consisting of multiple acini. Each terminal duct and its associated lobule are called *TDLU* the ducts and acini are lined by two layers of cells; a luminal layer of epithelia cells and basal layer of myoepithelial cells. In the larger ducts, the epithelial cells are columnar type and in small ducts and acini are cuboidal cells. [36]

2.5.1 Structure of the Breast:

The breast is a mass of glandular, fatty and connective tissue. The breast is made up of

- Lobule glands that produce milk
- ducts tubes that carry milk from the lobules to the nipple
- Fatty and connective tissue surrounds and protects the ducts and lobules and gives shape to the breast
- Areola is the pink or brown, circular area around the nipple that contains small sweat glands, which release (secrete) moisture as a lubricant during breast-feeding
- Nipple is the area at the center of the areola where the milk comes out.

Ligaments support the breast. They run from the skin through the breast and attach to muscles on the chest. There are several major nerves in the breast area, including nerves in the chest and arm. There are also sensory nerves in the skin of the chest and axilla. [64]

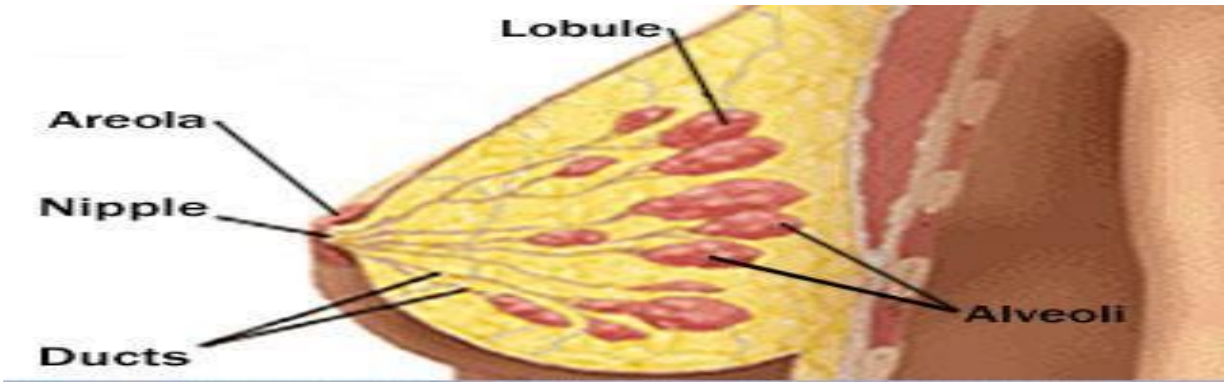

Figure (2.2) Shows different parts in the female breast stromata
(Image originally from the Choi**,** Seung Ho, Kasama, Kazunori Bariatric, Metabolic Surgery, London 2010)

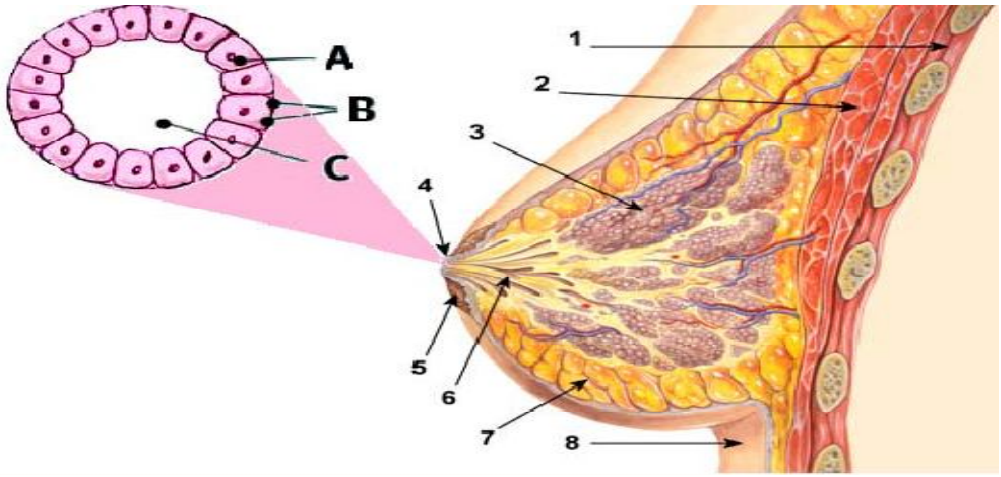

Figure (3.2) anatomy of normal breast: - 1. Chest wall 2. Pectoral muscles 3. Lobules 4.Nipple 5.Areola 6. Lactiferous duct, 7. Fat 8. Skin. A normal duct cells, B basement membrane, C lumen (this picture is reproduced from Snell growth anatomy)

2.5.2 The Lymphatic System of the Breast:

The breast has many blood vessels and lymph vessels. Lymph vessels are thin tubes similar to blood vessels. They collect and move lymph fluid away from the breast into small bean-shaped masses of lymphatic tissue, called lymph nodes, in the area around the breast. The lymph vessels and lymph nodes are part of the lymphatic system, which helps fight infections. The breast lymph nodes include:

- Supra clavicular nodes above the collarbone.
- Infra clavicle (or sub clavicle) nodes below the collarbone.
- Axillary nodes in the armpit (axilla).
- Internal mammary nodes inside the chest around the breastbone (sternum). [65]

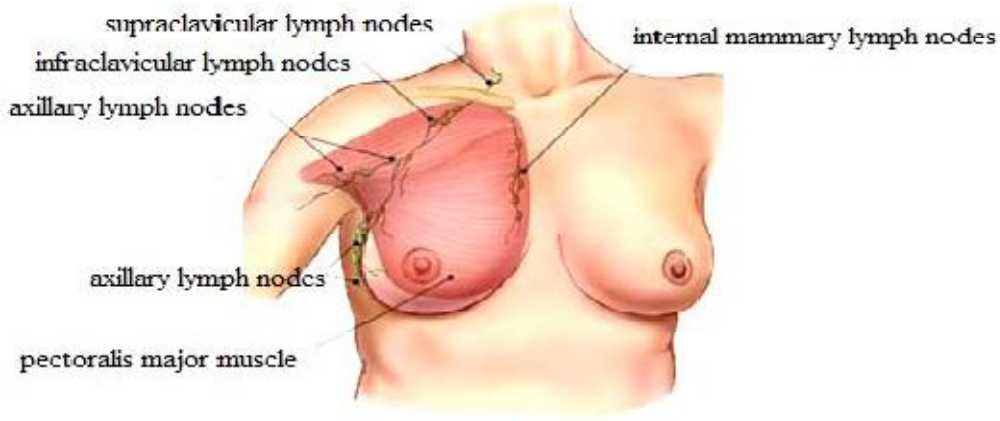

Figure (4.2) lymphatic system of female breast: (Image originally from CDC websites)

2.6 Hormones of the Breast:

Estrogen is the main female hormone. It influences female sexual characteristics such as breast development, and it is necessary for reproduction. The ovaries make most of the estrogen in a woman's body though a small amount is made by the adrenal glands. Progesterone is the other female sex hormone made in the ovaries.

Its role is to prepare the uterus (womb) for pregnancy and the breasts for producing milk for breast lactation. The breast tissues are exposed to monthly cycles of estrogen and progesterone throughout a woman's childbearing years. [59]

- In the first part of the menstrual cycle, estrogen stimulates the growth of the milk ducts.
- Progesterone takes over in the second part of a woman's menstrual cycle stimulating the lobules.

2.7 Development of the Human Breast: [57]

Breast tissue changes at different times during a woman's life it changes during puberty, during the menstrual cycle, during pregnancy and after menopause female breasts do not begin growing until puberty around (10–12) years of age, at this time the breasts respond to hormonal changes (mostly increased estrogen and progesterone) in the body and begin to develop. During puberty, the breast ducts and milk glands grow. [57] The breast skin stretches as the breasts grow creating a rounded appearance. Young women tend to have denser breasts (more glandular tissue) than older women. In older women, much of the glandular and ductal tissue is replaced with fatty tissue and breasts become less dense, ligaments also lose their elasticity when women age causing the breasts to sag the size and shape of women's breasts vary considerably. Some women have a large amount of breast tissue and have larger breasts. Others have a smaller amount of tissue with little breast fat. [58] The human breast consists of parenchymal and stromal elements. The parenchyma forms a system of branching ducts eventually leading to secretary acini development and the stroma consists mainly of adipose tissue, providing the environment for development of the parenchyma. [1,3]) These building blocks of the breast are recognized as early as the embryonic stage of human development. The process of development of the ductal system and acini is termed branching

morphogenesis and although it commences in the fetus, it halts in early childhood until puberty when hormonal stimulation triggers further differentiation under the influence of hormones, complex reciprocal interactions between the epithelium and mesenchyme lead to differentiation of the prenatally developed rudimentary structure to form a mature mammary gland.[59] Although the precise mechanisms are still unclear, our understanding of branching in the mammary gland is increasing.[60]

2.7.1 Prenatal Development:

Prenatal breast development can be classified into two main processes; formation of a primary mammary bud and development of a rudimentary mammary gland. The earliest stages of embryogenesis are largely hormone independent and hormones regulatory factors are important for development in the second trimester.[61]

2.7.2 First Trimester:

As early as (4 to 6) weeks of gestation, mammary-specific progenitor cells may be seen around day (35) of gestation, proliferation of paired areas of epithelial cells in the epidermis of the thoracic region occurs. These discrete areas of proliferation extend in a line between the fetal axilla and inguinal region and form two ridges called the mammary crests or milk lines. Most of the mammary crest atrophies except for paired solid epithelial masses in the pectoral region at the fourth intercostal space, which form the primary mammary buds. [116] Supernumerary nipples (polythelia) occur in (2 to 5%) of humans in a position from the groin to the axilla, supporting the existence of the mammary crest or ridge.[62] These supernumerary nipples can appear the stages of embryogenesis are largely hormone independent and hormones regulatory factors are important for development in the second trimester. The successive distinct stages of intrauterine breast development described below correlate loosely with gestational age and significant variations at similar stages can be seen. First trimester as early as (4 to 6) weeks of gestation,

mammary-specific progenitor cells may be seen. Around day (35) of gestation, proliferation of paired areas of epithelial cells in the epidermis of the thoracic region occurs. These discrete areas of proliferation extend in a line between the fetal axilla and inguinal region and form two ridges called the mammary crests or milk lines. Most of the mammary crest atrophies except for paired solid epithelial masses in the pectoral region at the (4^{th}) intercostal space, which form the primary mammary buds. [63] Supernumerary nipples (polythelia) occur in (2 to 5%) of humans in a position from the groin to the axilla, supporting the existing grows vertically into the mesenchyme surrounding the primary bud and has a slender stalk and bulbous end. [63] The secondary epithelial sprouts canalize and coalesce forming secondary buds that give rise to lactiferous ducts. The epithelial cells lining the lactiferous ducts are arranged in two layers, with the layer adjacent to the lumen gaining secretory function while the basal layer differentiates into myoepithelial cells. By (6) months of gestational age, the basic framework of the gland is established. A well-defined tubular architecture in a bed of dense fibro connective tissue stroma is noted at this stage. [64] This is around the time breast tissue in both boys and girls can be apparent. [65] These supernumerary nipples can appear in third trimester repeated branching of the secondary epithelial buds and canalization occurs in the third trimester. [66] Disagreement exists over the final morphology of the breast at birth although most sources agree these secondary processes end in rudimentary lobular structures or end buds. [67] Some argue that the breast at birth does not contain any evidence of lobules, only ductal structures with surrounding stroma. [68] The epidermis in the region of the future nipple becomes depressed, forming the mammary pit during the third trimester the lactiferous ducts drain into retroareolar ampulla that converge into this pit on the overlying skin. [69] The nipple is further delineated by proliferation of the mesoderm stimulated by the invagination of ectoderm in this region. The nipple is created with smooth muscle

fibers aligned in a circular and longitudinal fashion. [70] The surrounding areola is formed by the ectoderm during the (5^{th}) month of gestation. During the final weeks of gestation, the loose fibro - connective tissue stroma increases in vascularity. Due to a complex interplay between fetal, placental, and maternal hormones that has not yet been elucidated limited secretory activity in the late-term fetus and newborn infant may occur. [71] The failure of preterm infants to develop breast nodules or secrete milk after birth indicates that the intrauterine environment is essential for breast development. [72] Preterm infants do not develop breast nodules or secrete milk after birth, further lending evidence to the fact that the intrauterine environment is essential for breast development. At term approximately (15 to 20) lobes of glandular tissue have formed, each containing a lactiferous duct that opens onto the breast surface through the mammary pit both the surrounding skin and the fibrous suspensory ligaments of Cooper that anchor the breast to the pectoralis major fascia provide support to the Infant breast. The first (2) years of life are a critical period for some aspects of breast maturation as well as involution; the normal gland remains quiescent from (2) years of age to puberty. At birth, the breast is usually palpable in the newborn with varying amounts of tissue and no significant difference between the genders. Falling levels of maternal estrogens in the neonate stimulate the neonatal pituitary gland to produce prolactin, which results in unilateral or bilateral breast enlargement and/or transient secretion of milk in as many as (70%) of term neonates. [73] It has been speculated that the infant breast undergoes stimulation at approximately (3 to 4) months postnatal through a surge of the infant's own breast. Reproductive hormones, including estradiol breast tissue in female infants persists longer than in male infants due to higher estradiol levels in infancy in girls. [74] Soon after birth, the nipples become everted from proliferation of the underlying mesoderm and the areolae increase in pigmentation. Development of erectile tissue in the nipple areolar complex

increases response of the nipple to stimulation. Nipples that remain inverted until puberty are not uncommon. An increase in vascularity of the gland stroma soon after birth causes a visible difference between the light periductal connective tissue and the denser supporting stroma. (75)

2.8 Development of the Mammary Gland at Puberty:

Sexually dimorphic development of the breast first begins at puberty and unlike the preceding stages of development, pubertal changes are heavily under the influence of sex hormones, in particular estrogen. (76)

Table (2.2) morphological and functional changes in the infant breast (77)

Morphological type I	Branching ductal system with no or less than two dichotomous branching.
Morphological type II	Branching ductal system with more than two dichotomous branchings, but no terminal lobular units.
Morphological type III	Branching ductal system with number of branchings and well developed lobular system.
Table 1	Summary of functional changes.
Functional type I	All ducts and ductuless are lined by secretory type of epithelium.
Functional type II	Mixture of ducts lined by secretory and apocrine type epithelium.
Functional type III	Almost all ducts lined by apocrine type of epithelium.
Functional type IV	Mixture of ducts lined by apocrine type of epithelium and involuting ducts lined by multilayered epithelium.

2.9 Regulation of Breast Development:

Mutual and reciprocal interactions between epithelial components and mesenchymal or stromal cells are responsible for prenatal, infant, and pubertal breast development.[79] Evidence suggests the mesenchyme has inductive properties that lead to the local migration and changes in cell adhesion of epithelial cells. Hormonal influences on this paracrine interaction between the mesenchyme and parenchyma exist at all stages of development. The formation of lactiferous ducts is induced by placental hormones entering the fetal circulation. Other hormones implicated, but not completely elucidated in prenatal and pubertal breast development are progesterone, growth hormone, (*IGF-I) estrogen*, prolactin, adrenal corticoids, and tri-iodothyronine. [80]

2.10 Cell Cycle in the Breast Cancer:

Deregulation of *cell-cycle* is a distinguishing hallmark of tumor cells.[54] Regulation of *cell cycle* is a key mechanism for the maintenance of homeostasis of normal cell growth and viability, and this is a very tightly regulated process however, deregulation of *cell cycle* is well known to contribute to tumor development.[55] Normal cells possess an ability to arrest *cell cycle* after *DNA damage* in an attempt to maintain genome integrity whereas tumor-initiating cells are characterized by deregulated *cell-cycle* whereby the *DNA-damaged cells* proceed to undergo *DNA synthesis* and cell division, which leads to the development of tumor mass since cancer cells, including breast cancer cells, are known to exhibit uncontrolled cell growth and proliferation, one parameter to judge the efficacy of anti-cancer therapies is through their ability to arrest *cell cycle.* Therefore, it is not surprising that the acquisition of drug-resistance in cancer cells is often linked with defects in *cell cycle regulation*. *Cell cycle arrest* involves down-regulation of cyclins and *(CDKs)* and up-regulation of inhibitory *(p21 and p27)*. Several investigations

including those from the laboratory of practice have revealed that *cell cycle arrest* is an important mechanism responsible for apoptosis-inducing ability of cancer therapeutic agents in breast cancer cells. In particular functional loss of *G1-checkpoint inhibitors*, *(p21 and p27)*, is believed to be important during the progression of many human malignancies, and most therapeutic agents function via induction of these tumor suppressor proteins Loss of *(p21 and p27)* has also been implicated in the acquisition of drug-resistance phenotype, and conversely, their up-regulation has the ability to resensitize cancer cells to conventional therapeutics.[56]

2.11 Cell Cycle: An Overview:

The two main events phases of the *cell cycle* are - interphase and mitosis Interphase is the phase of *cell cycle* in which cell performs the majority of its purposes including preparation for the division of cell.[57] Mitosis is that phase of the *cell cycle* when cell prepares for and actually completes cell division. Interphase serves as the checkpoint to ensure that the cell is ready to enter into mitosis. Since the *cell cycle* is a "cycle", cells are continually entering and exiting the various phases of this dynamic cycle. Cells spend a majority of their time in interphase and this phase has three distinct stages – *G1 S andG*2. [57]

G1 phase G1:

Is the phase of *cell cycle* immediately following a round of cell division and occupies the time between mitosis and the beginning of *DNA replication* during *S phase*. In the *G1phase*, cells grow and function normally prior to another round of cell division. [58] Cell has to make sure that it is completely ready for division. *G1* is the phase when this monitoring takes place and if the cell is not ready yet to divide, it will continue to remain in this phase cells are even known to enter a phase called *G0* if they are not ready to continue in the cell cycle. *G0* can last for days, weeks,

or even years. However, if cell decides to divide, it grows in size during *G1 phase*, more cell organelles are synthesized protein synthesis occurs and cell prepares itself for *DNA replication*. [58]

S phase:

Immediately following *G1 phase* is the *S (synthesis) phase* it is the phase when *DNA replication* takes place. This phase represents a particular sensitive point in *cell cycle* because fidelity of *DNA replication* is required to ensure that the resulting daughter cells will have exactly the same genetic make-up as the dividing mother cell. Most of the events that occur during *S phase* are related to *DNA replication* and this phase is marked by synthesis of proteins/enzymes that are involved in *DNA replication* machinery. At the end of *S phase*, cells contain twice the normal number of chromosomes. [58]

G2 phase:

S- phase is followed by *G2 phase*. This phase is marked by further growth of cell in anticipation of mitosis. Since this phase occurs after the *duplication of DNA* and just before the commencement of cell division in mitosis, it represents another checkpoint in the *cell - cell cycle regulatory proteins* in B C. [58]

2.12 Regulation of Cell Cycle:

2.12.1 Cyclins:

Cyclins are so named because they undergo a constant '*cycle*' of synthesis and degradation during cell division. There are now several recognized classes or types of cyclins, active in different stages of the *cell cycle*. The *D- and E-type cyclins* are associated with the *G1-S phase* transition of the *cell cycle*; cyclins are proteins that play important roles in the functioning of *CDKs*. [59] *(CDKs)* as their names suggest are kinases that depend on cyclins for their kinase activity the *CDK* series was born with many of the pre-recognized *cell cycle regulators* named *CDK1 CDK2,*

CDK3 [60]. *CDKs* were classified as kinases based on the observation that the total amount of phosphorylated proteins increased following injection of *CDKs* into the oocytes of a variety of different organisms.[67] Cyclins bind to *CDK*s to form a *cyclin-CDK complex*. This complex, along with the various phosphorylated targets, acts as a signal for the cell to pass to the next *cell cycle* phase. *Cyclins and CDKs* are therefore, positive modulators of *cell cycle*. Synthesis of cyclins and *CDKs* marks the readiness of cells to divide. At the time when cell no longer wants to divide cyclins are degraded resulting in deactivation of *CDKs* and arresting the *cell cycle.* [60]

2.12.2 Inhibitors of Cyclin-Dependent Kinases:

Since *CDKs* are involved in the progression of *cell cycle*, molecules that inhibit *CDKs* are negative regulators of *cell cycle* and function to induce *cell cycle* arrest *Cyclin- CDK complexes* typically activate their downstream targets by phosphorylation therefore inhibitors of *cyclin-CDKs* modulate *cell cycle* by preventing or limiting *cyclin-CDKs* ability targeting new pathways and cell death in *B C* to phosphorylate their targets. There are two classes of *CDK inhibitors* members of the first class specifically bind to *CDK4 and CDK6* and inhibit their association with *D-type cyclins*. Members of the second class, also known as *(KIPs)* and include *(p21, p27 and p57),* are inhibitors of *cyclin A-CDK cyclin D-CDK* and *cyclin E-CDK complexes* Similar to existence of many *CDKs* many inhibitors of *CDKs* these are *(p21 and p27)* . [61]

2.12.3 P 21 (cyclin-dependent kinase inhibitor 1):

The *p21* is coded by human gene *CDKN1*and belongs to the *Cip/Kip* family of *CDKI inhibit*ors *proteins*. [60] These are number of biological effects of *p21* are mediated by its binding to and inhibition of *(CDKs)*. [62] Since these *cyclin-CDK complexes* play role in the progression through *G1 phase*, *p21* regulates the *cell*

cycle progression at *G1phase* of the *cell cycle*. It has been suggested that the ability to mediate *p53-dependent gene rep*ression might be a mechanism by which *p21* induces *cell cycle* inhibition. [62]

2.12.4 P27 (Cyclin-Dependent kinase Inhibitor 1B):

The *p27*is coded by human gene *CDKN1B*and, like *p21*, belongs to the *Cip/Kip* family of *CD Kin hibitor proteins*. Cloned *p27Kip1* inhibit *cyclin E-Cdk2*complexes leading to obstruction of entry of cells into *S phase*. It was *cell cycle* regulatory proteins in *BC*. [62]

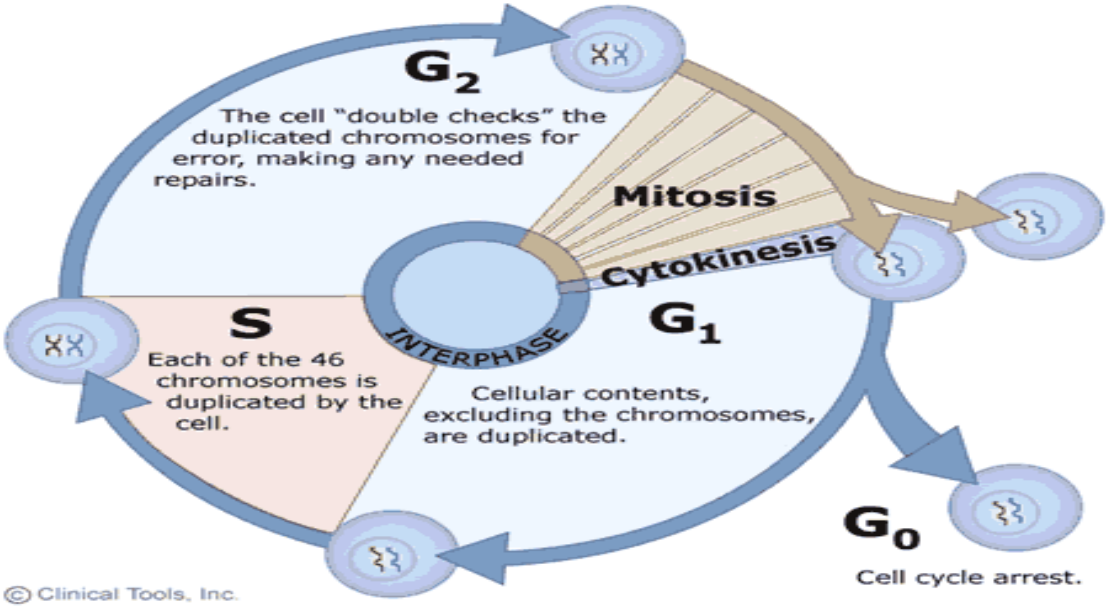

Figure (5.2) Illustrations of cell cycle: (Image originally from the MD consult website).

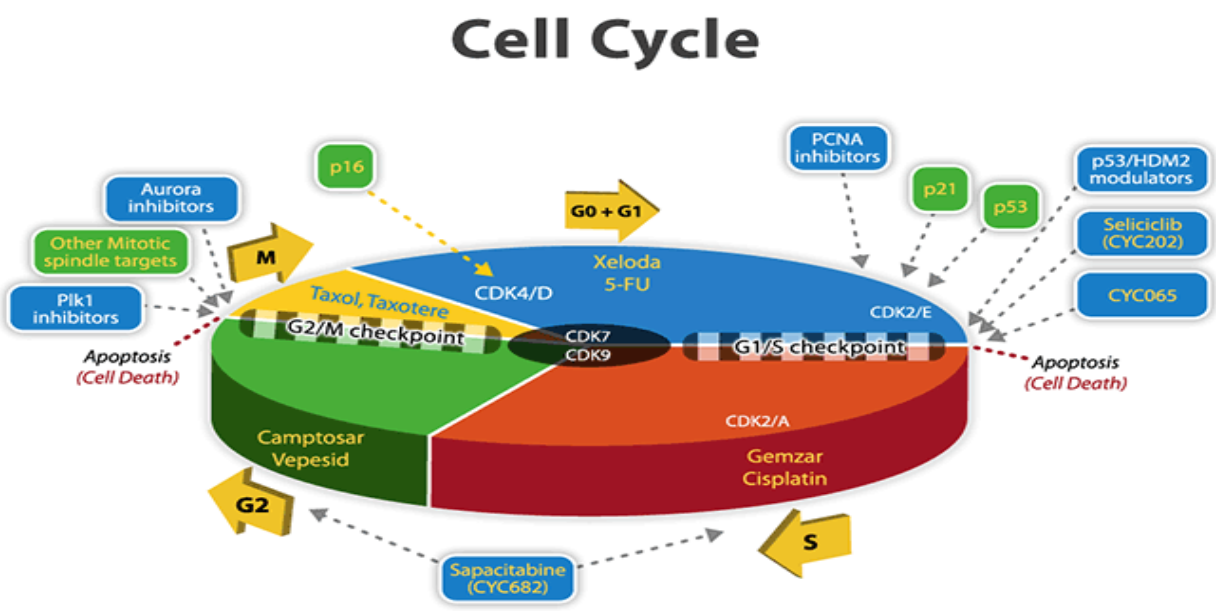

Figure (6.2) Illustrations of cell cycle (Image originally from the MD consult website).

2.13 Function of the Breast:

The breast's main function is to produce; store and release milk to feed baby milk is produced in lobules throughout the breast when hormones in a woman's body stimulate them after giving birth. The ducts carry the milk to the nipple milk passes from the nipple to the baby during breast-feeding. [63]

1.14 Breast Cancer:

BC is malignant tumor that originates in the breast tissue mainly from the inner lining of milk ducts or the lobules that supply milk to the ducts cancers that initiates from ducts are called ductal carcinomas and those originating from lobules are called as lobular carcinomas. [66] Cancer occurs due to abnormal changes or mutations in the genes responsible for regulating the growth of cells [68] the change in the genetic information causes a cell to no longer carry out its function properly[69].

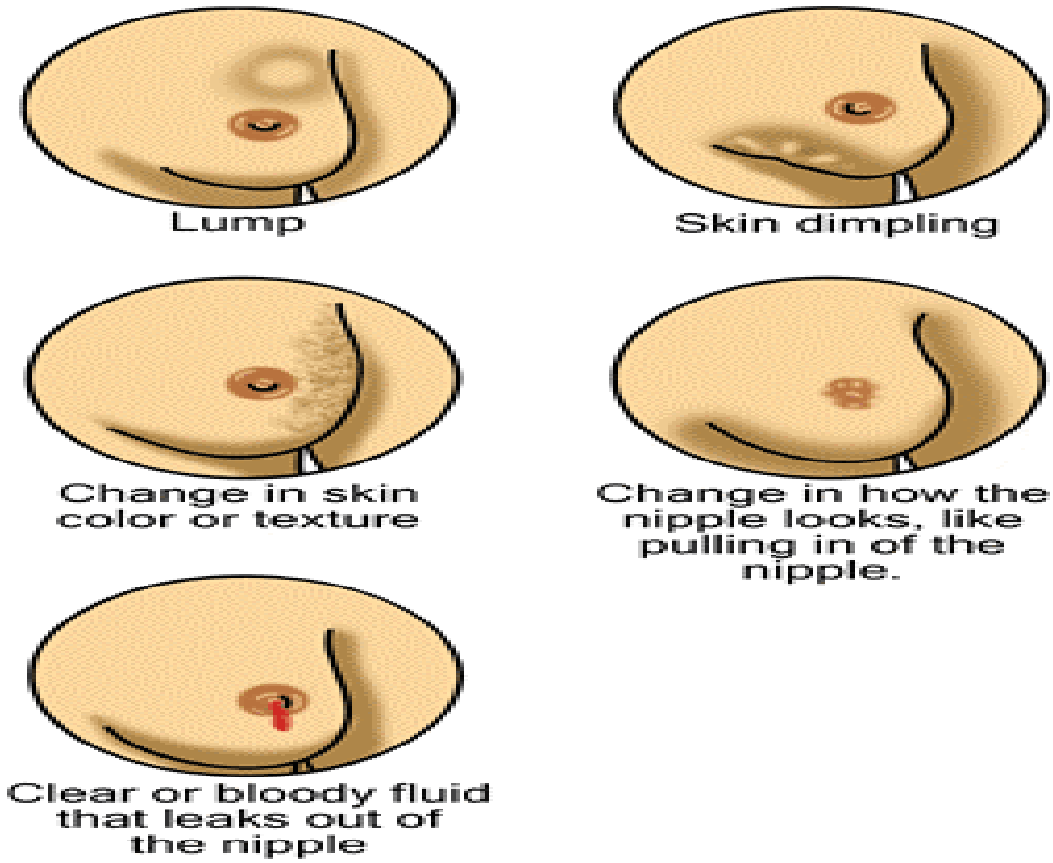

Figure (7.2) Illustrations of early signs of breast cancer (Image originally from the National Institutes for Health**).**

2.15 HPV as Risk Factor in Pathogenesis of Breast Cancer:

2.15.1 Molecular structure and Role of *high, low risk HPV* in *BC*:

(HPV) is about *(55) nm* in diameter. It has a single circular double stranded DNA molecule and belongs to the family papillomaviridae. Its genome is made up of (7,200 – 8,000) base pairs with a molecular weight of (5.2×10^6) *D*. On the basis of *DNA base pair (bp)* distribution, the *viral DNA* is divided into three parts: first a (4,000) *bp* region that responsible for *viral DNA replication* and cell transformation, second (3,000) *bp* region that encodes the structural proteins of the virus particles and last (1,000) *bp* Non-Coding Region *(NCR)* that contains the origin of *viral DNA replication.* [26.27] Suggested that the *genomic HPV DNA have* (9) *(ORFS*) -present on *single strand of DNA* and where divided into seven early *(E)* and two late-phase genes *(L)*. The transcription of *viral DNA was* regulated by early phase gene, while the capsid proteins (involved in viral spread) are regulated by late phase gene. The early-phase gene *(E)* encodes the *(E1, E2, E5, E6, and E7)* proteins. *(E1 and E2)* gene products regulated the transcription and replication of viral proteins and *E5gene* product transcribed from the episomal region of the *viral DNA*. [69] The *(E6 and E7) onco-proteins* are usually under control of *(E1 and E2) inhibitory genes* these genes have the ability to de-stimulate the tumor suppressor function and regulate the functions of the (*p21*, *p53) proteins* resulting in apoptosis, DNA *repair* and *cell cycle control* and finally lead to cellular immortalization. The non-coding, *(LCR)* contains binding sites for the *(E1 and E2) gene products* located just upstream of the promoter sequence *(P 97)* which controls the transcription of the *(E6 and E7) oncogenes.* [67] *HPV* must adhere to a specific receptor protein on the keratinocytes membrane. Once the virus entered into the cell, it transforms itself of its protein coat and the *viral DNA* may then utilize *host cell* themselves. These viruses elaborate early *gene proteins (E)* that are able to regulate the *host cell cycle*, or mitotic capabilities the *(E6 and E7) proteins* are most important in this

respect; they bind two host proteins that are regulators of the keratinocytes at the time of cell division. *E6* binds to a protein designated *p53*, a molecule that arrests cell division. However, once bound, it is degraded and this inhibition of keratinocytes mitosis is abrogated Likewise, *E7binds a protein* termed (*Rb)* and, similarly, *cell cycle* regulation is troubled. On the basis of their genotype, more than (120) types of *HPV viral DNA genotypes* have been fully sequenced it's classified on the bases of their infection in epithelial cells and the ability to effect cellular transformation. [68]

2.16 *(HPV)* Structure and Genome Organization:

The papillomaviruses are a big group of small, *non-enveloped DNA Viruses* which can induce squamous epithelial tumors (warts and papillomas) in much different anatomical localization. A strong correlation between *HPV* and BC cervix uteri, penis, vulva, vagina, anus and oropharynx (including base of the tongue and tonsils), oral cavity, larynx and hypo-pharynx has been recorded by the *(IARC)*. [69] Of the estimated (12.7) million new cancers occurring in 2008 worldwide, (4.8%) were attributable to *HPV infection*. [79] Cottontail *rabbit papillomavirus* was the first described papilloma virus. [80] In 1972 the connection between (*HPV)* and skin cancer in epidermal dysplasia verruciformis was suggested. [81] In 1977 some studies mentioned that (*HPV)* plays an important role in the cause of *BC*. [91] In 1983 there is some collaborators identified (*HPV16 and 18)* in cervical cancer and in the course of the next (12) years of research it has been recognized as a carcinogen influencing its development. Subsequent years confirmed the carcinogenicity of *HPV16* in relation to oropharynx and possibly to the oral cavity. The research was conducted by *IARC*. [88, 89] According to recent recommendations of the *(ICTV)*. [82] This virus belongs to the Papillomaviridae family, which contains (29) genera (30 genera according to (*ICTV)* formed by (189)(PV) types isolated

from humans (120 types), non-human mammals, birds and reptiles (64, 3 and 2) types respectively to accommodate the number of *PV genera* exceeding the Greek alphabet, the prefix ‘‘dyo’’ is used, the current set of *human PVs* is contained within five genera, whereas mammalian, avian and *reptile PVs* are contained within *(20, 3 and 1)* genera respectively.[83] The (*L1)(ORF)* is the most conserved region within the genome and has therefore been used for the identification of new *papilloma-virus types*. *A new papillomavirus* isolate is recognized if the complete genome has been cloned and the *DNA sequence* of the (*L1) (ORF)* differs by more than (10%) from the closest known type differences in homology ranging between (2%) and (10%) define a subtype and those of less than (1%) define a variant for each genus there are biological properties and characteristics genome organization. Some *Alpha-papilloma virus* (which among others include types *32, 10, 61, 2, 26, 53, 18, 7, 16, 6, 34, 1, 54)* are responsible for mucosal and cutaneous lesions in humans and primates high- and low-risk classification based on molecular biological data high-risk types (pre- and malignant lesions) immortalize human keratinocytes low-risk types (benign lesions) *beta-papilloma viruses types(5, 9, 49)* are responsible for cutaneous lesions in humans. [91] Infections exist in latent form in general population and are activated under conditions of immune suppression *gamma-papilloma viruses* are responsible for cutaneous lesions in humans histologically distinguishable by intra-cytoplasm inclusion bodies specific for the type of species. *Mu- papilloma viruses* are responsible for cutaneous lesions. Nu-papilloma viruses are responsible for benign and malignant cutaneous lesions. The genetic material is in the form of a *double-stranded, circular DNA* which accounts for (10–13%) of the virion mass. The viral genome consists of (7200–8000) base pairs. [95] And organized into (3) segments; early region *(E)* which comprises *(E1, E2, E4–E7)* and represents (50%) of the genome, the late region *(L)* consisting of (*L1 and L2)* which represents (40%) of the genome and the genomic regulatory

region (10%) of the genome). [86] All encoding protein fragments are located on a single *DNA* strand these *DNA fragments* were described as can be divided into early and late depending on the time of *viral DNA* replication occurrence. [88] Early fragments are involved in the regulation of *DNA replication* (E1, E2) transcription (E2) and cell transformation *(E5, E6, E7)* and late fragments neither encode structural proteins of the virion, no enzymes, and lipids nor were saccharides found within the *HPV structure*. [84] The virus is stable at pH = 3–7 becomes inactivated at (70 – 80) □ C and is killed after (30) min in the temperature above (50 - 80) □ C. It is resistant to solvents, acids and *X-ray*. In general, virus infection leads to the destruction of the cell; however, it may also cause cell transformation and tumor development. There have been over (100) different types of viruses identified up to date. [88] Within this group there are (118) types which nucleotide sequence has been elucidated.[89] They can be divided into two subgroups: those with low oncogenic potential, [86, 87] and with a high oncogenic potential (*16, 18, 31, 33, 35, 45, 51, 52, 55, 58, 59, 68, 73, 82, 83)* some authors also include *(39, 56 and 79)* in the high risk type.[90] It is essential to underline that this division in not clearly established as there are authors who propose a different qualification: low-risk types; *(6, 11, 42, 43, 44, intermediate risk types 31, 33, 35, 51, 52)*, and high risk types; *(16, 18, 45, 56 or high risk HPV types; 16, 18, 31, 33)*. [91]

2.16.1 E1 protein:

This protein is necessary for *viral DNA replication*. It is also a repressive agent in transcription and inhibits *DNA replication*, maintaining the episomal copies number within the cell at the same level. [91]

2.16.2 E2 protein:

Protein E2 is also involved in the *DNA replication* process; it combines with *E1 protein* and jointly they initiate it, especially *HPV(6, 11, 16)* it is also responsible for coding proteins which regulate *viral DNA transcription , E2* also plays an important role in cell transformation, initiating and inhibiting apoptosis transcriptional regulation, and in the modulation of the immortalizing and transformation potential of *HPV, E2 inactivation* affects the development of tumor lesions by promoting the expression of *(E6 and E7)*, and *active E2* inhibits the *transcription of(E6 and E7)*, causing an increase in *p53 expression* and apoptosis of the infected cells ,the (2) proteins are essential in maintaining the replication of the virus and synthesis of the genes through the course of the differentiation process of the epithelium. They are also necessary for the virus to complete its replicable cycle. [92]

2.16.3 E1 and E2 replications proteins:

The most highly conserved of all *HPV ORFs* are those encoding the *E1 protein*. *E1 proteins* are approximately *(68) KD* in size and are expressed at low levels in *HPV-positive* cells. *E1 proteins* function in origin recognition and exhibit both *ATPase* and (3′-5′) helicase activities. [65, 136, 157] They recognize *AT-rich sequences* at the origins of *HPV replication*, which are located proximal to the start sites of early transcription. [44, 45, 108] By itself, *E1* weakly binds origin sequences, but this binding is facilitated by complex formation with *E2 proteins*. [97, 143] *E2binding sites* are located adjacent to *E1 recognition sequences*, and *E2* acts to load *E1* onto the origin. Once bound, *E1 proteins* form hexamers that have a high affinity for *DNA*, as with other helicases, the *viral DNA* passes through the center of the *E1 hexameric ring*. These *E1 complexes* efficiently unwind *supercoiled DNA* with the help of chaperone proteins. *E1 proteins* also bind *DNA polymerase α* and help to

recruit cellular replication complexes to the viral origin of replication. [102] Chromatin-remodeling complexes may also be recruited by *E1* so as to modulate nucleosome positioning and allow for efficient procession of the replication fork. [144] the crystal structure of the *DNA binding domain* of *E1* has identified an extended loop and α-helix that are important for recognizing *DNA*. [139] One intriguing interaction is that of *E1 with cyclins A* and *E* and this may act to regulate *E1activity*, four cyclin kinase phosphorylation sites are present in *E1 proteins*, and mutation of these sites drastically reduces the replication *activity of E1*. [98] It is still not clear how *E1 activity* is regulated in differentiating cells, but studies with *HPV31* have shown that expression of *E1 transcripts shifts* from the early to late promoters, resulting in increased *E1 expression*. A shift in the absolute levels of *E1* or the ratios of (*E1 to E2)* is thought to result in high-level replication upon differentiation. The *E2 protein* is required for both the replication of *viral DNA* and transcriptional regulation. [90] *E2 proteins* are approximately *(50) kDa* in size and function as dimmers. The *C- terminus encodes a DNA binding domain* that has been crystallized and shown to consist of a dimeric β-barrel structure that *binds DNA at N terminus* contains a transactivation domain, while the *C terminus interacts with E1* the *N terminus* has also been crystallized and consists of a glutamine-rich α-helix packed against a β-sheet framework *E2 dimmers* bind to consensus palindromic sequences called *(E2B)*. [90] There are (4) (*E2B)* present in the (*URR)*, and three of these flank the (*E1) recognition sequences* in the origin of replication.[63] On infection, early-gene transcription is activated primarily by cellular transcription factors binding sequences in the *URR*.[139] At low concentrations, *E2* further activates early-gene expression, while at high concentrations it represses by interfering with the binding of transcription factors such as (*TFIID)* and *(Sp1*) to their recognition sequences, which overlap the (*E2B)*. This regulation of viral expression contributes to copy number control in

undifferentiated cells. On differentiation, there is a switch to the late promoter which is not repressed by E2, resulting in increased (E1 and E2) expression leading to *viral DNA amplification*.[83] (E2) may also form a complex with *C/EBP transcription factors*, which regulate many promoters of genes involved in differentiation Aside from its role in the regulation of transcription over expression of the *(E2) protein* can induce apoptosis by a *P53-independent* mechanism also over expression of *E2 in HeLa cells* was demonstrated to cause accumulation of *p53* but not transcription of downstream genes like *Bax*. *(E6)* overexpression in this system was unable to block the resultant apoptosis. furthermore, in *HeLa cells* introduction of heterologous expression vectors for *E2* results in a suppression of transcription of the endogenous *(E6 and E7*) genes, leading to senescence this indicates that the continued expression of *(E6 and E7)* is required to maintain the transformed phenotype of cervical cancer cells. [154]

2.16.4 E4 protein:

E4 - protein is a cytoplasmic protein disturbing the structural framework of keratin. It is sometimes detected in the cell nucleus, [124, and 126) and it influences the formation of the *HPV-1* triggered nodules, its role in the regulation of *cell cycle* may also be possible as a result of protein *E4 - activity*, the thickening of the spinous and horned layer of the epidermis and the koilocytosis of the epidermis occur. [126] *E4 protein* is expressed at increased levels in cells supporting the viral genome amplification. Its presence in lesions implies its role in the staging of the disease. This study authenticates previously made suggestions for the importance of the *E4 biomarker* in the diagnosis and disease- staging, and broadens the *E4* approach to include the confirmation of *HPV causality.* [126] *E4 proteins* are visible during the advanced stages of the infection approximately at the time of genome amplification initiation. *E4- proteins* of *HPVs* have an identifiable modular framework even

though they exhibit diversity at the primary amino acid sequence level and can be accrue to high levels in the upper epithelial layers where virus particles accumulate. Any analyses of *E4 - function* must take this into consideration. What is more, it has been stated that *E4's potential* to disrupt the cellular keratin network and the accumulation of cornified envelope may expedite the release of the virus and/or its transmission. [128]

2.16.5 E5 protein:

E5 protein is involved in the transformation and participates in *viral DNA replication.* [123, 124] This protein also allows for the infected cell to avoid being recognized by the immune system.

2.16.6 E6 and E7 proteins:

(*E6 and E7) proteins* play a central role in *HPV* dependent malignant transformation. They cause the impairment of the control of *cell cycle regulation* and cell maturation. [124, 125, 129] *E6* connects to the *p53protein*, leading to its proteolytic degradation. [125, 126] Previously, however*E6* connects to the *(AP)* which acts as ubiquitin ligase, and the combination of *(E6+E6+AP)* with the degradation area of *p53* causes *p53 proteolysis* and the elimination of all known functions of p53, this may result in an uncontrolled replication of *HPV 16 and/or 18 infected cells*, *E7 protein* binds and inactivates the (*Rb*) protein, leading to its degradation, which results in the cell's loss of control over the *cell cycle.* [130, 131] (*E6 and E7) onco-proteins* may undergo phosphorylation and to various degrees bind to the target proteins; (*E6 and E7)* proteins' expression is controlled by *E2 protein*, a host cellular protein *YY1* and pro-inflammatory cytokines. [125]

2.16.7 Function of the E6 Onco proteins:

The *E6 proteins* of both high- and low-risk types are approximately (150) amino acids in size and contain (2) zinc binding domains with the motif Cyst-X-X-cyst the *high-risk E6 proteins* are distributed to both the nucleus and the cytoplasm and have been reported to bind to over (12) different proteins. [162] Expression of high-risk *E6* alone leads to the transformation of *NIH 3T3* cells as well as the immortalization of human mammary epithelial cells. [79, 96] In contrast, efficient immortalization of human keratinocytes requires the expression of both (*E6and E7).* [58] Many of the first insights into the action of *E6* have come by studying its interactions with *p53. P53* is a well-characterized tumor suppressor that regulates the expression of proteins involved in *cell cycle control*, including the cyclin kinase inhibitor, *p21.* [84] On exposure to *DNA damage*, *p53* becomes activated and induces high-level expression of *p21*, resulting in *cell cycle arrest* as well as apoptosis. [84] One way in which infected cells act to prevent the spread of viruses to neighboring cells is through the activation of apoptotic pathways. Many viruses have evolved mechanisms to block the induction of apoptosis, but this can, in some cases, allow for the development of malignancies. To overcome the pro-apoptotic activities of *p53* and allow for cell cycle progression, *E6* binds to *p53* in a ternary complex with an ubiquitin ligase called *E6 AP* formation of this complex results in the ubiquitination of *p53* and subsequent degradation by the *(26S) proteasome* leading to a reduction in the half-life of *p53* from several hours to less than (20) min in keratinocytes.[64, 67]

2.16.8 L1 and L2 proteins:

L1 and L2 proteins are capsid proteins of the virus where L1 is the *major protein* and *L2* is the *minor capsid protein.* [133, 135]

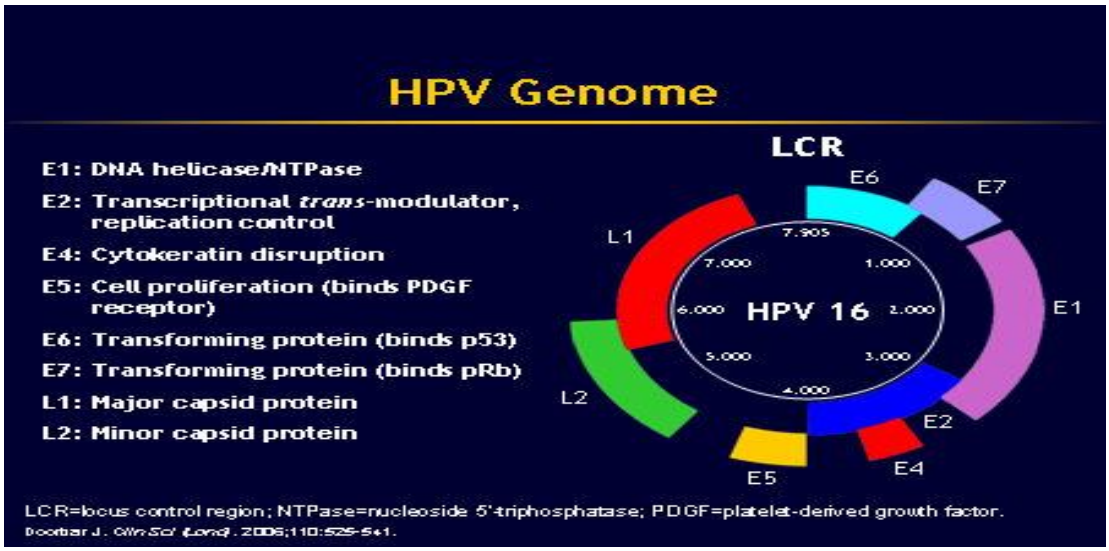

Figure (8.2): HPV genome (the image originated from Pubmed websites)

2.17 Pathogenesis of *HPVs:*

HPVs are the etiological agents of breast, cervical and other ano-genital malignancies. Over (100) different types of *HPVs* have been identified to date and all target epithelial tissues for infection. (1/3rd) of *HPV types* specifically infect the genital tract, and a subset of these is the causative agents of *BC* and ano-genital cancers. [70] Other *HPV types* that infect the genital tract induce benign hyper-proliferative lesions or genital warts The productive *life cycle* of *HPVs* is linked to epithelial differentiation *(HPVs)* are thought to infect cells in the basal layer of stratified epithelia and establish their genomes as multicopy nuclear episomes in these cells, *viral DNA* is replicated along with cellular chromosomes following cell division, and one of the daughter cells migrates away from the basal layer and undergoes differentiation. In highly differentiated supra-basal cells vegetative viral replication and late-gene expression are activated resulting in the generation of progeny virions.[70] Since virion production is restricted to differentiated cells,

infected basal cells can persist for up to several decades or until the immune system clears the infection. The *(E6 and E7) genes* encode viral onco-proteins that target *Rb and p53*, respectively during the viral life cycle these proteins facilitate stable maintenance of episomes and stimulate differentiated cells to reenter the *S phase*. The *E1 and E2 proteins* act as origin recognition factors as well as regulators of early viral transcription. The functions of the *E5 and E1E4proteins* are still largely unknown, but these proteins have been implicated in modulating late viral functions. The *(L1 and L2) proteins* form icosahedral capsids for progeny virion generation. The characterization of the cellular targets of these viral proteins and the mechanisms regulating the differentiation-dependent *viral life cycle* remain active areas for the study of these important human pathogens. [71, 72]

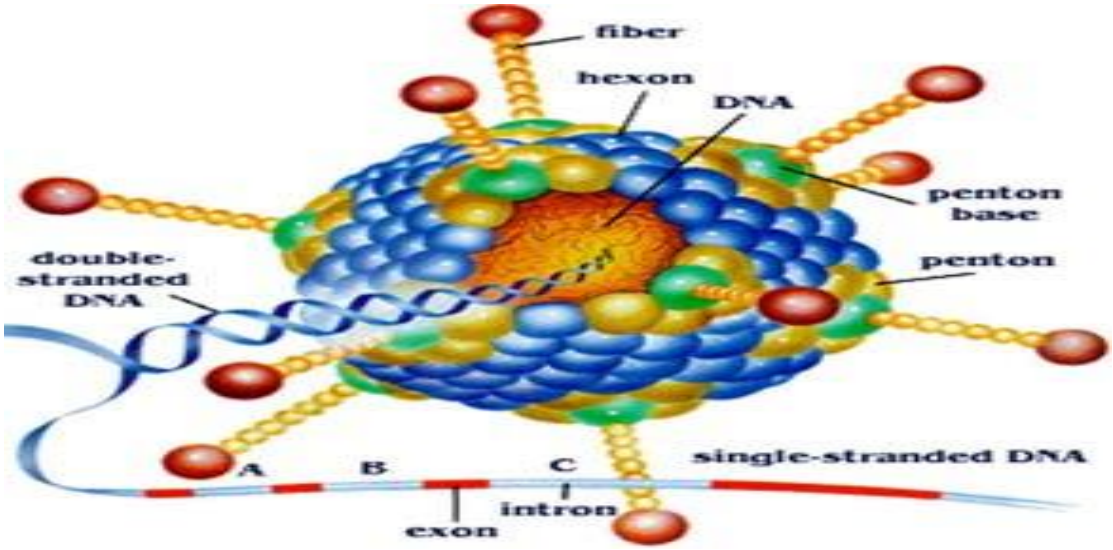

Figure (9.2): Condition that allow recognition and regression of *HPV* infected cells by effective immune cells (image originated form essential virology textbook)

2.18 Breast Cancer Pathogenesis (Carcinogenesis):

BC pathogenesis is a multi-step processes which arise as transformation of normal cells via the steps of hyperplasia, premalignant change, in situ carcinoma and invasive carcinoma. The mechanism of breast carcinogenesis interrelated by three factors: genetic mutations, hormonal stimulation of the mammary tissue and exogenous carcinogen exposure Therefore, breast carcinomas can be divided into sporadic cases, probably related to hormonal exposure, and hereditary cases, associated with germ line mutations. [112]

The multi-step progression mechanism of breast cancer at molecular level is genetic alterations, occurring in each step which gives the cell new properties for tumor progression. These genetic alterations range from small point mutations, via chromosomal deletions, translocations and amplifications to large-scale changes as whole chromosome losses or duplications. The early step is mutational activation of oncogenes coupled within activation of tumor suppressor genes. Subsequently, more independent mutations occurred in genes involved in regulation of mitosis and *DNA repair* mechanisms that lead to genomic instability. Aberrations that activate or inactivate various other genes can result of further progression to a malignant phenotype, during progression to a fully malignant, metastasizing phenotype, additional mutations are required. Finally, in a single cancer cell a few hundred human genes have an altered expression and it is difficult to determine which mutations start early. [112,113]

The most common mutated Genes involved in breast carcinogenesis are *BRCA1 and BRCA2* tumor suppressor genes. *BRCA1 gene*, located on *chromosome17q12-21*, is involved in many transcriptional processes and apoptosis. The encoded protein combines with other tumor suppressors, *DNA damage sensor*s, and signal transducers to form a large multi-subunit protein complex, known as the *BRCA1-associated genome* surveillance complex. Germline mutations in *BRCA1* are

scattered throughout the gene. It consists of insertions, deletions, frame-shifts, base substitutions and inferred regulatory mutations. In sporadic *BC* the gene is rarely mutated, but functionally inactivated by hyper-methylation of the promoter region. The *BRCA1* breast carcinomas are commonly poorly differentiated and have *P53 mutations*. The *BRCA2 gene* is located on *chromosome 3q12-13*, the gene codes for proteins involved in *DNA repair*, *cell cycle control* and transcription. Germline mutations can occur throughout the gene. The majority of mutations are frame-shifts, but there are a number of missense mutations. In sporadic breast cancer, mutational inactivation of *BRCA2* is rare and inactivation requires both gene copies to be mutated or totally lost. [114]

The *P53* on *chromosome (17p13.1)* is one of the most frequently mutated genes in sporadic human cancers. It has critical roles in *cell cycle control*, *DNA replication*, *DNA repair*, and apoptosis. Most mutations are point mutations leading to proteins defective for sequence-specific *DNA binding and activation* of *P53-responsive genes*. In sporadic breast carcinomas, the occurrence of *P53 mutations* is a late event. Rarely, a *P53 mutation* is associated with hereditary *BC (CHEK2),* is located on *chromosome 22q12.1*. The protein product of this gene is activated in response to *DNA damage* and plays an important role in transducing the *DNA damage signal* to downstream repair proteins. Moreover it activates *BRCA1 and P53* by phosphorylation. A particular germ line mutation occur *(CHEK2)*. In sporadic breast cancer mutations are rare but there is loss of protein expression by unknown mechanism. The *(PTEN)* which located on *chromosome 10q23* is a tumor suppressor gene act through regulating the *cell cycle*. Germ line mutations play a role in *BC*. [115]The *CDH1 gene (on 16q22.1)* encodes for the adhesion molecule *E-Cadherin* Patients with germ line *CDH1 mutations*, mainly splice-site and Messene mutations carry an increased risk of lobular breast carcinoma. *(STK11/LKB1)* is a tumor-suppressor gene important for mediation of apoptosis

and *cell cycle regulation*. Germline mutations in this gene associated with increased risk of BC. [114] *ATM gene* is encoded for a *PI3K-related protein kinase*. It has a central role in the repair of *DNA double-strand breaks ATM mutation* heterozygotes have a (2-fold) higher BC risk. This risk is elevated (5-fold) in women under the age of. [115] Hormonal exposure affect the cycles of proliferation that place cells at risk for *DNA damage*. Once premalignant or malignant cells are present, hormones can stimulate their growth, as well as the growth of normal epithelial and stromal cells that may aid the tumor development. Estrogen may also play a more direct role in carcinogenesis. Metabolites of estrogen can cause mutations or generate *DNA-damaging free radicals* in cell. The variants of genes involved in estrogen synthesis and metabolism could increase the risk of *BC*. [114,115]

2.19 Breast Cancer Invasion and Metastasis:

Breast carcinomas can infiltrate locally or metastasize to more distant sites via lymphatics and the blood stream. The mechanism of tumor Invasion occur in four steps; loosening of cell-cell adhesion, degradation of *(ECM)*, attachment to modified *ECM* components and migration of tumor cells.

2.19.1 First step:

Is losing of tumor cells adhesion by inactivation of *E-cadherin molecules* which its normal function keeps the cells together, the mutational inactivation of *E-cadherin genes* occurred by activation of *β-cateningenes* or, by inappropriate expression of the snail and twist transcription factors, which suppress the *E-cadherin expression*? [116]

2.19.2 Second step:

Is degradation of *(ECM)*, (basement membrane) by proteolytic enzymes of tumor cells or induced stromal cells. Multiple different families of proteases, such as (*MMPs)*, Cathepsin D and urokinase plasminogen activator, have been involved in tumor cell invasion, Moreover, cleavage products of proteoglycans and collagen have chemotactic, angiogenic and growth promoting effects. [117]

2.19.3 Third step:

Is modification of extracellular matrix to promote invasion and metastasis cleavage of basement membrane proteins collagen type (4) and laminin by *MMP-2* generates new sites that bind to receptors on tumor cells and stimulate migration. [118]

2.19.4 Finally:

Is propelling of tumor cells through degraded tumor basement membranes and zones of matrix proteolysis, migration is multi-step process that involves many receptors and signaling proteins such as *(AMF)*. Vascularization (angiogenesis) of tumors is essential in migration and invasion mechanism, it is controlled by balance between angiogenic and anti-angiogenic factors. [119]

2.20 Epidemiology of Breast Cancer:

Overall, BC accounts for more than (550,000) cases annually worldwide. [10] There are over (650,000) patients are diagnosed with *BC* and some (350,000) die from this disease worldwide every year.[11] The highest incidences of *BC* in the world are found in South Asia, and parts of central and southern Europe.[8] It represents a considerable burden worldwide, being the ($1/5^{th}$) most common cancer in 2008.[12] The *BCs* are the ($1/5^{th}$) most common cancer worldwide with more than (600,000) cases diagnosed each year. [10] Females are affected significantly more than Males with a ratio ranging from (10:2). The incidence rate in females exceeds

(20/100,000)in regions of France, Hong Kong and Indian subcontinent central and Eastern Europe, Spain, Italy, Brazil and among African Americans in the Unites States. *BC* is the (8^{th}) most common cause of cancer death worldwide. Its incidence varies widely among different regions In north America *BC* account for (3 to 4%) of all cancer diagnoses, the incidence in African American female during the years 1987 to 1991 was (24 /100,000) approximately (50%) higher than in white American female. [11] The mortality associated with BC in African Americans is also higher than in whites. [11, 12] Breast cancer is the second Nemours cancer in the world. [121] after the lung cancer, affecting one in eight women during their lifetime, but it is the leading cancer among women worldwide. [121] although various clinico-epidemiology genetic and epigenetic factors including mutations in breast cancer susceptibility genes *BRCA1* and *BRCA2*. [121,122] Sex-steroid hormones and lifestyle factors have been strongly implicated in the development of *BC*, these recognized risk factors may be absent in (50%–80%) of patients diagnosed with breast cancer. [123] which have created a heightened interest in identifying new risk factors that contribute to the pathogenesis of *BC* [123] BC is the most common cancer among females in Sudan. According to the (SCR) *BC* ranked first among women as it represented (26%) of all newly diagnosed female cancers in the year 2007. [125]*(HR-HPVs)* are carcinogenic viruses which are primarily associated with cervical cancer but are also linked with other ano-genital cancers and cancers of other organ sites such as oral cavity esophagus nasopharyngeal and laryngeal carcinoma and possibly in retinoblastoma.[126,127] In Sudan the rate of HPV genotype detection among cervical cancer samples was (95.5%) . The most common *HPV genotype* detected by both methods was HPV-16 (63.4%), followed by *HPV-18* (11.1%), HPV-*45* (4.5%), and *HPV-33* (3.3%).[121] The *HR-HPV onco-proteins(E6 and E7)* which have been found to interact and inactivate the two principal host cell tumor suppressor proteins *p53 and Rb* respectively are also

shown to immortalize human mammary epithelial cells in-vitro.[125,126] Several other viruses have also been implicated in the etiology of human BC.[125, 126] but these are not confirmed by other authors.[127, 128] Reports on the distribution of *HPV infection* in *BC* are not only limited but also highly controversial. Several studies did not find any (*HPV) infection* in BC. [132] A moderate frequency of (20-48) %*HPV infection* was reported by many authors. [135,144]Whereas a very high frequency of *HPV infection* ranging from (60% to 85%) occurrence of *HPV* in *BC* has been found. [145,148] Most interesting is recent demonstration of *high risk HPV18* in the *BC* cell lines by in-situ hybridization and observation of *HPV-specific koilocytes* in *BC* cells which reiterates the oncogenic role of *HPV* in BC.[149] A recent systemic review analyzed (29) primary studies including (2211) samples. It revealed that *HPV prevalence* in patients with *BC* was (23%) (95% CI, 21.2% 24.8%) According to this review, the prevalence of *HPV* ranged from 13.4% (95% CI, 10.2%–16%) in Europe to 42.9% (95% CI, 36.4%–49.4%) in North America and Australia. The prevalence of *HPV* in controls was (12.9%). Combinations of (9) case-control studies showed that *BC* was associated with *HPV* (odds ratio, 5.9; 95% CI, 3.26–10.67) considering the controversial reports on the etiology of *HPV* in breast carcinomas. [41]

2.21 Breast Cancer Current Statistics:

BC continues to be one of the most common cancers and a major cause of death among women worldwide. [44] According to the current statistics of the centers for disease control and prevention, *BC* is the most common cancer in women in the *USA* (excluding skin cancer) accounting for (32%) of all female cancers. [45] *BC* is responsible for (18%) of cancer deaths in women and is second cancer after lung cancer. The *(NCI)* estimates that about (1 in 8) women in the United States (approximately 13.3%) will develop *BC* during her life time. The *(ACS)* estimates

about (200 to 300) new invasive cases of *BC* will be diagnosed among women in the USA. [46]

2.22 Risk Factors in Pathogenesis of BC:

Several risk factors for BC have been well documented however, for the majority of women presenting with *BC* it are not possible to identify specific risk factors.[14, 15] A familial history of BC increases the risk by a factor of (2 or 3) the risk increases to (9-fold) for first-degree relatives of premenopausal women with bilateral *BC*. Up to (5-fold) increases in risk have been found for women with multiple first-degree relatives with *BC,* moreover there are rare familial syndromes such as Li-Fraumeni, in which there is an association with *BC* at a young age. Some mutations, particularly in (*BRCA1, BRCA2)* and (*P53)* result in a very high risk for *BC* women with *BRCA1* mutation are estimated to have lifetime risks about (80%) of developing *BC* and characterized by elevated cancer risk at younger ages.

Also the risk factors for *BC* may be divided into preventable and non-preventable *BC* like other forms of cancer can result from multiple environmental and hereditary risk factors. The term "environmental" means any risk factor that is not genetically inherited. For breast cancer, the list of environmental risk factors includes the individual person's development, exposure to microbes, "medical interventions dietary exposures to nutrients, energy and toxicants, ionizing radiation, and chemicals from industrial and agricultural processes and from consumer products reproductive choices, energy balance, adult weight gain, body fatness, voluntary and involuntary physical activity, medical care, exposure to tobacco smoke and alcohol, and occupational exposures, including shift work" as well as "metabolic and physiologic processes that modify the body's internal environment.[68] Some of these environmental factors are part of the physical environment, while others (such as diet and number of pregnancies) are primarily

part of the social, cultural, or economic environment.[69] Although many epidemiological risk factors have been identified, the cause of any individual *BC* was most often unknowable. Epidemiological research informs the patterns of breast cancer incidence across certain populations, but not in a given individual. Approximately (5%) of new *BC* is attributable to hereditary syndromes, and well-established risk factors accounts for approximately (30%) of cases. [70]

2.22.1 Sex:

In developed countries, about (99%) of *BC* cases are diagnosed in women. [71] The rate of breast cancer in men appears to be rising somewhat.[72] Men diagnosed with breast cancer tend to be older than women with breast cancer.[73] They are more likely to be diagnosed with hormone-receptor positive tumors, with about six out of seven cases being estrogen-receptor positive.[74] The overall prognosis is worse for men than for women.

2.22.2 Genetic risk factors:

About (5% to 10%) of *BCs* cases are thought to be hereditary meaning that they result directly from gene defects (called *mutations*) however *HPV* play an important role in gene mutation. [73]

BRCA1 and *BRCA2*:

The most common cause of hereditary breast cancer is an inherited mutation in the *BRCA1* and*BRCA2 genes*. In normal cells these genes help prevent cancer by making proteins that keep the cells from growing abnormally. [74] Inherited mutated copy of either gene from a parent, increase risk of developing BC during your lifetime. Although in some families with *BRCA1 mutations* the lifetime risk of breast cancer is as high as (80%), on average this risk seems to be in the range of (55% to 65%). For *BRCA2 mutations* the risk is lower, around (45%). BC linked to these mutations occurs more often in younger women and more often affect both

breasts than cancers not linked to these mutations. [74, 75] Furthermore, *BRCA2* in (5%) of the breast cancer cases there is a strong inherited familial risk two autosomal dominant genes, *BRCA1 and BRCA2* account for most of the cases of familial breast cancer. Women who carry a harmful *BRCA mutation* have a (60% to 80%) risk of developing breast cancer in their lifetimes. [74] Other associated malignancies include ovarian cancer and pancreatic cancer. If a mother or a sister was diagnosed *BC*, the risk of a hereditary *BRCA1 or BRCA2 gene mutation* is about (2-fold) higher than those women without a familial history. [75]

2.22.3 Changes in other genes:

Other gene mutations can also lead to inherited *BC*. These gene mutations are much rarer and often do not increase the risk of *BC* as much as the *BRCA genes*. They are not frequent causes of inherited BC. [75, 76]

- *ATM*: The *ATM* gene normally helps repair *damaged DNA*. Inheriting (2) abnormal copies of this gene cause the disease ataxia-telangiectasia. Inheriting (1) mutated copy of this gene has been linked to a high rate of *BC* in some families.
- *TP53*: The *TP53* gene gives instructions for making a protein called *p53* that helps stop the growth of abnormal cells. Inherited mutations of this gene cause *Li-Fraumeni syndrome.*

2.22.4 Family history of BC:

BC risk is higher among women whose close blood relatives have this disease. Having one first-degree relative (mother, sister, or daughter) with *BC* approximately doubles a woman's risk. Having (2) first-degree relatives increases her risk about (3-fold). The exact risk is not known, but women with a family history of *BC* in a father or brother also have an increased risk of *BC*

Altogether, less than (15%) of women with *BC* have a family member with this disease.

2.22.5 Other genes:

Hereditary *non-BRCA1 and non-BRCA2* breast tumors (and even some sporadic carcinomas) are believed to result from the expression of weakly penetrant but highly prevalent mutations in various genes. For instance, polymorphism has been identified in genes associated to the metabolism of estrogens and/or carcinogens *(CYP1A1 , CYP1B1 , CYP17 , CYP19 , COMT , NAT2 , GSTM1, GSTP1,GST)* to estrogen, androgen and vitamin D action *(ESR1, AR, VDR),* to co-activation of gene transcription *(AIB1) to DNA damage* response pathways *(CHEK2 , HRAS1, XRCC1, XRCC3 , XRCC5*). Sequence variants of these genes that are relatively common in the population may be associated with a small to moderate increased relative risk for *BC*. [76]

2.22.6 Alcohol:

The use of alcohol is clearly linked to an increased risk of developing *BC* associated with (*HPV)* because drinking alcohol interferes with the cell mediated immunity. The risk increases with the amount of alcohol consumed. Compared with non-drinkers, women who consume 1 alcoholic drink a day have a very small increase in risk. Those who have (2 to 5) drinks daily have about (1½) time the risk of women who don't drink alcohol. Excessive alcohol consumption is also known to increase the risk of developing several other types of cancer. [74, 75] There is sufficient scientific evidence to classify alcoholic beverages as a group (1) carcinogen that causes *BC* in women.[77] Group (1) carcinogens are the substances with the clearest scientific evidence that they cause cancer, such as smoking tobacco. The more alcohol a woman drinks, the more likely she is to get breast cancer.[78] The relationship is linear and dose-dependent. Even low levels of

alcohol consumption carry some risk. [79] A study of more than one million middle-aged British women concluded that each daily alcoholic beverage increases the incidence of *BC* by (11 cases per 1000) women.[80] This means that among a group of (1000) women who drink one alcoholic beverage per day, they will have (11) extra cases of *BC* when compared to a group of women who drink less than one alcoholic beverage per week a group of (1000) women who have four drinks per day will have an extra (44) cases of *BC* compared to non-drinkers. One or two drinks each day increases the relative risk to (150)% of normal, and (6) drinks per day increases the risk to (330%) of normal.

2.22.7 Phytoestrogens:

Some study supports the following conclusions:

- Plant estrogen intake in early adolescence may protect against breast cancer later in life.
- The potential risks of flavones on breast tissue in women at high risk for breast cancer are still unclear.

2.22.8 Vitamin D deficiency:

Vitamin D is related to reduce risk of breast cancer and disease prognosis Low vitamin D levels among women with *BC* correlate with more aggressive tumors and poorer prognosis and sub-optimal vitamin D levels with poor scores on every major biological marker that helps to predict a patient's *BC* outcome. [82]

2.22.9 Obesity and lack of Exercise:-

Gaining weight after menopause can increase a woman's risk factor. [83] Putting on (9.9) kg after menopause increased the risk of developing breast cancer by (18%). Lack of exercise has been linked to *BC*. [84] Physical activity after *BC* diagnosis has shown some associations with reducing breast cancer recurrence and mortality independent of weight loss. [85]

2.22.10 Hormones:

Persistently increased blood levels of estrogen are associated with an increased risk of breast cancer, as are increased levels of the androgens and rostenedione and testosterone (which can be directly converted by aromatase to the estrogens estrone and estradiol, respectively). Increased blood levels of progesterone are associated with a decreased risk of *BC* in premenopausal women.[87] A number of circumstances which increase exposure to endogenous estrogens including not having children, delaying first childbirth, not breastfeeding, early menarche (the first menstrual period) and late menopause are suspected of increasing lifetime risk for developing *BC*. [88] However, not only sex hormones, but also insulin levels are positively associated with the risk of *BC*. [89]

2.22.11 Contraception Pills Abuse:

A recent study find that patients with *HPV infections* who had used oral contraceptive pills for more than (5) years had a (4-fold) higher risk of developing BC. [112,113] However, most of the patients in the study were from developing countries where *BC*s screening (breast tests) is not performed regularly. Also, not everyone who is positive for *HPV infection* will develop *BC* this study find that about (20) million people in the *USA* are infected with the *HPV,* only about (12,000) women a year are diagnosed with *BC*. Additionally, about (60) % of patients with findings of *HPV* on their breast tests will clear the infection without additional treatment. Also remember that while a "4-fold higher risk" sounds like a lot, if the baseline risk is less than (1 in 100) patients, a 4-times higher risk is still less than (4 in 100). While this study shows that there may be some relationship between hormone use and promotion of *BC*, it cannot be interpreted to mean that all patients with *(HPV)* should discontinue their oral contraceptive pills. [112,113] Hormonal contraceptives may produce a slight increase in the risk of breast cancer diagnosis among current and recent users, but this appears to be a short-term effect.[114]

2.22.12 Obesity:

Being overweight or obese after menopause increases breast cancer caused by (*HPV)* as one of the risk factor in pathogenesis of breast cancer before menopause your ovaries produce most of your estrogen, and fat tissue produces a small amount of estrogen. After menopause (when the ovaries stop making estrogen) most of a woman's estrogen comes from fat tissue Having more fat tissue after menopause can increase your chance of getting breast cancer by raising estrogen levels. Also, women who are overweight tend to have higher blood insulin levels. [116]

2.22.13 Physical activity:

Evidence is growing that physical activity in the form of exercise reduces *BC*.

2.22.14 Endocrine disruptors:

Many xeno-estrogens (synthetic estrogenic compounds) and other endocrine disruptors are potential risk factors of *BC*. *DES* is a synthetic form of estrogen is also risk factor. [91] Pregnant women take *DES* to prevent certain pregnancy complications. However, it also increased their risk of breast cancer. It also increased the risk of *BC* in the prenatally exposed daughters after they have reached an age (40) years. [92]

2.22.15 Passive smoking:

Passive smoking increases breast cancer risk by (70%) in younger primarily pre-menopausal women. [93]

2.22.16 Age:

The risk of developing BC increased in elder patient by (1 out of 8) invasive breast cancers are found in women younger than (45) while about (2 of 3) invasive *BC* are found in women age (55) or older some study found there is relation between *HPV* and age of patient. [96, 97]

2.22.17 Personal History of Breast Cancer:

A woman with cancer in one breast has an increased risk of developing a new cancer in the other breast or in another part of the same breast. (This is different from a recurrence (return) of the first cancer.) This risk is even higher if *BC* was diagnosed at a younger age. [97]

2.22.18 Race and Ethnicity:

Overall, white women are slightly more likely to develop breast cancer than are African-American Women, but African-American women are more likely to die of this cancer. However, in women under (45) years of age, *BC* is more common in African- American women. Asian, Hispanic, and Native-American Women have a lower risk of developing and dying from *BC*.[98]

2.22.19 Dense Breast Tissue:

Some studies have found that the human papilloma virus found in women who have many fat in their body.[98, 99] Breasts are made up of fatty tissue, fibrous tissue and glandular tissue someone is said to have dense breast tissue (as seen on a mammogram) when they have more glandular and fibrous tissue and less fatty tissue. Women with dense breasts on mammogram have a risk of *BC* that is (1.2 to 2) times that of women with average breast density. Dense breast tissue can also make mammograms less accurate. A number of factors can affect breast density such as age, menopausal status, certain medications (including menopausal hormone therapy), pregnancy, and genetics.

2.22.20 Menstrual periods:

Women who have had more menstrual cycles because they started menstruating early (before age 12) and/or went through menopause later (after age 55) have a slightly higher risk of breast cancer. The increase in risk may be due to a longer lifetime exposure to the hormones estrogen and progesterone and this play an important role in activation of *HPV*. (100, 101)

2.22.21 Occupational exposure:

Many occupations or occupational hazards have been linked to *BC*. These include the dry cleaning agent perchloroethylene.[104] Asbestos pesticides, man-made mineral vitreous fibers, polycyclic aromatic hydrocarbons [105] Textile workers, wood workers manufacturers of mustard gas, plastic and rubber products, naphthalene refiners, ethanol, sulfuric acid mist, leather and paint workers, automobile mechanics, construction workers (cement) farmers and metal workers.[106]

2.22.22 Radiation:

Women who have received high-dose ionizing radiation to the chest for treatments for other cancers have a relative risk of *BC* between (2.1% to 4.0%) and the risk increases according to the amount of the dose. In addition, the risk is higher in women irradiated before age (30), when there is still breast development.[104] Some studies have found that exposure to radiotherapy or other radiation will help in the spread of (*HPV)* to others suffering from breast and other tissue, because (*X)*is working to laceration tissue, which helps the virus to spread [32]. Women who, as children or young adults, had radiation therapy to the chest area as treatment for another cancer (such as lymphoma) have a significantly increased risk for *BC*. This varies with the patient's age when they had radiation. If chemotherapy was also given, it may have stopped ovarian hormone production for some time, lowering the risk. The risk of developing *BC* from chest radiation is highest if the radiation was given during adolescence, when the breasts were still developing. Radiation treatment after age (40) does not seem to increase *BC* risk. [102, 103]

2.22.23 Other Viruses Cause Breast Cancer:

Chronic viral infection may be associated with *BC*, possibly by interfering with tumor suppressor gene function. Viruses may also participate as cofactors by enhancing activation, amplification, and overexpression of preexisting oncogenes within neoplastic tissues. [125]

2.22.23.1 Epstein-Barr Virus:

The strongest association between a virus and *BC* is that of *(EBV)* and nasopharyngeal carcinoma. A large body of evidence supports the role of *EBV* as the primary etiologic agent in the pathogenesis of nasopharyngeal carcinoma. *EBV* is the primary etiologic agent for oral hairy leukoplakia. While several investigators report finding (*EBV) in BC* others refute such a relationship. [109]

2.22.23.2 Herpes simplex virus:

(HSV) is less strongly correlated with the development of *BC* than (*EBV)* or (*HPV)*. [129.130] *HSV* can transform cells in vitro to a malignant phenotype. This may be due to an *HSV-encoded peptide* that increases mutagenicity of infected cells. [129.130]

2.22.23.3 Human immunodeficiency virus (*HIV*):

Patients infected with the *HIV* present with a variety of malignancies such as *BCs* in selected patients. [109.110]

2.23 Biomarkers of Breast Cancer:

2.23.1 Estrogen receptor:

*ER (*a) expression is undoubtedly the most important biomarker in breast cancer the Oxford overview confirms that patients with *ER-negative* disease have no benefit from (5) -year adjuvant treatment with tamoxifen, but some benefit may be derived in the uncommon group of *ER negative* and progesterone receptor *(PgR)-expressing* breast tumors (2005). In contrast, such treatment reduces the annual *BC*

death rate by (31%) in *ER-positive* disease. While the absence or presence of the ER is used to obtain treatment decisions, the Early *B C* Trialists' Collaborative group reported that higher levels of *ER* were associated with a lower risk of recurrence when receiving adjuvant tamoxifen. (1998) similar results were obtained in the *NSABP*-14 trial using the ligand-binding assay and *mRNA* expression of *ER*. [135] More recent analyses from the large prospective adjuvant trials anastrozole, tamoxifen, alone or in combination *(ATAC*) and *BIG 1-98* (letrozole versus tamoxifen) comparing aromatase inhibitors *(A*Is) with tamoxifen did not find a subgroup of *ER-positive* patients with different *ER expression* levels, which derives a greater benefit from AIs versus tamoxifen. [136] the trials revealed, however, that higher *ER levels* were related to improved outcome of both the endocrine treatments, it has been reported that *ER status* predicts for response to chemotherapy in the neoadjuvant setting. Multiple clinical studies have demonstrated that the *ER-negative BC* patients are more likely to achieve a pathological complete response *(pCR*) with neo adjuvant chemotherapy than the *ER-positive* patients, with *pCR rates* of 7–8 vs. (21%–33% respectively being reported. [136] this may be partly explicable by the *ER-negative* breast tumors tending to have higher proliferation rates, but this does not appear to provide a full explanation. [137] there have also been investigations concerning the amplification of the *ER gene (ESR1)*. An initial report indicated that *ESR1* gene amplification in *BC* could be detected inw20% of all invasive tumors, and that there was a correlation between the gene amplification and *ER expression levels*. [138] However, (3%) of invasive *BC* cases were reported as *(ESR1*) amplified by other independent groups. [139] Extensive research has been undertaken in trying to discover the function and relevance of different splice variants and point mutations of the *ER*. One *ER mutation* (*K303R)* which leads to a receptor that is able to induce proliferation even in conditions of low hormone levels, has been reported as being associated with

benign breast hyperplasia and breast cancer by one group. [140] But not confirmed by others. Despite much investigation of *(ESR1*) mutations and splice variants, their clinical role appears to be small. The recently released guideline of the *(ASCO)* and the *(CAP*) has the aim to improve hormone receptor testing for patients with *BC* and recommends *ER and* PgR testing in all newly diagnosed cases. [141]

2.23.2 Progesterone receptor:

The expression of the *PgR* is strongly dependent on the presence of *ER*. Tumors expressing *PgR* but not the *ER* are uncommon and represent (1%) of all breast cancer cases in some large series. [142] for this reason, tumors with *PgR expression* lacking ER expression should undergo a retesting of their *ER st*atus to eliminate false ER negativity. In the rare cases of solely *PgR-expressing* patients, some limited benefit from tamoxifen is described, but endocrine therapy is still widely recommended. [143] There is evidence that in metastatic *BC* the response to anti-estrogen treatment is better among patients with tumors expressing both *ER* and PgR versus those who only show *ER positivity* but lack the *PgR expression*. [144] Data from adjuvant trials comparing tamoxifen treatment with controls indicate a strong prognostic value for *PgR expression*, but indicate a little predictive significance. [145] Patients with high levels of *PgR* within their breast tumors have a better outcome than low expressers with tamoxifen, but the relative benefit from tamoxifen remains similar. [146] the impact of *PgR expression* on response to and outcome of treatment with AIs has been less clear. The *(ATAC*) trialists published a hypothesis generating report suggesting that patients with *PgR-negative* breast cancer would obtain a substantially greater benefit from anastrozole than from tamoxifen compared with *PgR-positive* patients. [148] However, this hypothesis was not confirmed in centrally analyzed material from *1856 ER- and/or PgR-expressing* patients, moreover, the *BIG 1-98* trial reported that the benefit from letrozole over

tamoxifen did not vary according to the *PgR status*. [149] Nevertheless, these adjuvant trials clearly supported the existence of a strong relationship between *PgR expression* levels and prognosis on endocrine therapy, which may be useful in estimating residual risk.

2.23.3 HER2:

The oncogene *HER2* was first identified to be an indicator of patient's prognosis. In cases of *HER*2 being overexpressed *(HER2 positive*), breast cancer patients are more likely to suffer from relapse and tend to have a shorter overall survival. Amplification of the *HER2 gene* and RNA/protein overexpression correlate strongly. [150] through the development of the monoclonal antibody trastuzumab, which is targeted at *HER2*, the amplification status of *HER2* became also a highly predictive biomarker. [151] Overexpression and amplification of *HER2* can be detected in about (15%) of all primary breast cancers, and this group of patients benefit significantly from *anti-HER2 therapies*. *HER2* status should be assessed in every diagnosed case of *BC. (152)* (HER2) status is currently assessed in most cases initially by immunohistochemistry, and in cases of equivocal protein expression levels, *HER2 gene* copy number is measured via fluorescence in situ hybridization *(FISH)* or chromatin in situ hybridization *(CISH)* techniques. *(153)*

2.23.4 Emerging biomarkers:

2.23.4.1Ki 67

The marker of proliferation *Ki67* was first identified by. [150] In the 1980s using a mouse monoclonal antibody against a nuclear antigen from Hodgkin's lymphoma cell line [150] *Ki67 i*s a nuclear non histone protein the characteristic that *Ki67* was universally expressed among proliferating cells and absent in quiescent cells led to the further evaluation of *Ki67* as a marker of proliferation. Although little is known about the exact function of the protein in cell division, *Ki67* is expressed during

G1, S, and *G2* phases of cell cycle with a peak during mitosis and an absence in *G0* phase. [153] Although initially the *Ki67* antibody was applied only to fresh frozen tissue the correlation of *Ki67* and other biomarkers in invasive *BC* has been studied intensively.

2.24 Signs and Symptoms of the breast cancer: [113]

- Change in the size or shape of a breast
- Lump or thickening in an area of the breast
- Dimpling of the skin
- Change in the shape of the nipple, particularly if it turns in, sinks into the breast or becomes irregular in shape
- Blood stained discharge from the nipple.

2.25 Types of the Breast Cancers:

2.25.1 Ductal Carcinoma in situ (DCIS):

BC in the duct cells that has not invaded deeper or spread through the body women diagnosed with (*DCIS)* have a high likelihood of being cured

2.25.2Lobular Carcinoma in situ (LCIS):

Although called a carcinoma *LCIS* which occurs in the milk-producing lobule cells does not invade or spread and is not a true cancer. However, women with *LCIS* have an increased likelihood of developing invasive *BC* in the future. [116]

2.25.3 Invasive Ductal Carcinoma:

BC that begins in the duct cells but then invades deeper into the breast, carrying the potential of spreading to the rest of the body (metastasizing). Invasive ductal carcinoma is the most common type of invasive *BC*. [123]

2.25.4 Invasive lobular Carcinoma:

BC that begins in the milk-producing lobule cells, but then invades deeper into the breast, carrying the potential of spreading to the rest of the body (metastasizing), invasive lobular carcinoma is an uncommon form of *BC*. [116, 117]

2.25.5 Simple Breast Cyst:

A benign (noncancerous) fluid-filled sac that commonly develops in women in their (30s or 40s) Breast cysts may cause tenderness and may be drained. [123]

2.25.6 Breast Fibroadenoma:

Avery common noncancerous solid tumor of the breast, a typical fibro adenoma creates a painless, mobile lump in the breast and most commonly occurs in women in their (20s or 30s). [124]

2.25.7 Fibrocystic Breast Disease:

Common condition in which noncancerous breast lumps may become uncomfortable and change in size throughout the menstrual cycle. [126]

2.25.8 Usual hyperplasia of the breast:

A breast biopsy may show normal-appearing, noncancerous ductal cells multiplying abnormally. The presence of usual hyperplasia may slightly increase a woman's lifetime risk of *BC* [125]

2.25.9 Atypical hyperplasia of the breast:

Abnormal-appearing cells multiplying either in the breast ducts (atypical ductal hyperplasia) or lobules (atypical lobular hyperplasia), sometimes discovered by a breast biopsy, although the condition is noncancerous, women with atypical hyperplasias are at (4 to 5) times' higher risk of developing *BC* compared to women with no breast abnormalities.[126]

2.25.10 Intra-ductal papilloma:

A noncancerous wart-like breast mass that grows inside the breast ducts intraductal papillomas may be felt as a lump or cause clear or bloody fluid to leak from the nipple. [126]

2.25. 11 Adenosis of the breast:

A non-cancerous enlargement of the breast lobules. [127]

2.25.12 Non-invasive carcinoma (carcinoma in situ):

This represents about (15%-20%) of all breast carcinomas. The term non-invasive means that the malignant cells are confined to either the ducts or the acini of the lobules, with no evidence of penetration of the tumor cells through the basement membrane into the surrounding fibrous tissue. There are (2) forms of non-invasive carcinoma; ductal carcinoma in situ and lobular carcinoma in situ. [116] *DCIS* can occur in both pre- and postmenopausal women. It present as palpable mass, especially if extensive and associated with fibrosis. The macroscopic appearances depend on the architecture of the *DCIS*. Creamy necrotic material can exude from the cut surface of the breast. Histologically, the changes are to be found in the small and medium- sized ducts, although, in older women, the larger ducts can be involved. The ducts may be completely filled with cells (solid pattern), or have central necrosis (comedo pattern). The cribriform pattern has numerous gland-like structures within the sheets of cells. Incomplete excision increases the risk of changing from non-invasive to invasive range from (1/3 to ½).[116] *LCIS* occurs more frequently in premenopausal women it does not present as palpable lump and is usually found in biopsies removed for other reasons. It is often multifocal within the one breast and is frequently bilateral. The changes are found in the acini, the normal cells are replaced by relatively uniform cells with clear cytoplasm that appear loose and non-cohesive. The overall size of acini increases, but the lobular

shape is retained. About (1/4 to 1/3) of all patients treated by biopsy alone will go on to develop an invasive carcinoma. [116,117]

2.25.13 Invasive (infiltrating) carcinoma:

The cells have broken through the basement membrane around the breast structure in which they have arisen, and spread into the surround tissues. This allows the cancer cells access to lymphatics and blood vessels, lead to distant metastasis and a fatal outcome. It is categorized into different histological types including; invasive ductal, invasive lobular, mucinous, tubular, medullary papillary, and others. [117,118] Invasive ductal carcinomas comprise the majority (up to 75%) of invasive carcinomas. They can occur in both pre- and post-menopausal women. Macroscopically, they usually have a scirrhous consistency (prominent fibrous reaction in the central part of the tumor). Histologically, the tumor cells are arranged in groups, cords and gland-like structures. They range from well differentiated carcinomas with abundant gland formation to poorly differentiated neoplasms with solid sheets of malignant cells. Invasive lobular carcinoma constitutes about (10%) of invasive breast carcinoma. Usually occurs in postmenopausal women. Macroscopically, they have scirrhous consistency histologically, the cells are small, uniform and dispersed singly, appear in columns one cell wide in a dense stroma (Indian files). About (80%) are classic type which is mostly histologic grade (2), with a minority grade (1). [117,118] Medullary carcinomas constitute about (3%) of invasive carcinoma. These tumors usually occur in post-menopausal women. They are circumscribed soft fleshy mass, composed of large tracts of confluent cells with little stroma, around the island of tumor cells there is a prominent lymphocytic infiltrate, predominantly lymphocytes, with macrophage. The patients have a significant better (10) years survival than women with invasive ductal carcinoma. [117,118] Mucinous carcinoma also known as

colloid, mucoid and gelatinous carcinoma comprise about (2% -3%) of invasive carcinoma, usually arise in postmenopausal women. The tumors are well circumscribed and have a soft, grey, gelatinous cut surface. These carcinomas comprise small nests and cords of tumor cells embedded in large amounts of mucin. The survival is better than those having invasive ductal or lobular. [117,118] Tubular or cribriform carcinomas comprise about (1%-2%) of invasive carcinoma. They are well differentiated carcinoma composed of cells arranged as tubules. Macroscopically, appear as small and firm gritty tumors with irregular outline. Patients with tubular carcinomas have a good prognosis, with a very low risk of metastasis. [117,118,119] Papillary carcinomas form about (2%) and occur in postmenopausal women. They are circumscribed and focally necrotic, with little stromal reaction. The tumors are in the form of papillary structures. The patients have good prognosis. [117,118]

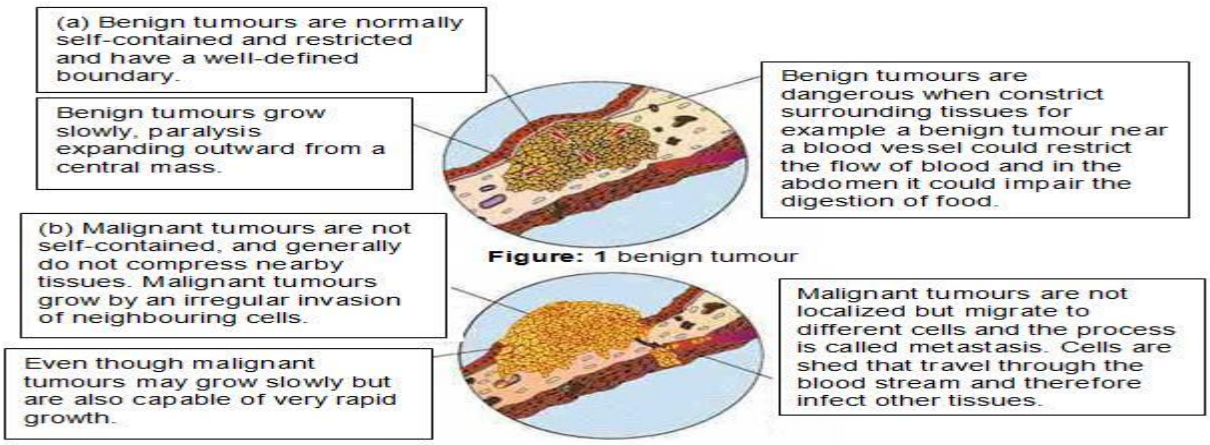

Figure (10.2): Shows benign vs. malignant cancers ((Image originally from the *Henary Website*).

(A). A benign tumor is a mass of cells that remains within the tissue in which it originally developed.

(B) The invasion of cancer cells into surrounding tissues is the hallmark of a malignant tumor. (Malignant cells may break free from the tumor and travel to other locations in the body through the process of metastasis). [20]

2.25.14 Paget's disease:

This is a rare form of breast cancer that is characterized clinically by eczematoid changes of the nipple. The Paget's disease represents the migration of malignant cells from subjacent mammary ducts in the nipple. The prognosis of Paget's disease depends on the underlying breast carcinoma type. [117,118,119]

2.26 Molecular Basis of Breast Cancer:

Genetic factors, though not so well understood mechanisms, play important roles in the initiation and development of *BC*. Traditionally, *BC* is divided into sporadic and familial (i.e. hereditary). The majority of *BC* cases are sporadic whereas (25%) of cases occur in patients with a family history of the disease moreover, major known genes causing syndromes predisposing to *BC* only account for a small percentage of total cases (5% - 6%).[140]

2.27 Genes Involved in BC Progression

The first key genetic step in *BC* development is the mutational inactivation of the *tumor suppressor gene*. *APC* is an essential negative regulator in the conserved *Wnt /Wingless (Wg)* signal transduction pathway during normal development, *Wnt* signaling plays roles in many processes such as induction of cell proliferation, cell fate specification, and induction of apoptotic cell death. *APC* is the primary gatekeeper of cell proliferation and survival in the colonic epithelium since the loss of both functioning copies of *APC gene* allows for uncontrolled cell growth. [154] the

second key genetic step in (*CRC)* development is the inactivation of the *TP53* gene that encodes for the *transcription factor p53*. *P53* plays an important role in *cell cycle regulation* apoptosis, and is involved in regulating cellular responses to oxidative stress and many other pathways Hence, mutations in both alleles of *TP53* and the loss of its function are critical *BC promoting* events as they enable the conversion of adenomas to carcinomas. [155, 156] The Third gene, associated with the development of cancer is *K-ras*. The activation of the *K-ras proto-oncogene* by a point mutation is one of the most frequent genetic alterations associated with *BC*. [57]

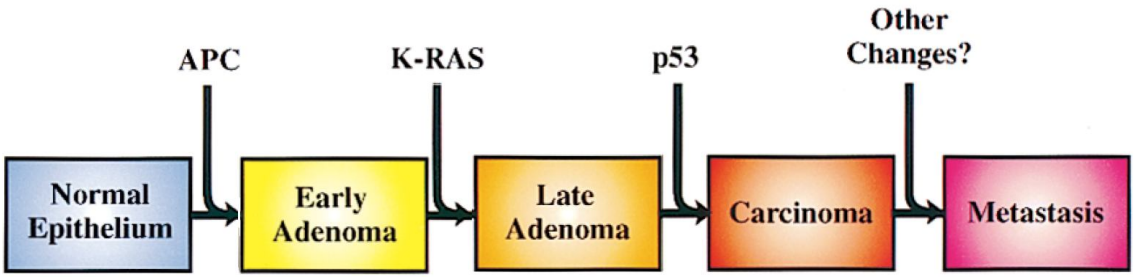

Figure (11.2) Genetic changes associated with the progression of breast cancer

2.28 Diagnosis of Breast Cancer:

The triple test is the recommended approach for the investigation of palpable and impalpable breast lesions. It comprised of; clinical examination and medical history, imaging, and fine needle aspiration cytology or biopsy it has (99.6%) sensitivity and specificity of (93%). ([112,113])

2.28.1 Clinical Examination (Symptoms and Signs):

Although, widespread use of screening mammography has increased the number of breast cancers found before they cause any symptoms. Some breast cancers are not

found by mammography, even under ideal conditions mammography cannot find every *BC*. The most common sign of *BC* is a new lump or mass. A mass that is painless, hard, and has irregular edges is more likely to be cancerous, but some rare cancers are tender, soft, and rounded. Other signs of breast cancer include a generalized swelling in part of a breast (even if no distinct lump is felt), skin irritation or dimpling, nipple pain or retraction redness or scaliness of the nipple or breast skin, or a discharge. Moreover, breast cancer can spread to axillary lymph nodes that are obviously enlarged, even before the original tumor in the breast tissue is large enough to be felt. [114,115]

2.28.2 Imaging tests:

Breast imaging, ultrasound and diagnostic mammography can improve the pre-operative diagnostic assessment, and permits image-guided needle sampling of suspicious lesions. Diagnostic mammography can be used to evaluate changes in breast tissue that are difficult to detect by screening mammography. It's different from screening mammography because more *X-rays* are needed to obtain views of the breast from several angles. *(CT) scan* is an *X-ray* technique that gives information about the body's internal organs in 2-dimensional slices (*CT) s*cans are not used routinely to evaluate the breast, but used to assess the spread of cancer into the chest wall. This helps to determine whether or not the cancer can be removed with mastectomy. Moreover, it can be used to examine other parts of the body where *BC* can spread such as; the lymph nodes, lungs, liver, and brain. *(MRI)* is a technology that uses magnets and radio waves to produce detailed cross-sectional images of the inside of the body. Breast (*MRI*) has a number of different uses for breast cancer, including: screening high-risk women, gathering more information about an area of suspicion found on a mammogram, and monitoring for

recurrence after treatment.*(US)* based diagnostic imaging is a technique can be used for visualizing internal organs for possible pathology or lesions.[115,116]

2.28.3 Fine Needle Aspiration (FNA) Cytology:

This is a percutaneous procedure that uses a fine needle and a syringe to sample fluid from a breast cyst or remove clusters of cells from a solid mass the reliability of (*FNA) cytology* depends on the skills of the aspirator histological type of the lesion, age of the patient, and size of the lesion. The advantages of *(FNA)* includes: quicker procedure, does not require local anesthetic, less traumatic and relatively inexpensive. While the disadvantages are: requires training in the preparation of quality smears, considerable cytology expertise is required for interpretation, inappropriate for the assessment of micro-calcifications, also does not enable to distinguish between carcinoma in situ and invasive carcinoma.[115]

2.28.4 Biopsy:

Is a surgical method used to remove tissue from a suspected lesion in the body different techniques can be used to perform breast biopsy include core needle biopsy, vacuum assisted biopsy and open surgical biopsy. The core needle biopsy is a procedure that removes small solid samples of tissue using a cutting (core) needle under local anesthesia. In cases where the lump cannot be felt imaging tools used to guide the core needle to the right location, such as ultrasound and mammogram. Vacuum assisted biopsy is a version of core needle biopsy using a vacuum technique to assist the collection of the tissue sample. In this technique, the needle normally has a lateral opening and can be rotated allowing multiple samples to be collected through a single skin incision. However, surgical biopsy requires an approximately (3 to 5) centimeters incision and performed in an operating room in sterile conditions under general anesthesia. Core needle biopsy is a minimally invasive biopsy type and achieved better sensitivity and specificity. [115,116]

2.28.5 Histopathology:

Histologically, *BC* classified primarily from the epithelium that lining the ducts or lobules. Carcinoma of the breast arises from the ductal epithelium in (90%) of cases while the remaining (10%) originate from the lobular epithelium histopathological evaluation of a *BC* is necessary for conclusive diagnosis of the tumor, as well as, determine patient's prognosis and understand the nature of the tumor to determine the appropriate clinical management. [115]

2.28.6 Grading:

Carcinomas are assigned a histologic grade, according to how well they resemble the normal tissue that they are derived from. Invasive ductal carcinomas are graded using the bloom and Richardson (Nottingham scheme). There are three points system is given to each of three histologic parameters; tubular formation nuclear features and mitotic rate. The summation score for the three parameters determines the grade. Carcinomas are graded as well differentiated (grade1) moderately differentiated (grade 2) and poorly differentiated (grade 3), and there is progressively increasing aggressiveness from well to poorly differentiated carcinomas. [119,120]

2.28.7 Staging:

Cancer staging is based on the size of the primary lesion, spread to the regional lymph node and the presence or absence of metastasis. It is important factor in the treatment plan. The most common system used to describe the stages of *BC* is the (*TNM) system*. This system takes into account the tumor size and spread *(T),* whether the cancer has spread to lymph nodes *(N),* and whether it has spread to distant organs *(M*, for metastasis). Numbers after the *T, N, and M* give details about the cancer. It is expressed as a Roman numeral, after stage 0 (carcinoma in situ),

the other stages are I through IV. As a rule, the lower the number, the less the cancer has spread. [119,120]

2.28.8 Immunohistochemistry (IHC):

Estrogen and progesterone hormonal receptors status *(ER/PR)* and human epidermal growth factor receptor 2 expression *(Her2/neu),* are important determinants of the treatment plan. *ER and PR positive BC* tend to have a better prognosis with highest rate of response to anti-estrogen therapy Over-expression of *Her2/neu* is associated with poorer prognosis. However, it cans response for targeted therapy such as the monoclonal antibody trastuzumab and this has improved the prognosis significantly. Tumor cells that do not express any of these three receptor types, are named triple-negative, which characterized by poor prognosis and more aggressive treatment is required. [121,122]

2.28.9 Molecular methods:

Analyzing differences in gene expression patterns across individual patients with a certain type of cancer may reveal molecular differences that permit refinements in their classification, prognostication, and treatment selection. At least some data suggests that primary *BC* may carry specific molecular changes that are capable of predicting the presence of (or potential for) cervical nodal metastases however, these approaches remain investigational. [148,149] Genetic and epigenetic alterations may lead to protein changes including decreased or increased expression. The accumulation of these alterations in oncogenes, proto-oncogenes and tumor suppressors can lead to the formation of a malignancy critically altered pathways in (*HNSCC)* include *p53*, *(EGFR),(STAT3)* and *(VEGFR),* among other important molecules that may serve as therapeutic targets.[148,149]

2.28.9.1 Immunohistochemistry:

Histological examination plays a central role in diagnosis, classification, grading and staging of malignancy. Difficulties arise from the subjective nature of histological analysis that are influenced by the practitioner's experience, bias and training. With poorly-differentiated neoplasms, inter- and intra-observer variability can be high. [148,149] Immunohistochemistry has greatly assisted in the identification of tumors that cannot be accurately identified using routine histopathological procedures.[148,149] In some undifferentiated tumors, subtle features of epithelial versus mesenchymal differentiation can often be appreciated which assist the immunohistochemical approach to these tumors. Some tumors, however, may not fit into either of these two categories because of their overlapping histological features; nevertheless, making the correct histopathological diagnosis is essential in deciding the appropriate therapy. The immunohistochemical evaluation of undifferentiated tumors should first aim at a broad lineage determination of the neoplasia. Based on the result of the screening panel, a more detailed or specific panel should then be applied to further sub classify the tumor or to confirm a particular diagnosis. [148,149]

2.28.9.2 Polymerase Chain Reaction (PCR):

Work flow started with sample collection, followed by *PCR* and gel electrophoresis (methodology).

2.29 Prevention and Control of Breast Cancer:

(WHO) recommend *BC* control within the context of comprehensive national cancer programs? Its involve prevention, early detection, diagnosis and treatment, rehabilitation and palliative care. Raising general public awareness on the *BC* problem and the mechanisms to control as well as advocating for appropriate policies and programs are key strategies of population-based *BC* control *(WHO)*

(2013). Control of specific modifiable *BC* risk factors as well as prevention, which promotes healthy diet, physical activity limit of alcohol intake, obesity and breast feeding, can reducing the incidence of *BC* in the long term. These risk modifications might prevent (38%) of *BC* in the *USA,* (42%) in the *UK*, (28%) in Brazil and (20%) in China. [146, 147] Women with family history of *BC* or *BRCA1and BRCA2 mutations* are highly recommended for screening regularly. Prophylactic bilateral mastectomy may be considered in people with *BRCA1 and BRCA2 mutations*. [148, 89] The selective estrogen receptor modulators such as tamoxifen can reduce the risk of *BC*, but may increase the risk of thromboembolism and endometrial cancer. [150, 151]

2.29.1 Early detection:

Although some risk reduction might be achieved with prevention, these strategies cannot eliminate the majority of *BC* that develops in low- and middle-income countries. Therefore, early detection in order to improve *BC* outcome and survival remains the cornerstone of *BC* control. There are (2) early detection methods; early diagnosis or awareness and screening method. Awareness of early signs and symptoms is mandatory in symptomatic populations' in-order to facilitate diagnosis and early treatment. Early diagnosis remains an important early detection strategy, particularly in low and middle income countries where the diseases is diagnosed in late stages and resources are very limited. There is some evidence that this strategy can produce increasing in proportion of *BC* detected at early stages that are more amenable to curative treatment. Screening is a systematic application of a screening test in a presumably asymptomatic population. It aims to identify individuals with an abnormality suggestive of cancer. Screening mammography is the only screening method that has proven to be effective. It is an *X-ray photograph* of the breast used to check for *BC* in women who have no signs or symptoms of the

disease. Screening mammograms can detect tumors that cannot be felt physically also can find micro-calcifications that may indicate the presence of *BC*. The population-based mammography screening programs can reduce *BC* mortality by about (20%) in the screened group versus the unscreened group across all age groups. *(BSE)* is a Checking one's own breasts for lumps or other unusual changes. There is no evidence on the effect of screening through breast self-examination. However the practice of *BSE* has been seen to empower women, taking responsibility for their own health. Therefore, (BSE*)* is recommended for raising awareness among women at risk rather than as a screening method. *(CBE) screening* is a physical exam of the breast performed by a health care provider to check for lumps or other changes. It has been shown that the age-standardized incidence rate for advanced-stage breast cancer is lower in the screened *CBE* group compared to the unscreened group. [151, 152]

2.29.2 HPV Vaccine:

HPV vaccine is known to be very effective in protecting younger women who have not been exposed to *HPV* against cervical anal and oral *HPV infections* [148,149] Now a new study is the first to show that vaccination protects against infection at all three anatomic sites in women ages (18 to 25) including some who were previously exposed to the virus. Researchers conducted the analysis as part of the NCI-funded Costa Rica Vaccine Trial, in which (4186) women were randomized to receive the (*HPV 16/18) vaccine* a bivalent vaccine that protects against two *HPV strains* that cause (70%) of cervical cancers or a control vaccine against hepatitis A at enrollment. In addition, participants had blood tests to detect *HPV antibodies* and contributed cervical samples to test for *HPV, DNA* oral and anal samples were taken at a follow-up visit (4) years later overall efficacy after (4) years was (65%) for all sites and (91%) for at least (2 of the 3) sites. Notably, the vaccine appeared

to protect against concordant infections. In women diagnosed with cervical infections at follow-up, (40%) in the control group had the same type of infection at oral or anal sites compared with (15%) of those who received the *HPV vaccine*. [148,149] Not only do we see multisite vaccine efficacy in this population, we actually see that the vaccine may provide some protection against [infection at] the other sites in previously infected women. Vaccine efficacy was (84%) among women with no evidence of previous exposure, (58%) among those with evidence of infection prior to enrollment, and (25%) among women with active infection at the time of vaccination, the vaccine has no therapeutic effect for women with active infections, but there are multiple *HPV types* that can cause cancer, and women with *HPV infections* could still derive protection from subsequent infections, no vaccine for *HPV* in breast cancer till now but there is some trials. [151]

2.30 Management of the Breast Cancer:

The awareness of early detection and progress in treatment has improved the survival of *BC* patients in all ages, races and all stages. *BC* mortality has been decreased by (34%) and there are (3) million *BC* survivors in the *U.S.A.* [153,154] the management of *BC* depends on various factors, including; stage, grade, *ER/PR* status and *Her2/neu expression* of the cancer. *BC* treatment can includes some combination of surgery, radiation therapy, chemotherapy, hormone therapy and targeted therapy. [125]

2.31 Treatments of the *BC* Patients:

2.31.1 Surgery:

This is usually the first line of treatment against breast cancer. Surgery types include**:** lumpectomy, also known as breast-conserving surgery, is the removal of only the tumor and a small amount of surrounding tissue mastectomy is the removal of all of the breast tissue. Lymph node removal or axillary lymph node

dissection, can take place during lumpectomy and mastectomy if the biopsy shows that *BC* has spread outside the duct. Breast reconstruction is the rebuilding of the breast after mastectomy and sometimes lumpectomy reconstruction can take place at the same time as cancer-removing surgery, one month to years later. [126,127]

2.31.2 Chemotherapy treatment

Chemotherapy is a treatment by cytotoxic drugs that kill the cancer cells and interfere with their ability to grow and divide. Its target the cancer cells in the primary lesion or any cancer cells that may have distant spread and metastasis Chemotherapy can be used to treat early-stage invasive *BC*; helps lower the risk of recurrence by killing of cancer cells that might still is present in the body after surgery. Moreover, it can be used in advanced stage *BC* to destroy the cancer cells and given before surgery to shrink the tumor chemotherapy medicines are given in combination, this known as chemotherapy regimens e.g. *(AT*) chemotherapy have systemic side effect because it destroys normal cells in blood, mouth, intestinal tract, nose nails, vagina, and hair.[127.128]

2.31.3 Radiation therapy

Radiation is a highly targeted, effective method to destroy cancer cells in the breast that may remain after surgery. Radiation can reduce the risk of *BC* recurrence by about (70%). It is relatively easy to tolerate and the side effects are limited to the treated area. It uses a special kind of high-energy *X-ray* beam to damage the *DNA* of cancer cells; radiotherapy has an important role in treating stage 0 through stage III of *BC*. There are (2) main types of radiation: external radiation is the most common type, typically given after lumpectomy and sometimes, mastectomy. Internal radiation, delivered inside the breast, is a less common method used after lumpectomy. [128,129]

2.31.4 Hormonal therapy:

Hormonal therapy is systemic treatment for hormone receptor positive breast cancers. About (80%) of *BC* are estrogen receptor positive, (65%) of them are also progesterone receptor positive. However, about (13%) of *BC* is estrogen receptor positive and progesterone receptor negative. Hormonal therapy medicines treat *B C* by two methods: by lowering the amount of the hormone estrogen in the body through *(ERDs)* e.g. Faslodex. The other method, by blocking the action of estrogen on *BC* cells this group includes: aromatase inhibitors (Arimidex, Aromasin, and Femara) and *Selective Estrogen Receptor Modulators (Tamoxifen)*. In some cases, the ovaries and fallopian tubes may be surgically removed to treat hormone-receptor-positive *BC* or as a preventive measure for women at very high risk of *BC.* [129,130]

2.31.5 Targeted cancer therapies:

A targeted therapy is a drug designed to attack a certain molecular agent or pathway involved in the progression of cancer. It is less likely than chemotherapy to harm normal cells as well as, it can be effective in early stage and metastatic *Her2/neu* over-expressed breast cancer. Some targeted therapies are antibodies; these types are called immune targeted therapies. Currently, there are (3) targeted therapies available: *Herceptin (Trastuzumab)* works against *Her2/neu positive BC*by blocking the ability of the cancer cells to receive chemical signals that tell the cells to grow. *Tykerb* works against *Her2/neu positive breast cancers* by blocking certain proteins that can cause uncontrolled cell growth. *Avastin* works by blocking the growth of new blood vessels that cancer cells depend on to grow and function. [131]

2.31.6 Gene therapy:

Cancer gene therapy is a treatment that transfers *DNA* to cancer cells through vectors, resulting in the suppression of breast tumor growth or death of cancer cells. Many types of vectors have been developed for gene therapy such as chemical vectors and viral vectors. The most efficient gene delivery agents are viral vectors such as Adenoviruses that can carry and transduce the inserted gene to the host cell efficiently. Chemical vectors such as lipids and polymers can bind with *DNA* to form aggregates, which interact with cell surface receptors or fused with the plasma membrane to deliver *DNA*. Gene therapy can act by block of oncogenic signaling, block tumor induced angiogenesis, kill tumor cells directly or enhance tumor immunogenicity. There are many regulatory elements can be used to target *BC* cells in gene therapy include; promoters and the 5'- and 3'-untranslated regions *(UTRs)* of *mRNA*. [131,132]

2.32 Others Cancers Caused by *HPV:*

2.32.1 Head and neck cancers (HNCs):

(HNCs) are a group of malignant tumors originating from the upper aero digestive tract [101]. The vast majority (90%) of these cancers are *(SCCs)*. [151] *(HNSCCs)* have relatively common features relating to their etiology and classification. Consequently, the *(EGFR*) is frequently expressed in at least (80%) of *HNSCCs*. [150] other less common types of *HNCs* are adenocarcinomas, lymphomas sarcomas, melanomas and others. [152] each year approximately (560,000) cases of *HNCs* are diagnosed worldwide and (300,000) patients die annually. Incidence rates are more than twice as high in men as in women.[151] Oral cancer is the most common type of *HNCs* with (240,000) cases diagnosed each year, while cancers of the larynx, pharynx, thyroid and nasopharynx are less common.[152] Oral cancer has the highest rates of incidence in Western Europe India, South Africa and Australia. There is a particularly high incidence of oral cavity cancer in males in France,

whereas in females the highest incidence is in India.[153] Increased incidence of oral cavity and pharynx cancer has been reported in Germany, Denmark, Scotland, Central and Eastern Europe.[153] This is thought to be due to an increase in alcohol consumption.[153] Many risk factors were well established in etiology of (*HNCs)* most of which related to lifestyle and environmental factors tobacco and alcohol are the main risk factors, accounting for (7 out of 10) cases of (*HNSCs)*heavy alcoholic who are also heavy smokers have a (35) times increased risk of developing oral cancer compared to those who do not drink or smoke another potent risk factor is *(HPV)* particularly; *high risk (HR) types 16, 18* many studies have strongly proved the link between *HPV and HNCs HPV* must adhere to a specific receptor protein on the keratinocytes membrane.[150] once the virus entered into the cell, it transforms itself of its protein coat and the *viral DNA* may then utilize host cell themselves. These viruses elaborate early *gene proteins* that are able to regulate the host *cell cycle*, or mitotic capabilities. The *(E6 and E7) proteins* are most important in this respect; they bind two host proteins that are regulators of the keratinocytes at the time of cell division. (E6*) binds* to a protein designated *p53*, a molecule that arrests cell division. However, once bound, it is degraded and this inhibition of keratinocytes mitosis is abrogated. Likewise, *(E7)* binds a protein termed *Rb* and, similarly, *cell cycle regulation* is troubled. On the basis of their genotype, more than *(120) types* of *HPV DNA genotypes* have been fully sequenced, it is classified on the bases of its infection in epithelial cells and the ability to effect cellular transformation for e.g. *HPV 1* is responsible for the infection in cutaneous cells while *HPV (6, 11, 18)* for mucosal epithelial cells of the oral cavity, oropharynx, anogenital tract and uterine cervix, the potentially oncogenic *HPV* is divided into high and low-risk types. The *high-risk HPV* such as *(16, 18, 31, 33, 35, 52, 58, 59, 68, 73, and 82)* are responsible for malignancies while the *low-risk sub types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81)* are rarely

found in carcinoma and frequently connected with benign and potentially malignant lesions of the head and neck.[154] Early diagnosis in *HNCs* is vital as patients who present with early-stage disease have significantly better outcomes than those who present with late-stage disease. Routine physical examination, including a thorough oral examination, is the best way to detect *HNCs* before they become symptomatic. Definitive diagnosis usually requires a biopsy. Additional information is obtained from a combination of imaging tests, such as *(CT), (MRI) or (PET*), endoscopy and fine-needle aspiration of any neck mass.[153] Many methods can be applied for diagnosis of (*HNCs)*, as well as, identifying some related etiological agents such as *HPV*. *HPV* can be identified in cytological smears or biopsy using different techniques e.g. immunocytochemistry, *PCR and ISH*. On the basis of cytology and histopathology, *HPV infection* is characterized by koilocytosis, perinuclear cytoplasmic haloes, nuclear dysplasia, atypical immature metaplasia and binucleation. These methods show limited sensitivity and are unable to determine which types of (*HPV)* are involved in the infection of the epithelial cells.[153]*ISH techniques* employ the use of type-specific radioactively *labeled DNA probes*, which are complementary to *HPV DNA sequences* used for detection of viruses in the premalignant and malignant lesions of the *HNCs*. *ISH* and immunohistochemistry have low sensitivity because these tests only detect the virus when it is present in more than (10) copies of the *viral DNA per cell*. [153,154,155] *PCR* is highly sensitive detection method for *specific subtypes of (HPV)* because it detects the virus in less than (1) copy of the viral *DNA per cell*' currently, the main treatment options for head and neck cancers are surgery, radiotherapy and chemotherapy. The types of treatment used will depend on the site and disease stage as well as on the patient's overall health status. For most early-stage tumors, surgery is carried out to remove the tumor. However, for certain anatomical sites, such as the base of the tongue, radiotherapy is used, either alone

or combined with surgery.[153,154,155] Radiotherapy in combination with chemotherapy (most often cisplatin) - administered either as a definitive treatment or after surgery - plays a role in the management of locally advanced and/or inoperable head and neck cancers; this is known as radio-chemotherapy however is associated with significant toxicities In addition, locally advanced head and neck cancer is associated with a poor prognosis due to high recurrence rates for patients with advanced (metastatic) or recurrent disease, treatment options include systemic chemotherapy.[153,154,155] Despite the introduction of chemotherapy treatment in this setting approximately (30) years ago patients with advanced (metastatic) or recurrent head and neck cancer still have a poor prognosis, with median survival of (6-10)months [153,154,155]

***HPV Infection*:**

It has been implicated in a variety of papillomatous and malignant squamous proliferations, including those seen in the head and neck area such as sinonasal papillomas, laryngeal papillomas, and squamous cell carcinoma.[153,154,155] *Benign HPV types* induce lesions characterized by hyperplasia, parakeratosis and papillomatosis. The differences in these features vary between *HPV types*. *High risk HPV types* can potentially induce lesions with intraepithelial neoplasia characterized by disorganized architecture of the epithelia, abnormal mitotic figures and nuclear atypia. These lesions are graded depending on how much of the epithelia that are affected. In addition, in *HPV infected cells halos* appear around the nucleus, a phenomenon that known as koilocytosis.

2.32.2 Cervical cancers:-

(HPVs) are small, *double-stranded DNA viruses* that infect cutaneous and mucosal epithelial tissues of the ano-genital tract the hands, or the feet a subset of *HPV types* are the causative agents of cervical cancer, since (99%) of tumors are positive

for *HPV DNA*. [150] to date over (100) different viral types have been identified, and about (1/3rd) of these infect epithelial cells in the genital tract. The viral types that infect the genital tract fall into two categories: high risk and low risk. The high-risk types are associated with the development of ano-genital cancers including those of the cervix, while infections by the *low-risk HPVs* induce only benign genital warts. The *high-risk types* include (*HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45)*, while the *low-risk types* are (HPV-6 and HPV-11 HPVs) that infect the genital tract are sexually transmitted, and it is estimated that about (2/3rd) of individuals who have sexual relations with an infected partner will themselves become infected. However, the majority of infections are subclinical Infection by *high-risk HPVs* is not limited to the genital tract since approximately (20%) of cancers of the oropharynx contain *DNA* from these *HPV types*. [154]

Infection of the genital tract by *HPVs* can initially result in low-grade lesions termed dysplasias or cervical intraepithelial neoplasia grade (I). These lesions exhibit only mildly altered patterns of differentiation, and many of them are cleared by the immune system in less than a year.[152, 14] The mechanisms by which the cellular immune response clears *HPV infections* are still not clearly understood. Some of these lesions, however, are not cleared by the immune system and can persist for periods as long as several decades. Persistence of infection by *high-risk HPV types* is the greatest risk factor for development of genital malignancies such as squamous cell carcinoma or, less commonly adenocarcinoma of the cervix.[154] Cervical cancer is the second most prevalent cancer worldwide and is the (1/5th) leading cause of cancer deaths in women [153, 154]. Approximately (470,000) new cases of cervical cancer are diagnosed yearly, with the mean age for the development of malignancy being (52) years.[154] Risk factors for tumor development include persistent infection with high-risk viral types, a large number

of lifetime sexual partners, co-infection with *(HIV)* immunosuppression, and cigarette smoking.[157]

2.32.3 Colorectal Cancer:

Cancer is a general term given for a group of diseases in which host cells begin to grow out of control. There are many types of cancers, all of which start as single cells that have lost control of their normal growth and replication processes. Untreated cancer can lead to serious illness and even death [153,154,155] Colon cancer is defined as cancer that forms in the tissues of the colon. Most colon cancers are adenocarcinomas (cancers that begin in cells that make and release mucus and other fluids), while rectal cancer is the cancer that forms in the tissues of the rectum. [153,154,155] *(CRC)* usually originates from epithelial cells lining the colon and rectum; these cells replicate at a relatively high rate leading to increased risk of mutation and consequent carcinogenesis of the epithelial cells. *CRC* begins as a benign adenomatous polyp which develops into an advanced adenoma with high-grade dysplasia and then progresses to an invasive cancer. [153,154,155]

2.33 Association between *(CRC)* and *(HPV):*

Viruses are now accepted as genuine etiologic factors of human cancer. Such viruses include *hepatitis B and C virus*, *EBV, and HPVs*. [153,154,155] It is well accepted that *HPVs* are causative agents of cervical and most anal cancers and are also associated with several other cancers such as those of the head and neck furthermore, the gut and intestines have hundreds of bacterial species constituting their normal flora and are occasionally exposed to various viruses under some circumstances this normal flora can be disrupted and may cause diseases including cancer of the colon. Previous studies trying to establish a link between colorectal cancer and *HPV infections* failed to identify any relationship this was attributed to several reasons, including small sample size ranging from (10 to 50) cases) and

poor choice of diagnostic techniques. However, with the development of techniques such as *PCR*, a clear link between *CRC and HPV infection* was established. [151, 154] Despite the evidence of an association between (*HPV) and CRC*, there were numerous studies indicating the absence of such a relationship, therefore, due to the conflicting reports concerning the association between *CRC and HPV*, this hypothesis remains controversial and in need of further investigation. Hence, the major aim of our current study is to investigate the presence of *HPV infection* in colorectal cancer tissue as indicated by the identification of *HPV E6 DNA and HPV proteins* in colorectal cancer tissues. [157]

2.34 Breast Cancer in Men:

BC is a malignant tumor that starts from cells of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body breast cancer occurs mainly in women, but men can get it, too many people do not realize that men have breast tissue and that they can develop *BC*.[155,156]

2.35 Normal Breast Structure:

To understand breast cancer, it helps to have some basic knowledge about the normal. The breast is made up mainly of lobules (milk-producing glands in women) 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). Until puberty usually around *(13 or 14)* young boys and girls have a small amount of breast tissue consisting of a few ducts located under the nipple and areola (area around the nipple). At puberty, a girl's ovaries make female hormones, causing breast ducts to grow, lobules to form at the ends of ducts, and the amount of stroma to increase. In boys hormones made by the testicles keep breast tissue from growing much. Men's breast tissue has ducts, but

only a few if any lobules. Like all cells of the body, a man's breast duct cells can undergo cancerous changes. But breast cancer is less common in men because their breast duct cells are less developed than those of women and because their breast cells are not constantly exposed to the growth-promoting effects of female hormones. [146,147]

2.38 Benign Breast Tumors:

Men can also have some benign (not cancerous) breast disorders there are many types of benign breast tumors (abnormal lumps or masses of tissue) such as papillomas and fibro-adenomas. Benign breast tumors do not spread outside the breast and are not life threatening. Benign tumors are common in women but are very rare in men.

2.39 Gynecomastia:

Gynecomastia is the most common male breast disorder. It is not a tumor but rather an increase in the amount of a man's breast tissue. Usually men have too little breast tissue to be felt or noticed. A man with gynecomastia has a button-like or disk-like growth under his nipple and areola, which can be felt and sometimes seen. Although gynecomastia is much more common than breast cancer in men, both can be felt as a growth under the nipple, which is why it's important to have any such lumps checked by your doctor. Men can also have some benign (not cancerous) breast disorders is common among teenage boys because the balance of hormones in the body changes during adolescence. [150] it is also common in older men due to changes in their hormone balance. In rare cases gynecomastia occurs because tumors or diseases of certain endocrine (hormone-producing) glands cause a man's body to make more estrogen (the main female hormone). Men's glands normally make some estrogen, but it is not enough to cause breast growth. Diseases of the liver, which is an important organ in male and female hormone metabolism, can

change a man's hormone balance and lead to gynecomastia. Obesity (being extremely overweight) can also cause higher levels of estrogens in men. Some medicines can cause gynecomastia. These include some drugs used to treat ulcers and heartburn, high blood pressure, and heart failure. Men with gynecomastia should ask their doctors if any medicines they are taking might be causing this condition.

Chapter Three

Materials and Methods

3.1 Study Design:

A retrospective hospital-based case-control study will be conducted.

3.2 Study Center:

Radiation & Isotopes Center in Khartoum (RICK), in Sudan.

3.3. Study population:

3.3.1 Patients:

143 formalin fixed paraffin wax embedded breast tissue blocks were used in this study. Of the (143) breast tissue blocks, (100) were patients' blocks with breast cancer (ascertained as cases) and the remaining (43) were with breast inflammatory conditions ascertained as control).

Tissue blocks were collected from different histopathology laboratories in Khartoum State and other states which mostly received by *RICK* for treatment plan. Full clinical data were obtained from the laboratory information system and reports and general hospital registry office including: age, diagnosis, menopausal status, histological type, grading, age, socio-economic status, marital status, radiation, family history, inflamed breast tissue samples detected at the safety margin of the malignant breast cases was considered as controls the samples diagnosed in institute of endemic disease university of Khartoum labs.

3.3.2 Inclusion Criteria:

All Sudanese female patients at any age diagnosed as having breast carcinoma of any type stage or grade in the study period. Patients should not receive chemotherapy or hormone therapy before surgery.

3.3.3 Exclusion Criteria:

Any patients with breast carcinoma who receive chemotherapy or hormone therapy before surgery.

3.3.4 Control:

Sudanese women will be included. They will be matched to the patients with respect to their age.

3.4 Data Collection:

Sources of data will include: clinical examination (done by the physician in the respective hospitals), a questionnaire and laboratory investigations.

3.4.1 Questionnaire:

A simple questionnaire is designed and used for each patient; included complete demographic characteristics, present and family history of cancer, personal habits and medical history. Another questionnaire will be designed for control subjects including same questions.

3.4.2 Duration of the research:

The study conducted from June 2013 to March 2016.

3.5. Sample Size:

The formula to calculate the sample size is:

$$\mathbf{n = \frac{t^2 \times p\,(1-p)}{M}}$$

Where:

N = required sample size.

t = confidence level at (95%), (standard value of 1.96).

p = estimated prevalence of the disease in the area.

m = margin of error at (5%), (standard value of 0.05).

3.6. Sampling Techniques:

3.6.1 Specimens:

Breast tissues will be collected from Formalin – Fixed, Paraffin - embedded tissues. For the control group tissue inflammatory breast lesion; tissues will be obtained from Formalin – Fixed, Paraffin - embedded tissues. Tissues must be diagnosed as inflamed breast lesions.

3.6.2 Laboratory Tests:

1. *DNA* extraction.
2. *PCR* technique.

3.6.3 DNA Extraction procedure:

(10)µm sections were cut from the formalin fixed paraffin embedded *BC blocks*, and then pooled in eppendorf tubes. Next the following procedure was used:

1- (1) ml of xylene was added to each tube and the tubes were vortexed vigorously for (10) sec to deparaffinize the tissue. Next, the mixture was centrifuged at full speed for (2) min at room temperature and the supernatant was removed by pipetting while the pellet remained in the tube; this step was repeated two times.

2- (1) ml ethanol (96%-100%) was added to each pellet, mixed by vortexing centrifuged at full speed for (2) min at room temperature and the supernatant was removed by pipetting. This step was repeated two times to remove residual xylene.

3- Next, eppendorf tubes containing the tissue pellets were opened and incubated at (37) °C for (10-15) minutes until the ethanol evaporated.

4- Each pellet was resuspended in (180) µl ATL lysis buffer and (40) µl proteinase K, mixed by vortexing, and incubated at (56) °C in a shaking water bath until the tissue was completely digested.

5- (200) µl AL buffer and (200) µl of ethanol (96%-100%) were added to each tube, and mixed thoroughly by vortexing.

6- The mixture was transferred to a minelute column placed in a collection tube. This assembly was centrifuged at (8000) rpm for (1) min and the flow-through were discarded.

7- Each minelute column was washed two times using the washing solution (AW1 and AW2).

8- Finally, *DNA* was eluted from each minelute column (placed into a new eppendorf tube) using (20-100) µl AE elution buffer by centrifugation at full speed for (1) min. Solution obtained containing the *DNA* was stored at (20) C° for later use.

3.6.4 Selection of Primers:

The primers were designed to detect the *HPV types 16, 18.31.33* using the *NCBI* primer design tool.[154] (Primer sequences and the expected *PCR* products sizes of each pair are available in **(Table 2.3)** *GAPDH (Glyceraldehydes 3-phosphate dehydrogenase)* gene primers were used as controls, to demonstrate the integrity and the quality of the isolated *DNA*.

Table (1.3): Sequences and properties of designed *PCR* primers used to amplify the *HPV16, 18, 31, and 33* used in this study.

Primers	Primer sequence(5'-3')	Length	TM(^{O}C)	Product size (bp)
HPV16				
Forward	5-CCACAGGAGCGACCCAGAAAGTT-3	23	61.3	390
Reverse	5-ACCGGTCCACCGACCCCTTATAT-3	23	61.7	
HPV18				
Forward	5-GCGCGCTTTGAGGATCCAACAC-3	22	61.4	323
Reverse	5-TGGCACCGCAGGCACCTTAT-3	20	62.2	
HPV31				
Forward	5-GGCCTCCAAGGAGTAAGACC-3	20	57.2	157
Reverse	5-CCCCTCTTCAAGGGGTCTAC-3	20	56.7	
HPV33				
Forward	5- CAC AGT TAT GCA CAG AGC TGC-3	21	61.4	321
Reverse	5- CAA CGA GGT AGA A GA AAG CAT C-3	22	62.2	

3.6.5 Polymerase Chain Reaction *PCR* Genotyping of *HPV*

To amplified (4) types of *HPV (16, 18, 31 and 33)*, specific primers were used to detect them in *BC* tissues (inflammatory as control, cancers as cases). Amplification was performed according to *HPV* kit from (Sacace technologies-Casera –Italy). The *PCR* was carried out in a total reaction volume of (40) µl containing between (20) µl mix-1(contained in PCR tubes), (10) µl of mix-2 and (10) µl of *extracted DNA* (sample). Negative control and positive control of high risk *HPV DNA* tubes contained (10) µl of *DNA buffer*, (10) µl of *high risk HPV DNA*. Samples and controls were amplified using Gene Amp *PCR system (9700)* (reagents and primers provided by Sacace technologies-Casera –Italy). The *PCR* protocol was described in **table (2.3)**

Table (2.3): PCR protocols of amplification product for samples.

Primer sequence (5'-3')		Cycling profile	25 µl PCR reaction mixture
HPV16	F: 5-CCACAGGAGCGACCCAGAAAGTT-3 R 5-ACCGGTCCACCGACCCCTTATAT-3	95°C for 5 min 40x 95°C for 45 sec 54°C for 1 min 72°C for 45 sec 72°C for 5 min	12.5 µl master mix 1 µl F.P 1 µl R.P 7.5-9.5 µl NFW 1-3 µl DNA
HPV18	F: 5GCGCGCTTTGAGGATCCAACAC-3 R: 5-TGGCACCGCAGGCACCTTAT-3	95°C for 5 min 40x 95°C for 45 sec 54°C for 1 min 72°C for 45 sec 72°C for 5 min	12.5 µl master mix 1 µl F.P 1 µl R.P 7.5-9.5 µl NFW 1-3 µl DNA
HPV31	5-GGCCTCCAAGGAGTAAGACC-3 5-CCCCTCTTCAAGGGGTCTAC-3	95°C for 5 min 40x 95°C for 1 min 54°C for 1 min 72°C for 1 min 72°C for 5 min	12.5 µl master mix 1 µl F.P 1 µl R.P 7.5-9.5 µl NFW 1-3 µl DNA
HPV33	5- CAC AGT TAT GCA CAG AGC TGC-3 5- CAA CGA GGT AGA AGA AAG CAT C-3	95°C for 5 min 40x 95°C for 1 min 54°C for 1 min 72°C for 1 min 72°C for 5 min	12.5 µl master mix 1 µl F.P 1 µl R.P 7.5-9.5 µl NFW 1-3 µl DNA

3.7 Agarose Gel-electrophoresis:

The *PCR* products were visualized in (1.5%) Agarose gel with (0.5) µg/ml Ethidium bromide staining, the gel was prepared by dissolving (0.7) gm of agarose powder in (35) ml of 1X TBE buffer and heated at (65)°C 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 (1000) bp *DNA ladder* and *PCR* product was loaded on the gel. Gel-electrophoresis was performed at (120V) and (36 Am) for (60) minutes. Pictures were taken by Gel documentation system (Gel mega, digital camera and software in a computer).

3.8 Interpretation of PCR results:

According to manufacture *high risk HPV* kit (from *Sacace technologies- Casera – Italy*) manual, the *PCR* product length for (*HPV16*) should be (457) bp, *HPV 18* should be (322) bp, *HPV 31* should be (452) bp and *HPV 33* should be (398) bp to be visualized after staining with ethidium bromide.

3.9 Ethical Considerations:

Ethical clearance to conduct this study has been obtained from the Ethics Committee of the Ministry of health, Khartoum state; Initial consent will be obtained from authorities of the study areas.

3.10 Data Analysis:

1. Deceptive analysis type
2. Analytical data analyses are used in this study.

   The data will be cleaned and checked for consistency before entering it for analysis. Analysis will be conducted using Epi Info Version (6) (The Epi Info computer programs produced by *CDC* and the World Health Organization. *(WHO)* provide public-domain software for word processing, database management, and statistics work in public health) the alpha (α) level of significance will be set at (0.05). Descriptive analysis will be done by using ***(Chi square test)***.

Chapter Four

Results

4.1 PCR results of *HPV and genotypes:*

Table (1.4) Distribution of *HPV* infection with *BC* types*:*

Type of breast cancer	No of sample	Positive sample	Negative sample	p.value
Invasive ductal carcinoma	41(29%)	23 (16.1%)	18(12.9%)	0.00022
Invasive lobular carcinoma	21(15%)	14(9.8%)	7(5%)	0.000016
invasive micropapillary carcinoma	12(8%)	9(6.3%)	3(2.1%)	0.000095
Medullary carcinoma	26(18%)	11(7.7%)	15(10.5%)	0.098
Inflammatory breast Sample	43(30%)	10(7%)	33(23.1%)	
Total	**143**	**67(49.9%)**	**76(53.6%)**	
P=0.00022				

In above table invasive ductal carcinoma is more common among each type of breast cancer in relation to HPV infection **table (1.4)**

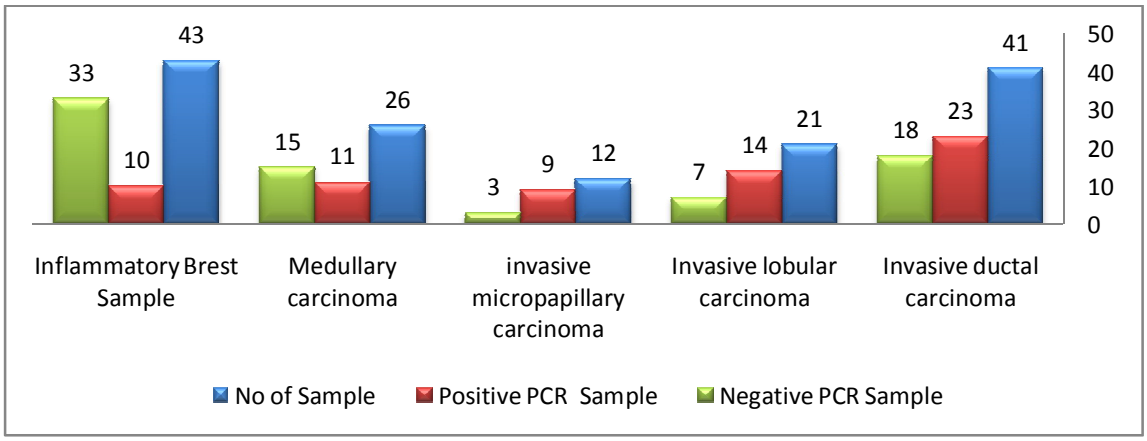

Figure (1.4) Description of *HPV* infection with *BC* types:

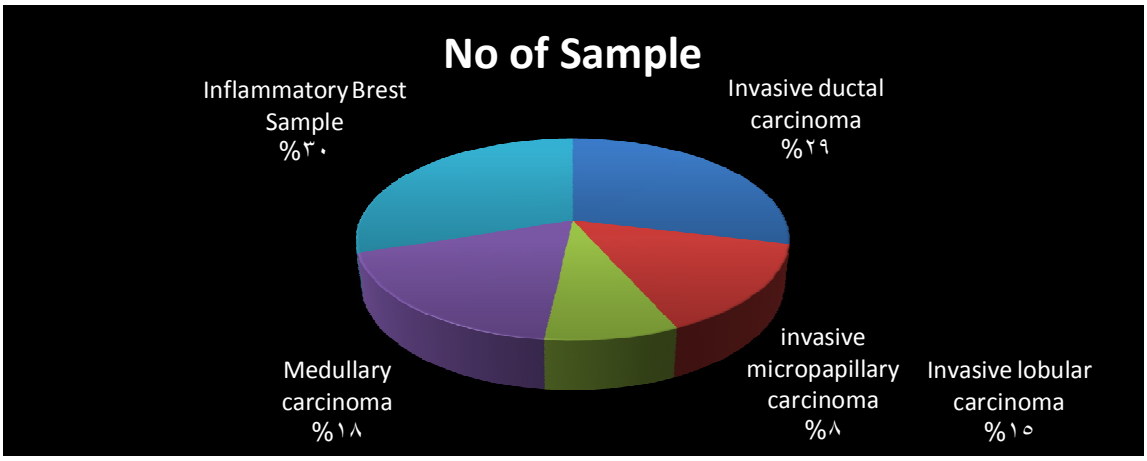

Figure (2.4) Description of *HPV* infection with *BC* types:

Table (2.4) Distribution of *HPV* genotypes in BC patients*:*

PCR result of HPV	No of positive Sample	%
HPV genotype 16	22	33%
HPV genotype 18	21	31%
HPV genotype 31	10	15%
HPV genotype 33	14	21%
Total of Sample	67	100%

The above table shows that HPV genotype 16 is most prevalent in breast cancer specimens.

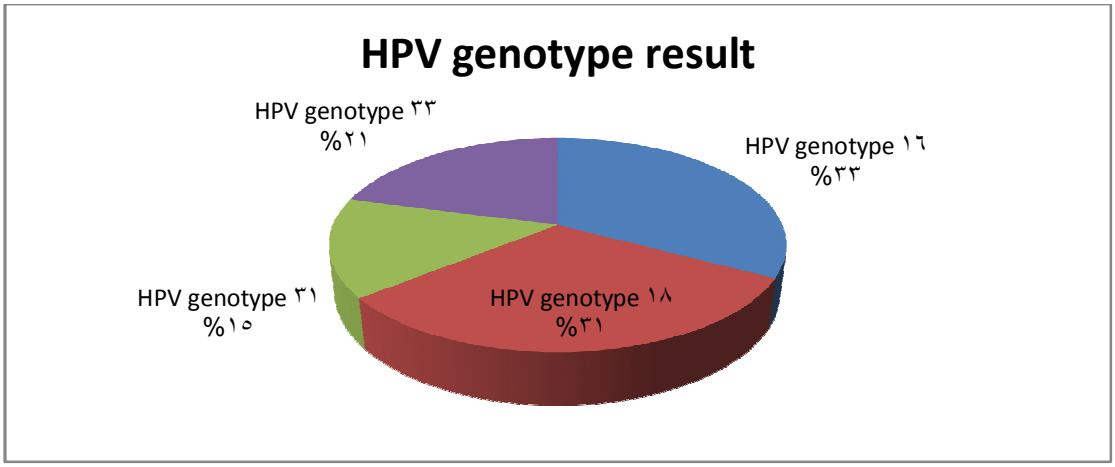

Figure (3.4) Description of *HPV* infection with genotypes*:*

Table (3.4) Distributions of HPV genotypes with different histopathological types of BC:

Type of breast cancer	*HPV* genotype			
	16	18	31	33
Invasive ductal carcinoma	13(54%)	9(45%)	0(0%)	2(14%)
Invasive lobular carcinoma	5(25%)	6(30%)	0(0%)	2(14%)
invasive micropapillary carcinoma	0(0%)	0(0%)	(0%)0	9(65%)
Medullary carcinoma	5(25%)	5(25%)	(0%)0	1(7%)
Inflammatory breast Sample	0	0	10(100%)	0

In this table HPV genotype 16, 18 are more dominant in breast cancer specimen when compares with inflammatory breast conditions as control group **table (3.4).**

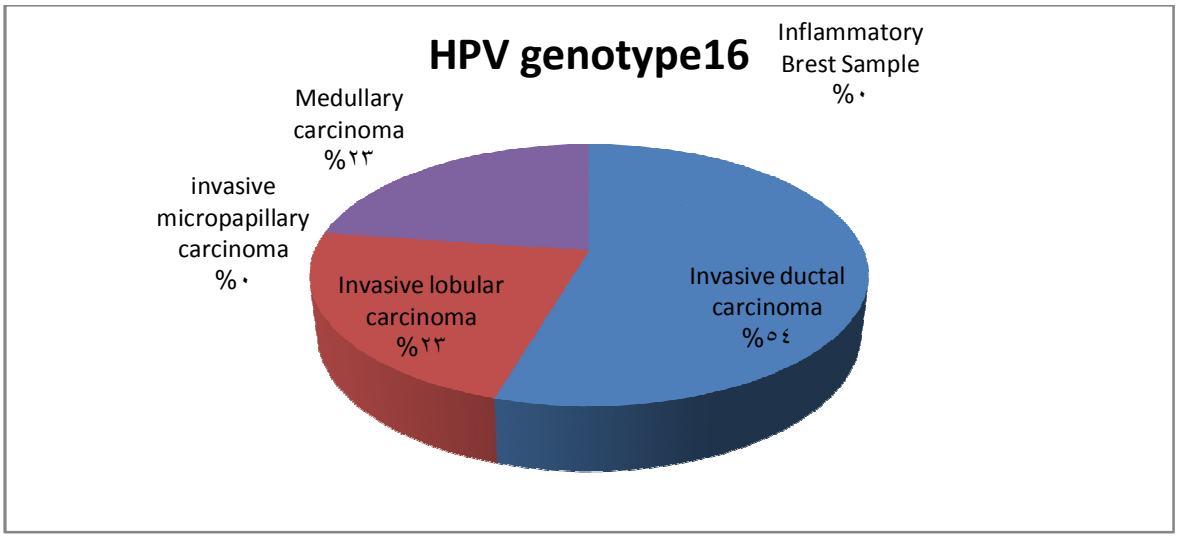

Figure (4.4) Description of *BC* types by *HPV* genotype 16

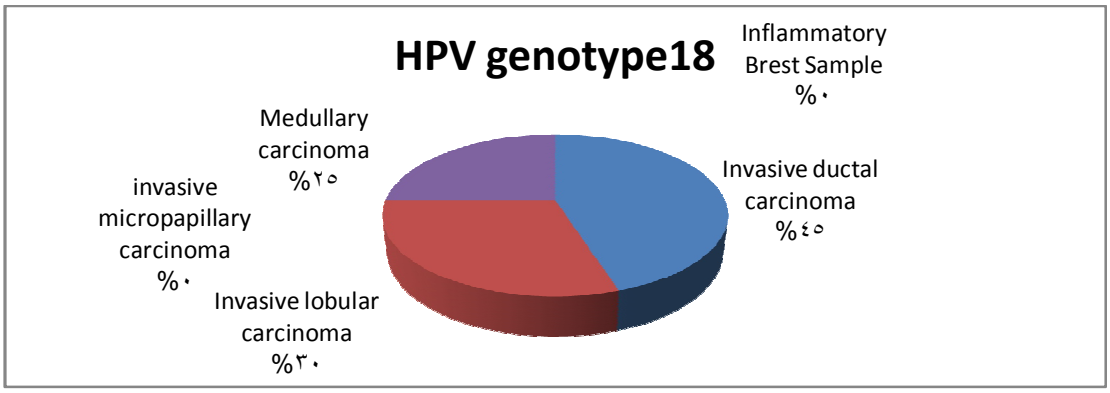

Figure (5.4) Description of *BC* types by *(HPV)* genotype 18:

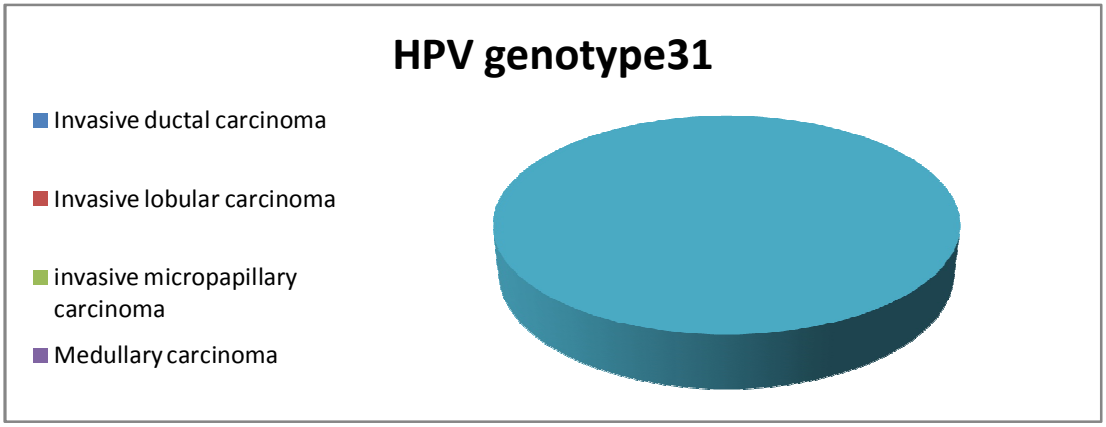

Figure (6.4) Description of *BC* type by *HPV* genotypes 31

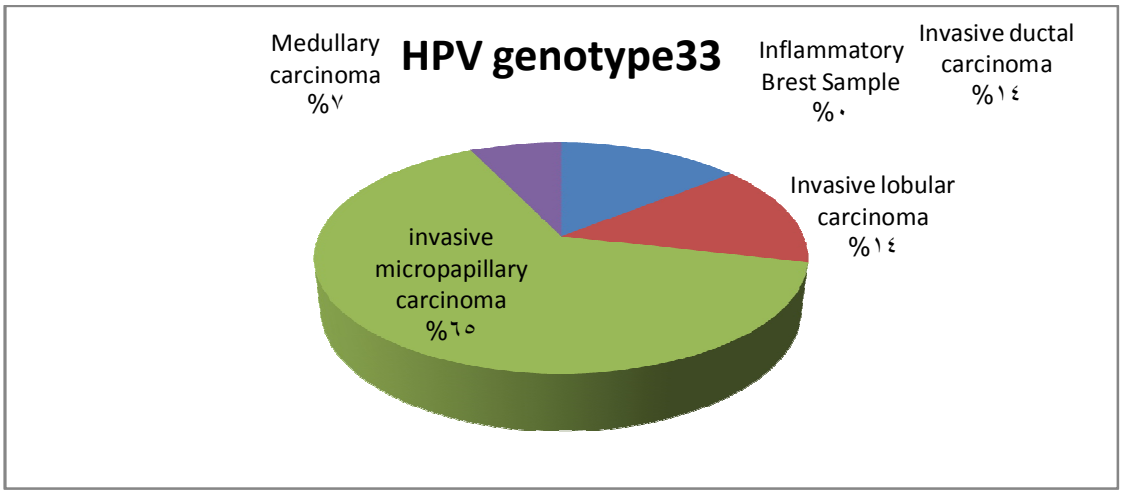

Figure (7.4) Description of *BC* type by *HPV* genotypes 33

Table (4.4) Distribution of *HPV* infections in BC patients with reference to age group*:*

Age group /years	*HPV* infection		
	Positive	**Negative**	**Total**
(31-40)	13(88.81%)	13(5.6%)	**26**
(41-50)	36(63.16%)	23(53.5%)	**59**
Over (50)	8(14.04%)	7(16.28%)	**15**
Total	**57**	**43**	**100**
Chi surges P= 0.0012			

HPV infection is more common among age group (41-50) this indicates there is strong relation between age and HPV and this math with literature **table (4.4).**

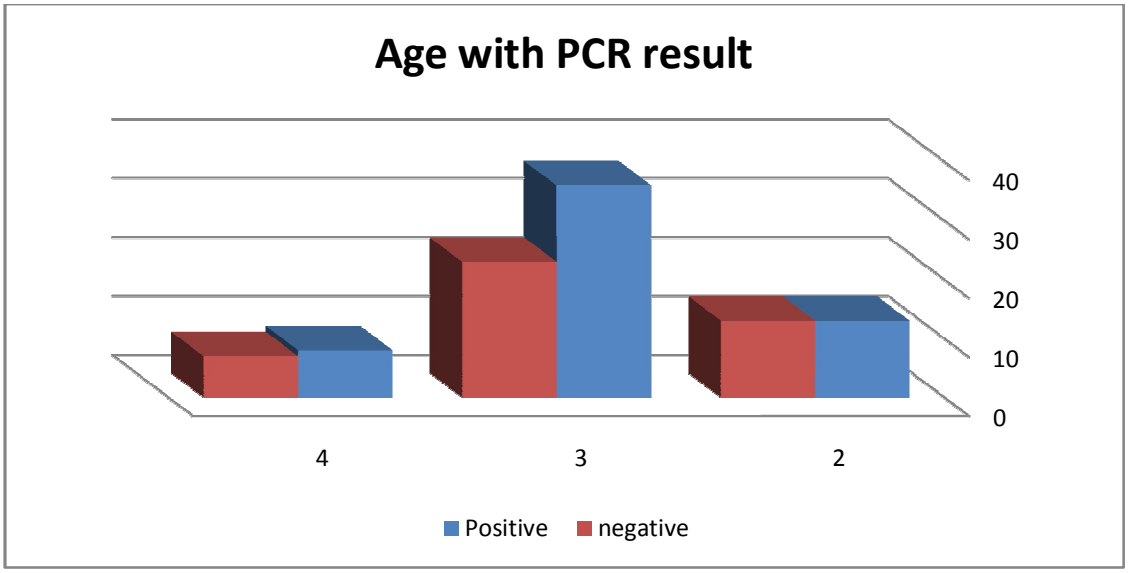

Figure (8.4) Description of *HPV* infection with age group*:*

Table (5.4) Distribution of *HPV* infection in relation to stage of *BC:*

PCR result of *(HPV)*	Stage of breast cancer			
	Stage 0	**Stage I**	**Stage II**	**Total**
Positive	10(17.5%)	18(31.6%)	29(50.9%)	**57**
Negative	11(25.6%)	11(25.6%)	21(48.8%)	**43**
Total	**21**	**29**	**50**	**100**
P = 0.5864 (Chi square)				

In this table HPV infection is prevails in stage II 29(50.9%) than other breast cancer stages this explained the relation between HPV infections and the chronicty of the disease **table (5.4)**

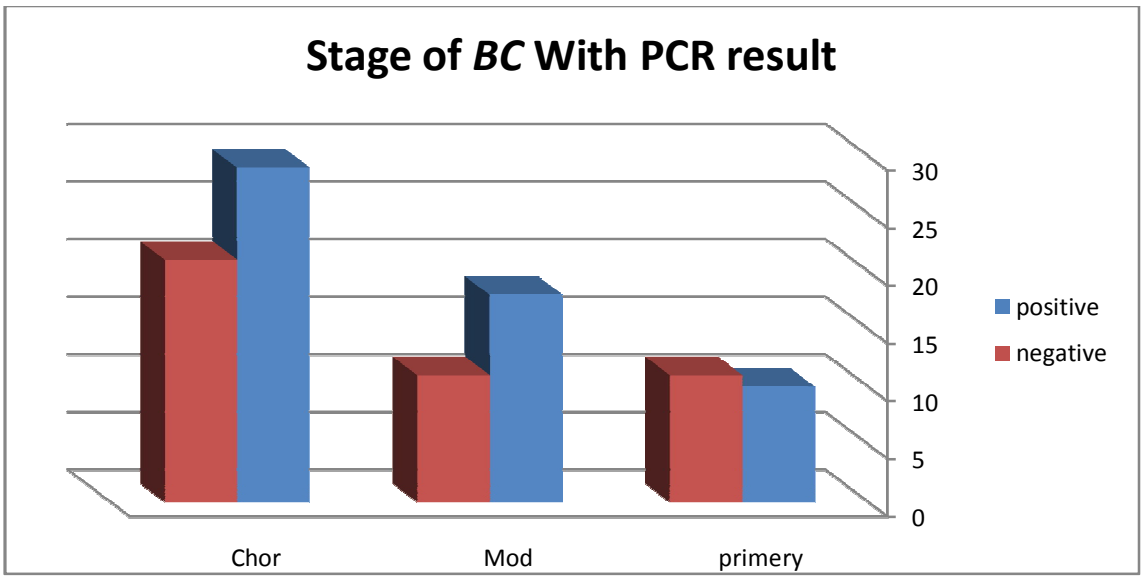

Figure (9.4) Description of *HPV* infection with the stage of *BC*:

Table (6.4) Distribution of *HPV* infection in relation to menstrual cycle*:*

PCR result of *HPV*	menarche			
	Early	**Normal**	**Late**	**Total**
Positive	7(12.3%)	21(36.8%)	29(50.9%)	57
Negative	4	24	15	43
Total	**11**	**45**	**44**	**100**
(Chi square=0.1207) P= 0.1697				

The above table shows the positive HPV infection cases are common in late menarche patients**.**

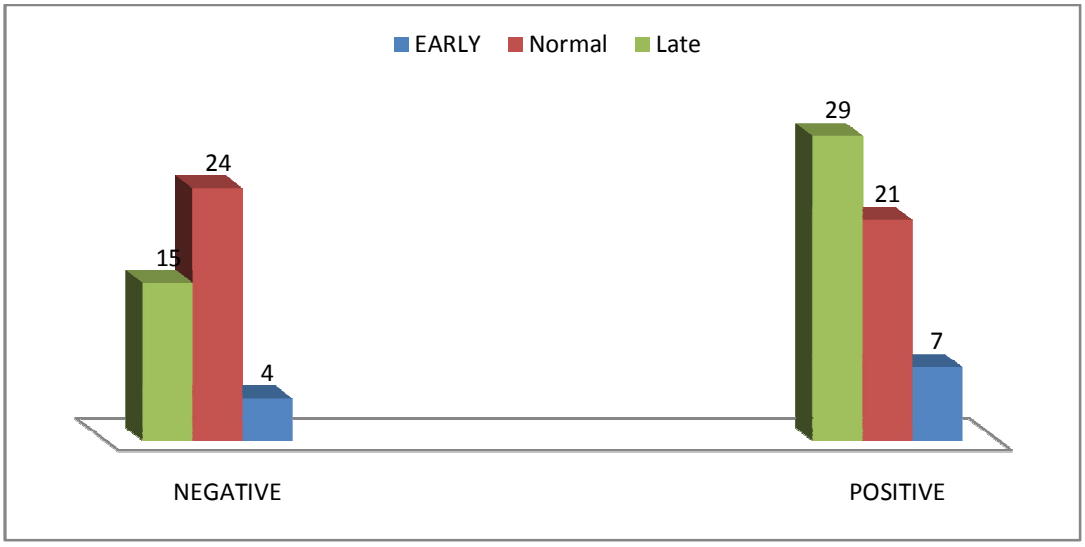

Figure (10.4) Description of *HPV* infection with menstrual period*:*

Table (7.4) Distribution of *HPV* infection with practicing of exercises*:*

PCR result of *HPV*	Practicing of exercises		
	Yes	**No**	**Total**
Positive	3(5.3%)	54(54.7%)	57
Negative	3	40	43
Total	**6**	**94**	**100**
(Chi square =0.1263)P=07223			

Table (7.4) shows that HPV is more common in no exercises practicing breast cancer patient.

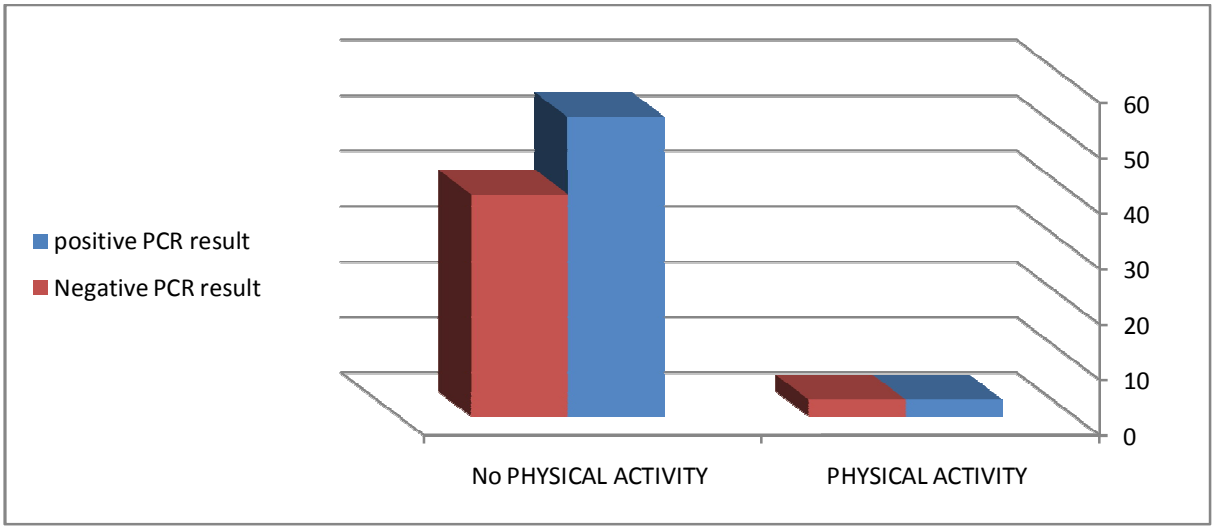

Figure (11.4) Description of HPV infection with exercise:

Table (8.4) Distribution of *HPV* infection with the duration of illness:

PCR result of *HPV*	Duration of illness in years			
	(1-2)	(3-4)	(5-6)	Total
Positive	23(40.4%)	23(40.4%)	11(19.2%)	57
Negative	15	23	5	43
Total	**38**	**46**	**16**	**100**
(Chi square3.234)		**P= 0.034**		

The above table shows that the duration of illness does not increase the risk of infection with HPV in BC patients.

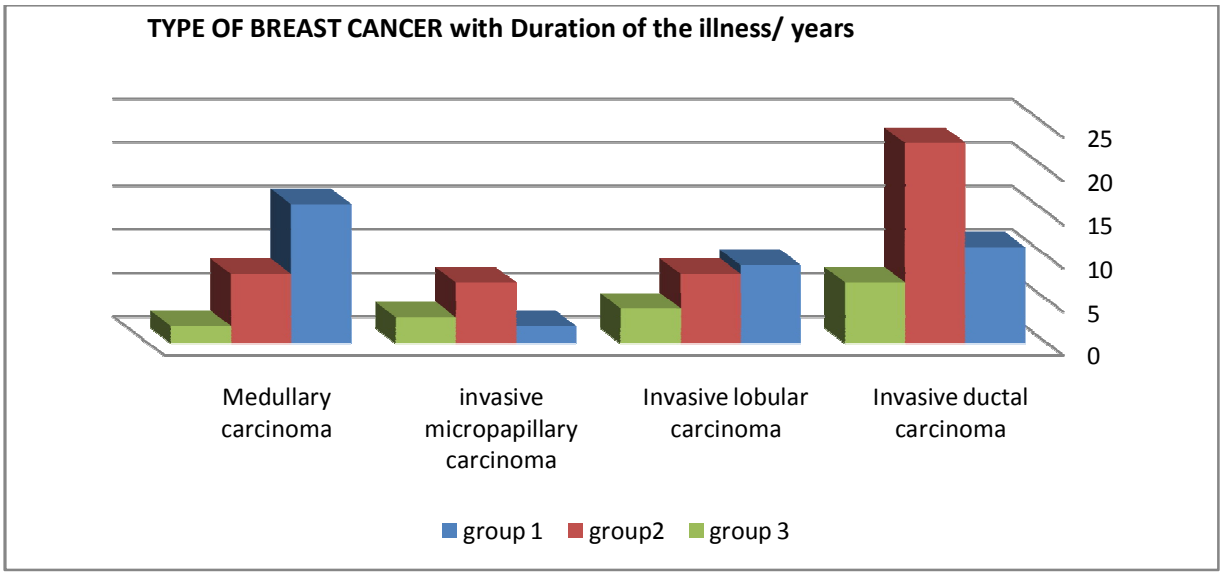

Figure (12.4) Description of *HPV* infection with the duration of illness:

Table (9.4) Distribution of *HPV* infection in relation to lymph nodes involvement:

PCR result of *HPV*	Lymph nodes involvement		
	Yes	**No**	**Total**
Positive	57(100%)	0(0%)	57
Negative	42	1	43
Total	**99**	**1**	**100**
(Chi square=1.3256) P=0.2496			

The above table shows the infected BC individual with HPV have more advance disease than others without infection.

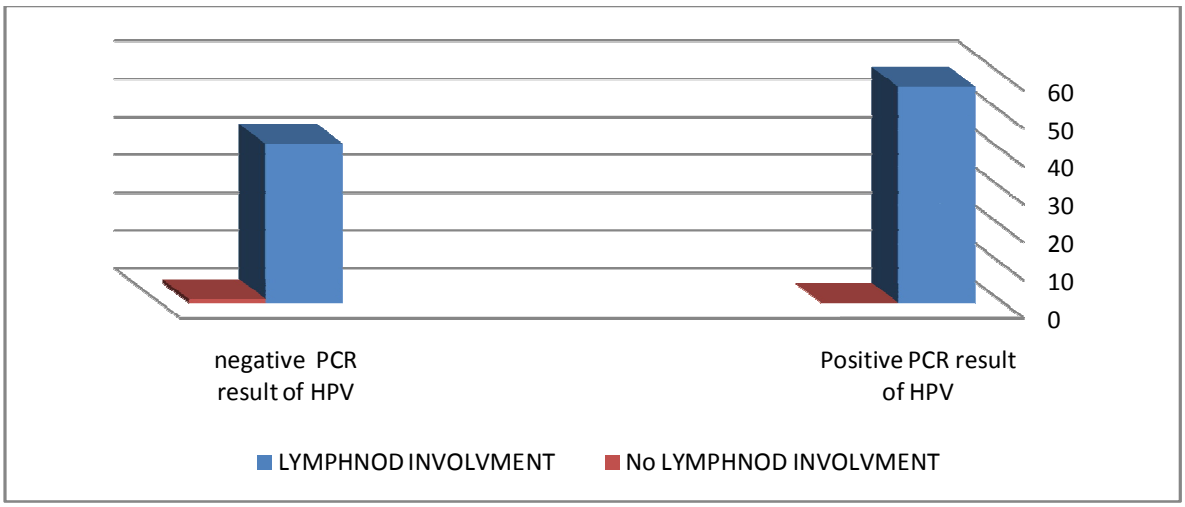

Figure (13.4) Description of HPV infection with the lymphoid involvement:

Table (10.4) Distribution of *HPV* infection in BC patients in relation family history of the disease*:*

PCR result of *(HPV)*	Family history of BC		
	Yes	**No**	**Total**
Positive	23 (60.5%)	34 (54.8%)	57 (57.0%)
Negative	15 (39.5%)	28 (45.2%)	43 (43.0%
Total	**38**	**62**	**100**
Chi square=.3078. P=0.5790			

This **table (10.4)** shows the no familiar susceptible to get infection with HPV

Table (11.4) Distribution of *HPV* infection with marital status*:*

PCR result of *HPV*	Marital status			
	Married	**Single**	**Divorce**	**Total**
Positive	6(10.5%)	31(54.4%)	20(35.1%)	57
Negative	8	23	12	43
Total	**14**	**54**	**32**	**100**
(Chi square=1.5257) P=0.4663				

The above shows no obvious relationship to marital status.

Table (12.4) Distribution of *HPV* infection in some socio-economic status*:*

PCR result of *HPV*	socio-economic status				
	House wife	**Student**	**Employee**	**Others**	**Total**
Positive	25(43.9%)	2(3.5%)	14(24.6%)	16(28.1%)	57
Negative	16	1	12	14	43
Total	**41**	**3**	**26**	**30**	**100**
	P= 0.8900				

Table (12.4) house wives girls 25(43.9%) more infected with HPV it may be due to low socio-economic status.

Table (13.4) Distribution of *HPV* infection in relation to breast tissue consistency*:*

PCR result of *HPV*	breast tissue consistency			
	Fatty	**Fibrous**	**Granular**	**Total**
Positive	25(43.9%)	7(12.3%)	25(43.9%)	57
Negative	22	7	14	43
Total	**47**	**14**	**39**	**100**
P=0.5099				

HPV infection is found in each types of breast tissue with atropism to fatty 25(43.9%) and granular 25(43.9%) than fibrous 7(123%) tissue **table (13.4)**

Table (14.4) Distribution of *HPV* infection with body weight*:*

PCR result of HPV	Body weight			
	Obese	**Normal**	**Underweight**	**Total**
Positive	42(73.7%)	9(15.8%)	6(10.5%)	57
Negative	6	30	7	43
Total	**15**	**72**	**13**	**100**
(Chi square=.7239) P= 0.6963				

The above **table (14.4)** shows the relations between body weight and HPV infection whereas HPV infection is more common among obese patients 42(73.7%) than others body weight.

Table (15.4) Distribution of *HPV* infection with family planning*:*

PCR result of *HPV*	family planning			
	Contraceptive	**Natural control**	**No control**	**Total**
Positive	22(39.0%)	5(8.8%)	30(52.6%)	57
Negative	15	2	26	43
Total	37	7	56	100
(Chi square=.9449) P=0.6235				

The above table shows no afferent differences between family planning procedures.

Table (16.4) Distribution of *HPV* infection with the previous exposure to radiations*:*

PCR result of *HPV*	Previous exposure to radiation		
	Yes	**No**	**Total**
Positive	13(22.8%)	44(77.2%)	57
Negative	14	29	43
Total	**27**	**73**	**100**
(Chi square=1.1706) P=0.2793			

This **table (16.4)** explains the relation between the previous exposures to radiation with HPV infection whereas more common in no previous exposure to radiation than other one and this indicated the association of HPV with breast cancer.

Table (17.4) Distributions of *HPV* infection with metastasis *BC:*

PCR result of *HPV*	Metastasis breast cancer		
	Yes	**No**	**Total**
Positive	39(22.2%)	18(31.5%)	57
Negative	28	15	43
Total	**67**	**33**	**100**
(Chi square=.1199) P=0.7292			

The above **table (17.4)** found that in metastasis breast cancer patient HPV is more common.

The relations between *BC* and associated risk factor:

Table (18.4) Distribution of *BC* types with mean age:

Type of breast cancer	Obs	Mean age	Variance	Std Dev
Invasive ductal carcinoma	41	3.0976	.3902	.6247
Invasive lobular carcinoma	21	3.0000	.3158	.5620
Invasive micropapillary carcinoma	12	2.9167	.2652	.5149
Medullary carcinoma	26	2.4615	.3385	.5818
P= 0.0012				

This **table (18.4)** find out the statistical significant between breast cancers and the mean age of the patients.

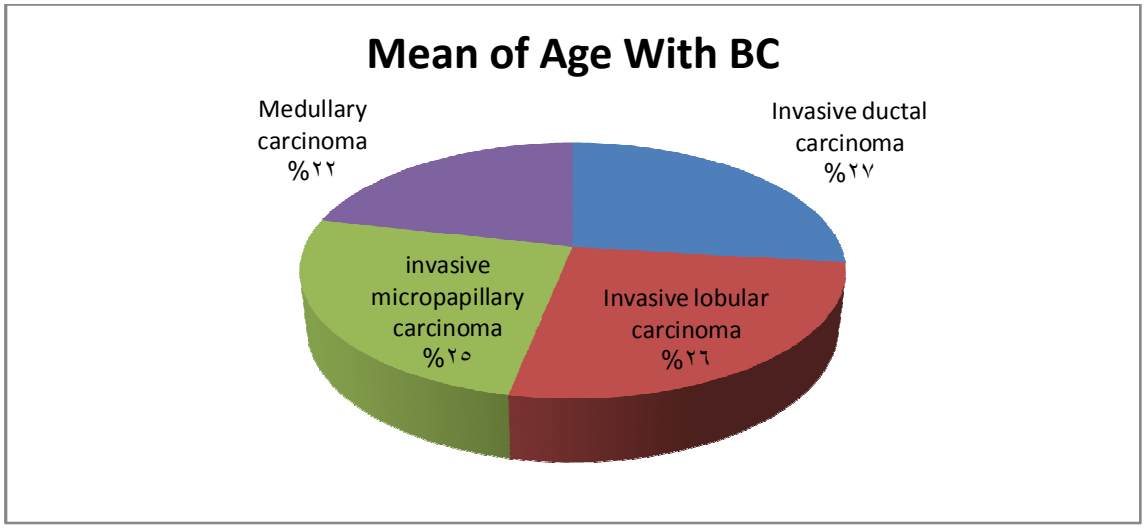

Figure (14.4) Description the mean age with *BC* types

Table (19.4) Distribution of lymphoid involvement with the stage of *BC*:

Stage of breast cancer	Lymphoid involvement		
	Yes	No	Total
Stage 0	21(21%)	0(0%)	21
Stage I	29(29%)	0(0%)	29
Stage II	49(49%)	1(2%)	50
Total	**99**	**1**	**100**
(Chi square =0.8914) P= 0.3451			

This table shows the relation between stages of breast cancer and lymphoid involvement; whereas in Stage II there is more lymphoid involvement positive cases **table (19.4)**

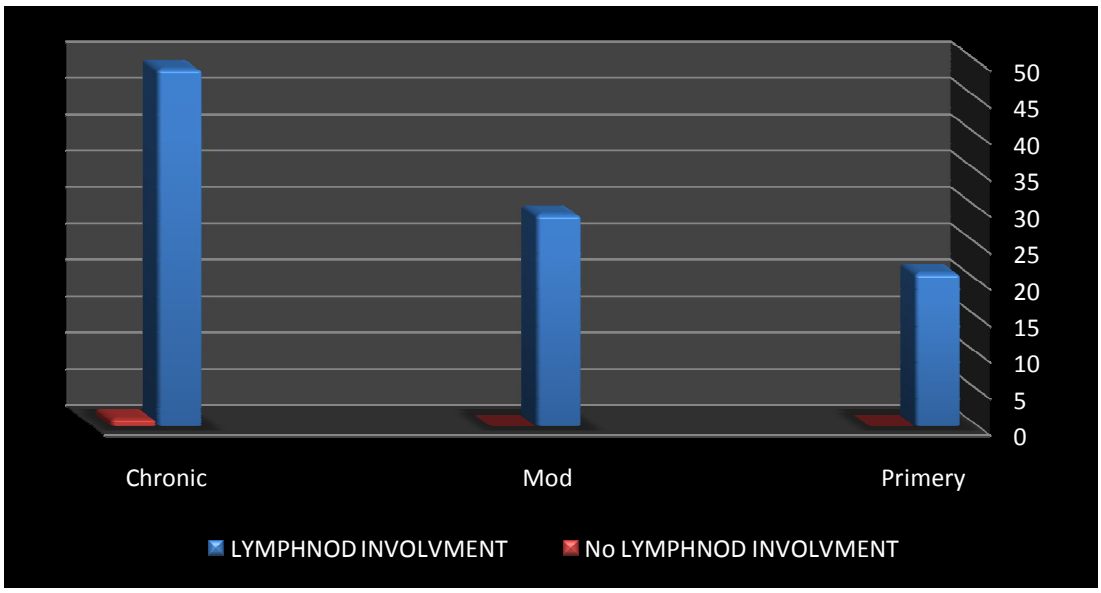

Figure (15.4) Description the lymphoid involvement with *BC* stages:

Table (20.4) Distribution of *BC* types by breast feeding:

Breast feeding criteria	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
less than 1 year	26(37.7%)	11(15.9%)	9(13.04%)	23(33.3%)	69
One year	12(46.2%)	8(30.8%)	2(7.7%)	3(11.5%)	26
More than one year	3(60%)	1(20%)	1(20%)	0(0%)	5
Total	**41**	**20**	**12**	**26**	**100**
(Chi square= 9.0052) P= 0.0430					

This table shows the relation between the types of breast cancer and breast feeding and there is high incidence rate of each breast cancer types in less breast feeding patients **table (20.4).**

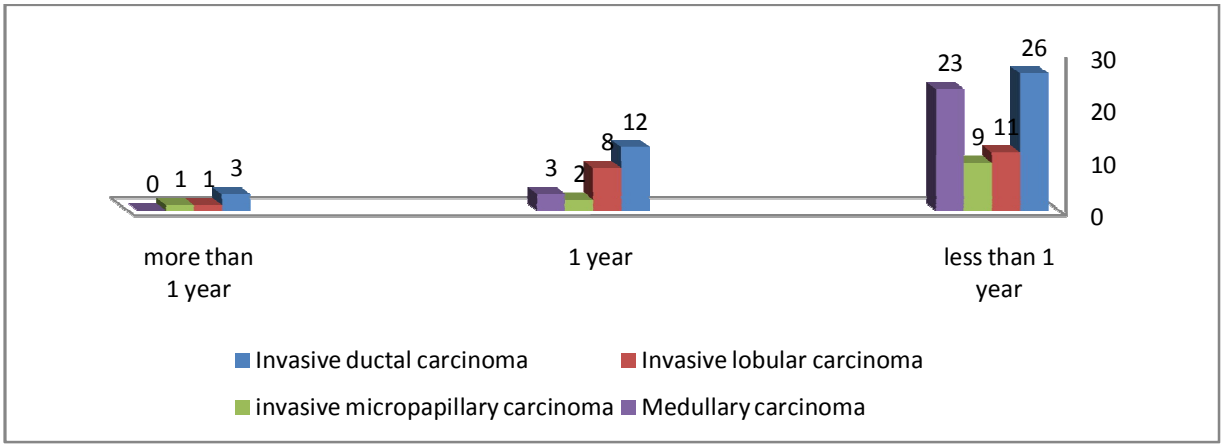

Figure (16.4) Description of *BC* type with breast feeding:

Table (21.4) Distributions of *BC* types by menstrual period:

Menstrual period	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Early	1(9.1%)	3(27.3%)	2(18.2%)	5(45.5%)	11
Normal	21(46.7%)	7(15.6%)	5(11.1%)	12(26.7%)	45
Late	19(43.18%)	10(22.7%)	5(11.4%)	9(20.5%)	44
Total	**41**	**20**	**12**	**26**	**100**
(Chi square =3.5995) **P=0.4629**					

The above table describing the relationship between menstrual period and breast cancer types whereas there is high percentage of each types of breast cancer in late menstrual period patients **table (21.4)**

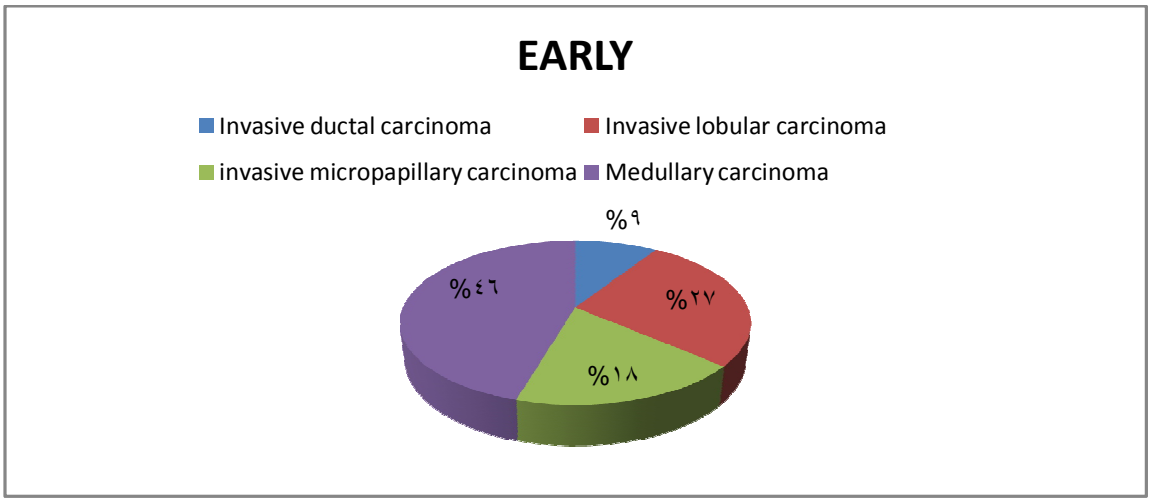

Figure (17.4) Description of *BC* types with menstrual period:

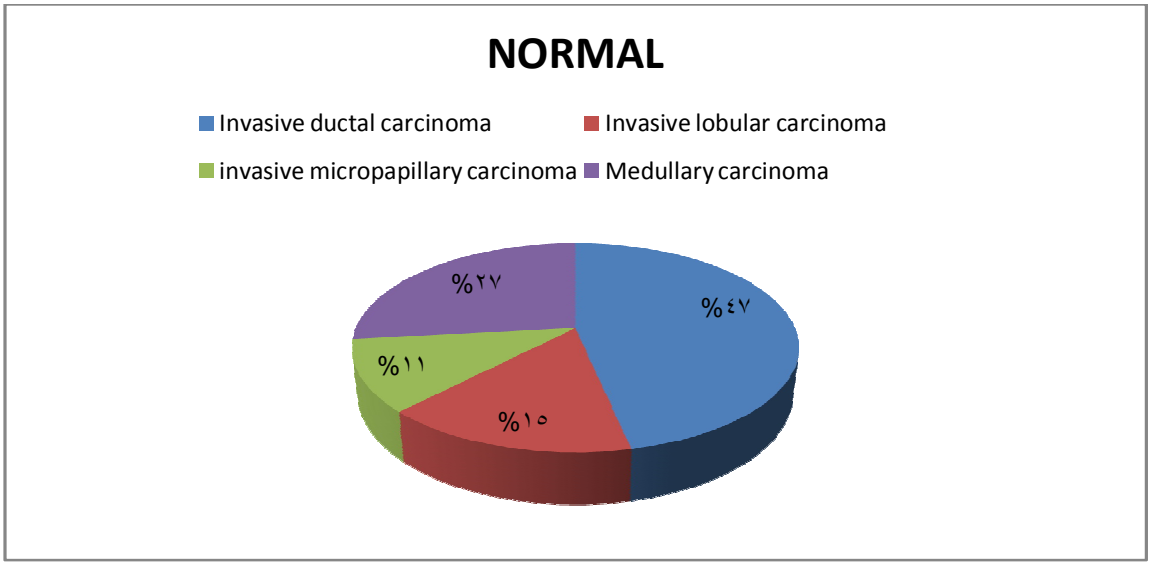

Figure (18.4) Description of *BC* type with menstrual period:

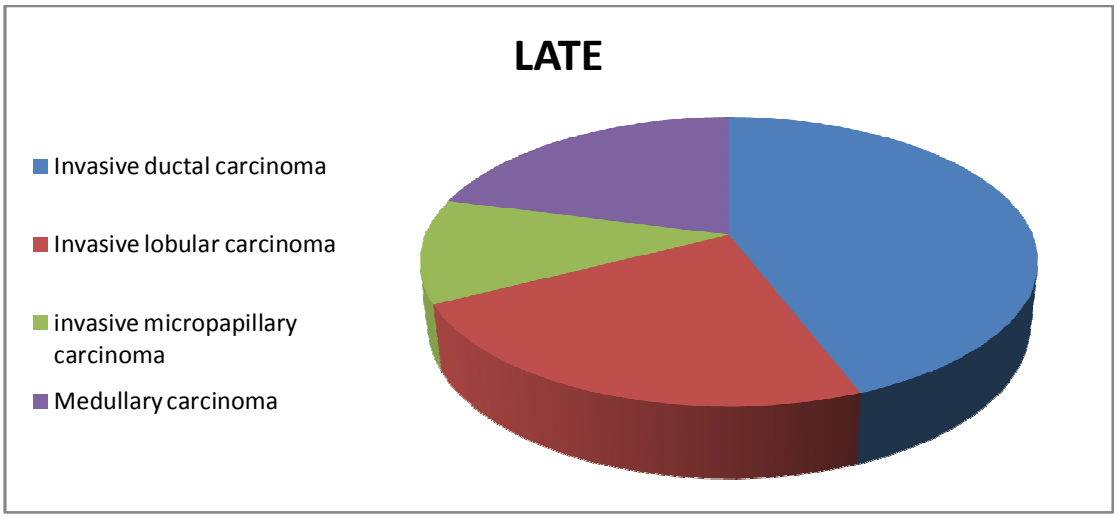

Figure (19.4) Description of *BC* types with menstrual period:

Table (22.4) Distribution of *BC* types with lymphoid involvement:

Lymphoid involvement	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	Invasive micro papillary carcinoma	Medullary carcinoma	total
Yes	41(41.4%)	21(21.2%)	12(12.1%)	25(25.3%)	99
No	0	0	0	1	1
Total	**41**	**21**	**12**	**26**	**100**
(Chi square =2.8462) P=0.5839					

In the above table invasive ductal carcinoma is more common among patient with lymphoid involvement than other breast cancer types **table (22.4).**

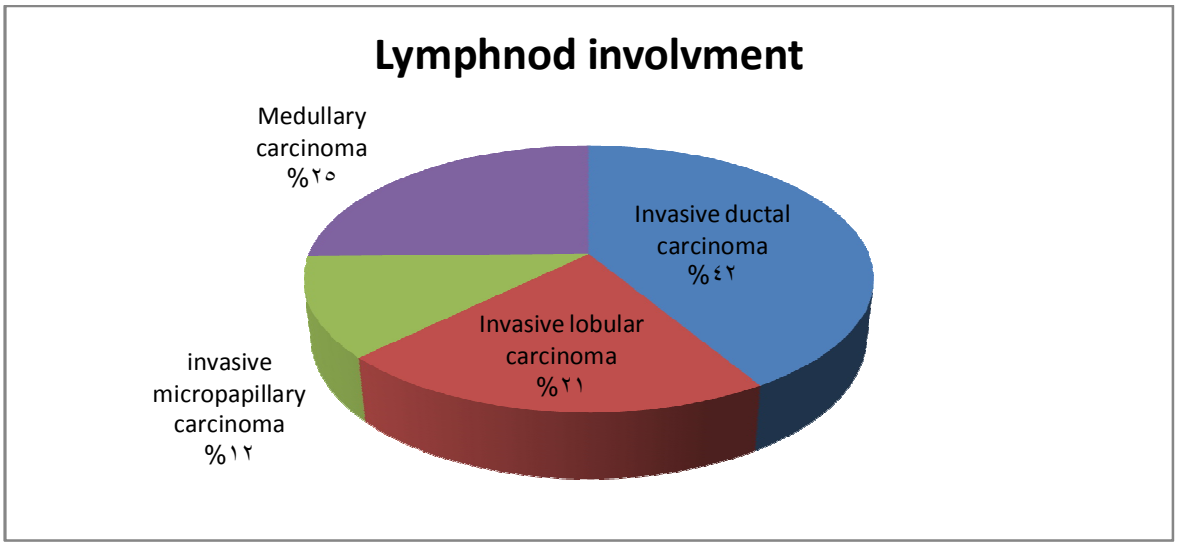

Figure (20.4) Description of *BC* types with lymphoid involvement

Table (23.4) Distribution of *BC* stages with exercises practicing:

Stage of breast cancer	Exercises practicing		
	Yes	No	Total
Stage 0	6(28.6%)	15(71.4%)	21
Stage I	2(10%)	18(90%)	20
Stage II	1(1.7%)	58(98.3%)	59
Total	**9**	**91**	**100**
(Chi square = 9.2368) P=0.0024			

In this table high incidence of breast cancer found in no exercises practicing patients table **(23.4).**

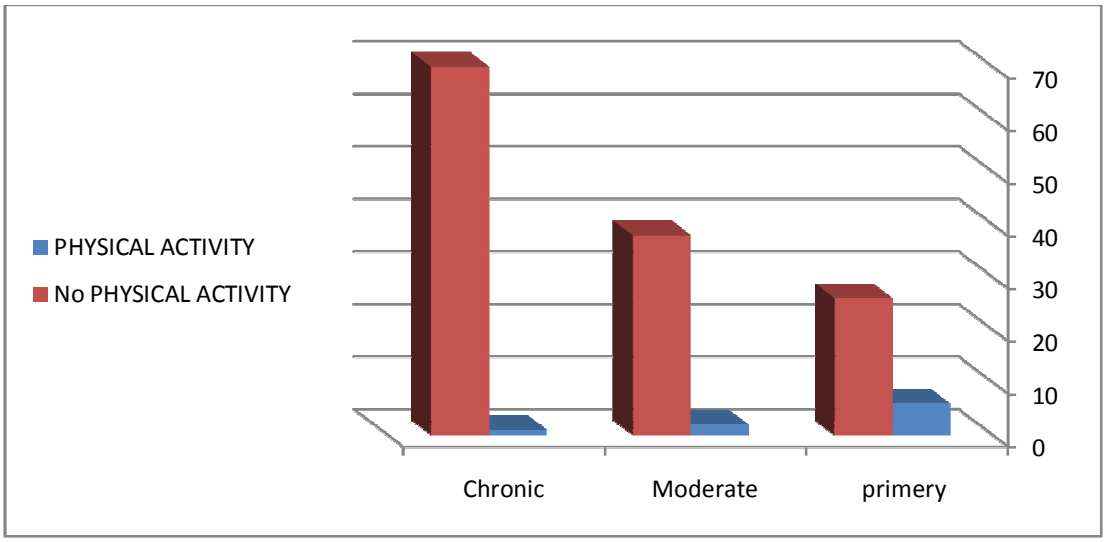

Figure (21.4) Description of Exercises practicing with the *BC* stages:

Table (24.4) Distribution of *BC* types by the duration of illness in years:

Duration of illness/ years	Type of the breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
(1-2)	11(28.9%)	9 (23.7%)	2(5.3%)	16 (42.1%)	38
(3-4)	23(50%)	8(17.4%)	7(15.2%)	8(17.4%)	46
(5-6)	7(43.8%)	4(25%)	3(18.8%)	2(12.5%)	16
Total	**41**	**21**	**12**	**26**	**100**
P= 0.0367 (T .test)		**(Chi square=10.8332) P=0.0285**			

The above table explains the relation between duration of illness and types of breast cancer, invasive ductal carcinoma 23(50%) is more common **table (24.4)**

Table (25.4) Distribution of *BC* types by family history:

Family history	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Yes	10(26.3%)	9(23.7%)	4(10.5%)	15(39.5%)	38
No	31(50%)	12(19.4%)	8(12.9%)	11(2%)	62
Total	**41**	**21**	**12**	**26**	**100**
(Chi square=8.5562) P=0.0432					

This table shows the relations between family history and breast cancer **table (25.4).**

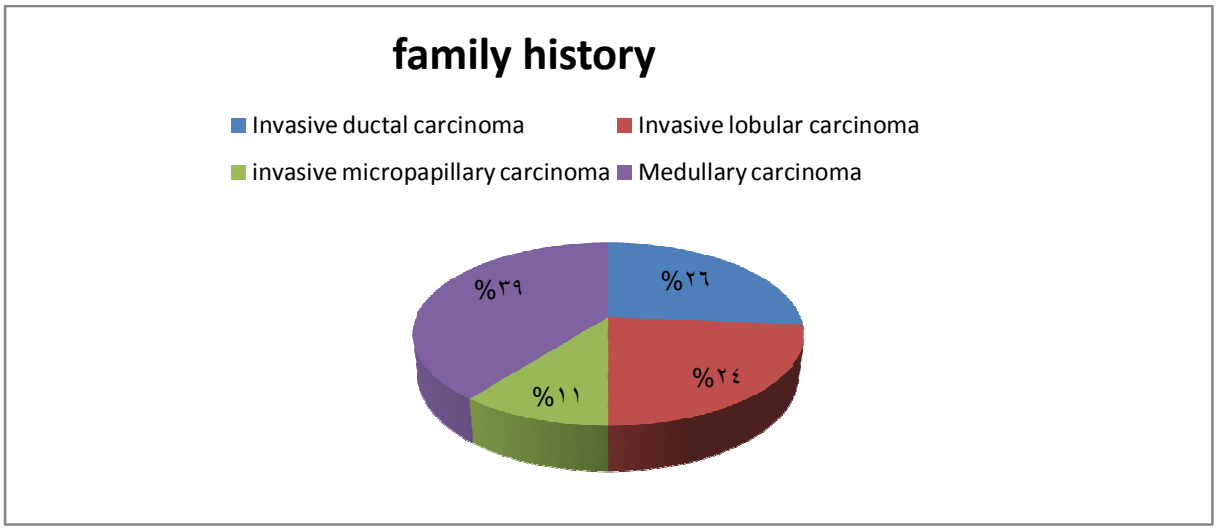

Figure (22.4) Description of *BC* types with family history:

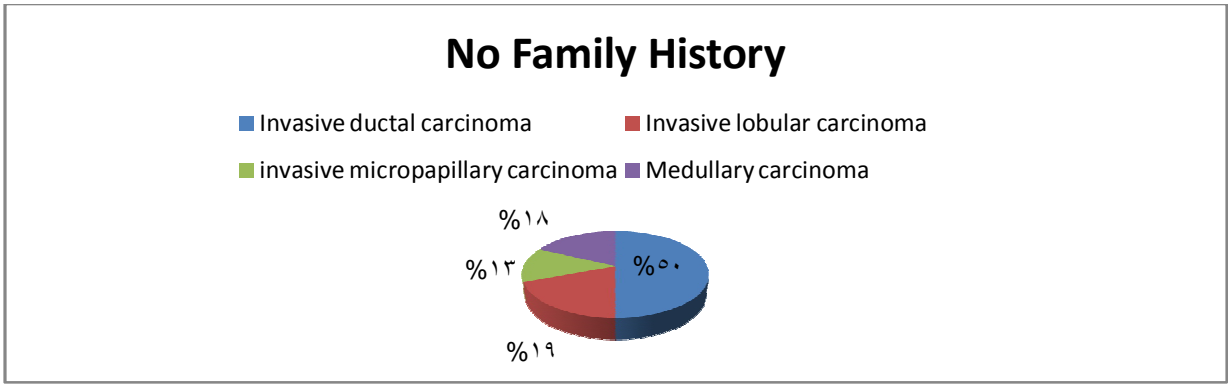

Figure (23.4) Description of *BC* types with no family history:

Table (26.4) Distribution of *BC* types with marital status:

Marital status	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Single	21(38.9%)	10(18.5%)	7(13.0%)	16(29.6%)	54
Married	2(14.3%)	4(28.6%)	0(0%)	8(57.1%)	14
Divorce	18(56.3%)	7(21.9%)	5(15.6%)	2(6.3%)	32
Total	**41**	**21**	**12**	**26**	**100**
(Chi square= 17.4189) P=0.0016					

The above table describing the relation between marital status and types of breast cancer, higher incidence rate of Invasive ductal carcinoma found in single patient than other marital status **table (26.4).**

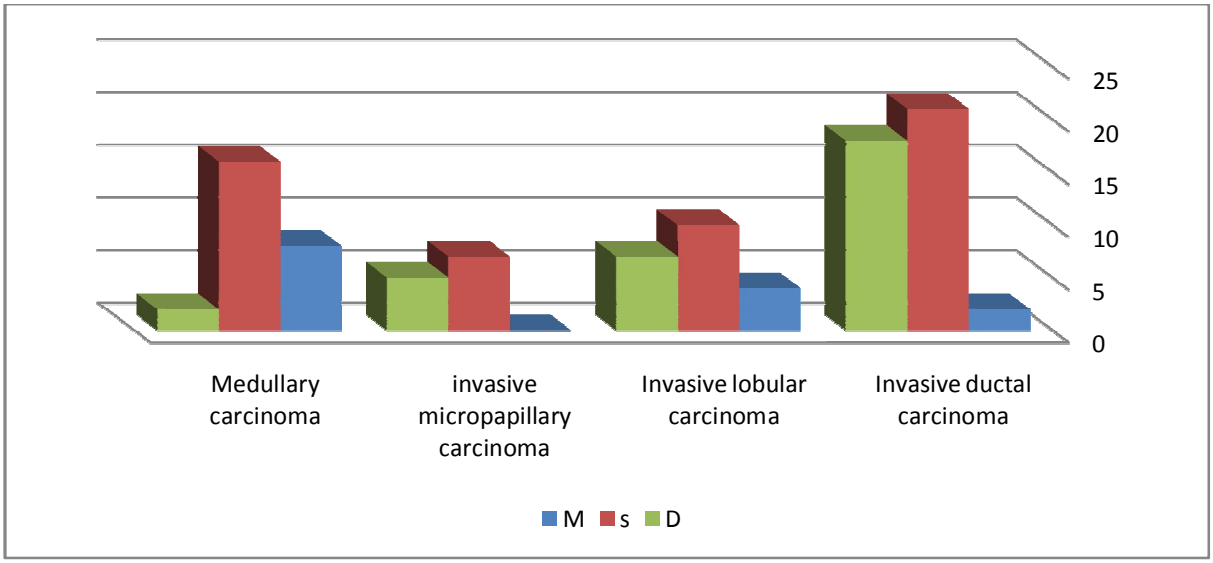

Figure (24.4) Description of *BC* types with marital status:

Table (27.4) Distribution of *BC* types by socio-economic status:

socio-economic	Type of breast				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
House wife	17(41.5%)	6(14.6%)	6(14.5%)	12(29.3%)	41
Employee	11(42.3%)	6(23.1%)	2(7.7%)	7(26.9%)	26
Student	1(33.3%)	1(33.3%)	0(0%)	1(33.3%)	3
Others	12(40%)	8(26.7%)	4(13.3%)	6(20%)	30
Total	**41**	**21**	**12**	**26**	**100**
(Chi square= 21.2483) P=0.5661					

This table is explain the relation between socio-economic status and types of breast cancer and Invasive ductal carcinoma as a type of breast cancer is prevails in house wives than others **table (27.4)**

Table (28.4) Distribution of *BC* types with dense of breast tissue:

Dense of breast tissue	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Fatty	24(51.1%)	9(19.1%)	1(2.1%)	13(27.7%)	47
Fibrous	4(28.6%)	1(7.1%)	3(21.4%)	6(42.9%)	14
Granular	13(33.3%)	11(28.2%)	8(20.5%)	7(17.9%)	39
Total	**41**	**21**	**12**	**26**	**100**
(Chi square=10.7716) P=0.0293					

The above table describing the relationship between dense of breast tissue and breast cancer types, higher incidence rate is found in fatty and granular than fibrous breast tissue **table (28.4)**

Table (29.4) Distribution of *BC* types with body weight:

Weight	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Normal	4(26.7%)	3(20%)	2(13.3%)	6(40%)	15
Obese	33(45.8%)	11(15.3%)	8(11.1%)	20(27.8%)	72
Underweight	4(30.8%)	7(53.9%)	2(15.4%)	0(0%)	13
Total	**41**	**21**	**12**	**26**	**100**
(Chi square =7.4878) P=0.1122					

Table (29.4) is shows the relationship between body weight and breast cancer types, invasive ductal carcinoma and other types of breast cancer is more common in obese patients.

Table (30.4) Distribution of *BC* types with family planning:

family planning	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Contraceptive	17(46.0%)	4(10.8%)	2(5.4%)	14(37.8%)	37
Natural control	3(42.9%)	2(28.8%)	2(28.8%)	0(0%)	7
No family planning	21(37.5%)	15(26.8%)	8(14.0%)	12(21.4%)	56
Total	**41**	**21**	**12**	**26**	**100**
(Chi square=6.1559) P=0.1878					

The above table is explains the relation between family planning and breast cancer **table (30.4)**

Table (31.4) Distribution of *BC* types with previous exposure to radiation:

Previous radiation	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Yes	10(37.0%)	7(26.0%)	1(3.7%)	9(33.3%)	**27**
No	31(42.5%)	14(19.2%)	11(15.1%)	17(23.3%)	**73**
Total	**41**	**21**	**12**	**26**	**100**
(Chi square= 5.7649) P=0.2174					

Table (31.4) is describing the relation between previous exposures to radiation and breast cancer, high incidence rates of each type of breast cancer is found in no previous exposure to radiation patients.

Table (32.4) Distributions of *BC* types with metastasis:

Metastasis	Type of breast cancer				
	Invasive ductal carcinoma	Invasive lobular carcinoma	invasive micro papillary carcinoma	Medullary carcinoma	Total
Yes	35(52.2%)	17(25.3%)	11(16.4%)	4(6.0%)	67
No	6(18.2%)	4(12.1%)	1(3.0%)	22(66.7%)	33
Total	**41**	**21**	**12**	**26**	**100**
(Chi square=42.4781) P=0.0000					

Table (32.4) is shows the relation between metastasis and types of breast cancer, Invasive ductal carcinoma 35(52.2%) is found more common in metastasis.

Figure (25.4): Separation of the GAPDH PCR product by (1.5%) agarose gel electrophoresis Lane.

1. 100 bp ladder. Lane

2. PCR negative control. Lanes

3-9: 157 bp amplified *GAPDH* product in 7 breast samples.

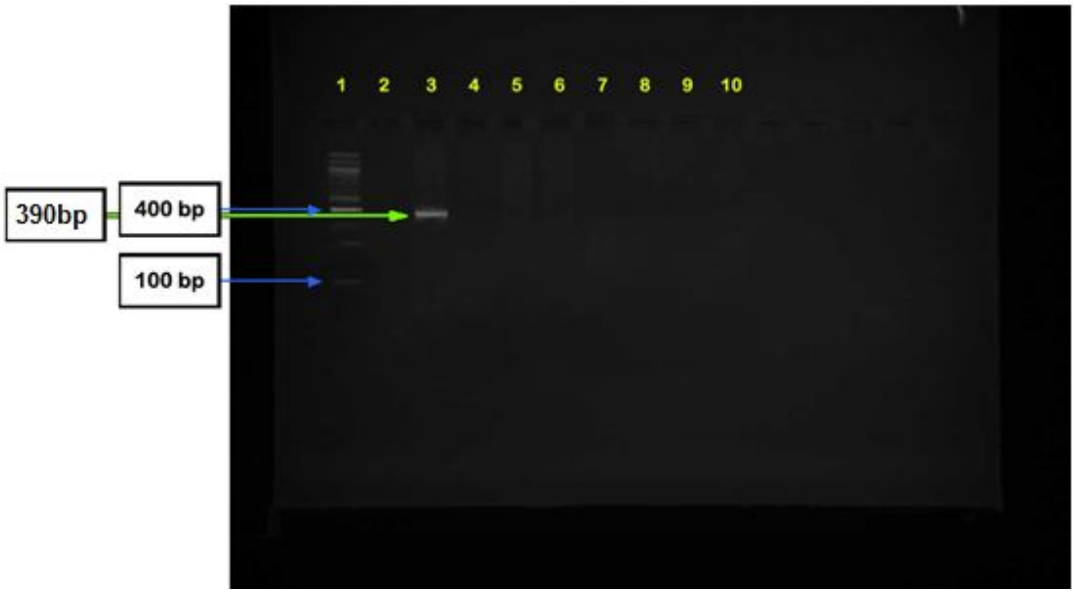

Figure (26.4) Separation of *PCR* product for the *HPV* 16 by (1.5%) agarose gel electrophoresis Lane

1. 100bp ladder Lane.

2. PCR negative control Lane.

3. Positive control for *HPV* Lanes.

4-10. Breast samples are negative for *HPV*.

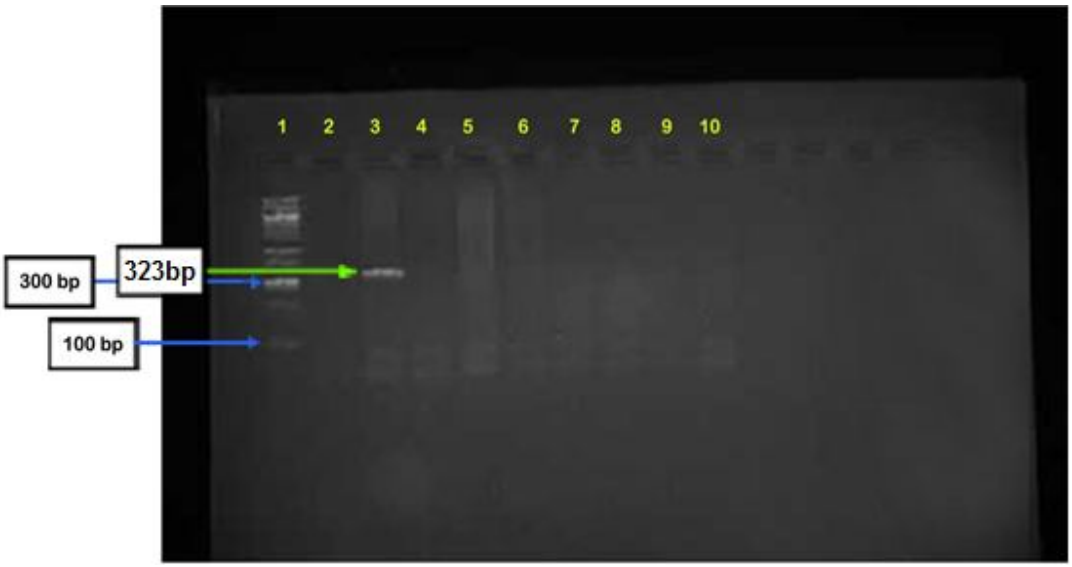

Figure (27.4) Separation of *PCR* product for *HPV* by (1.5%) agarose gel electrophoresis lane.

1. 100 bp ladder lane.
2. *PCR* negative control lane.
3. Positive control for *HPV* 18 lanes.

4-10: breast cancer samples were negative for *HPV* 18**.**

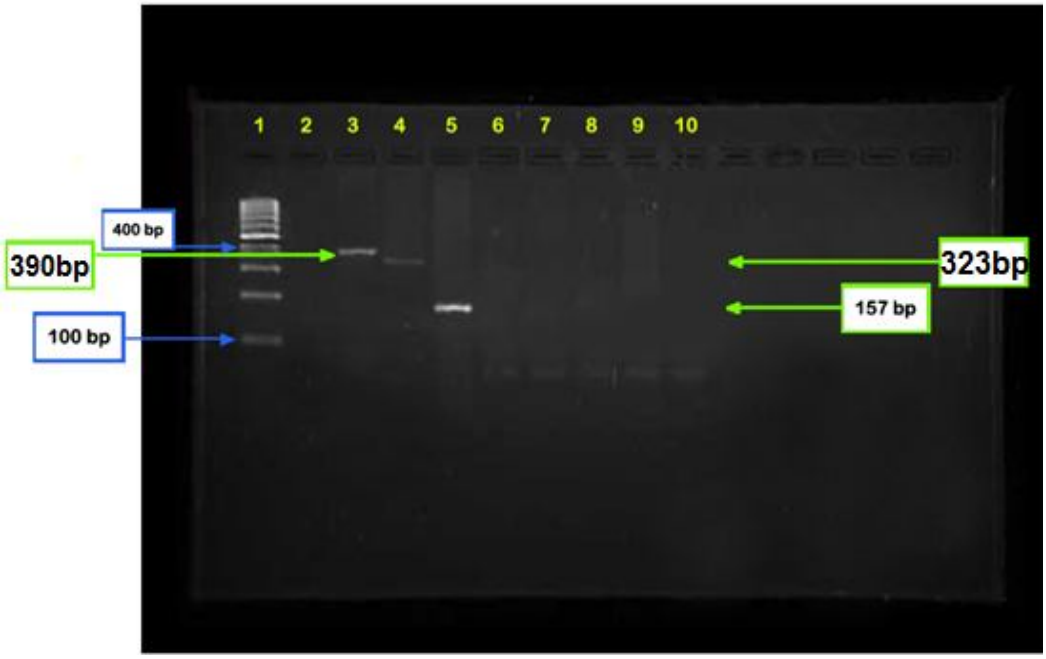

Figure (28.4) Separation of *PCR* products of control samples by 1.5% agarose gel electrophoresis Lane.

1.100 bp ladder Lane.

2. *PCR* negative control Lane.

3. Positive control for *HPV16* Lanes.

4. Positive control for *HPV18* Lane.

5. GAPDH product to check *DNA* quality lanes.

6-10. control samples negative for *HPV*.

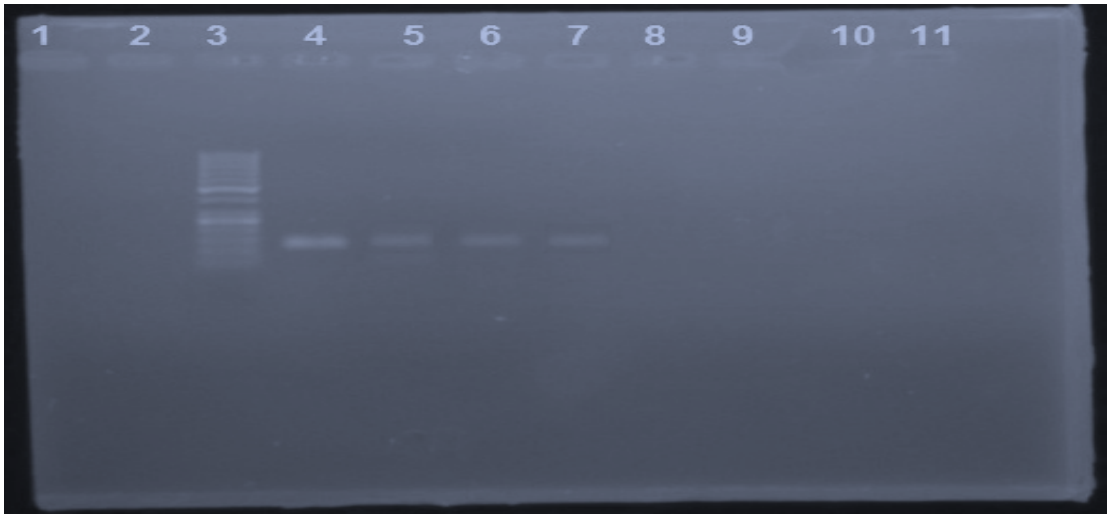

Figure (29.4) Separation of *PCR* products of control and samples by (1.5%) agarose gel electrophoresis Lane

1- Negative control.

2- *HPV* negative Sample.

3- 100pb *DNA marker*.

4- 4-7 positive samples for *HPV*.

5- 8- 11 negative sample for *HPV*.

Figure (30.4): Separation of *PCR* products *HPV genotypes* to control and samples by 1.5% agarose gel electrophoresis Lane.

1- Ng Control.
2- 100 bp *DNA* marker.
3- *HPV* positive sample 267 bp.
4- *HPV* negative sample.
5- *HPV* genotype 16 (457 bp).
6- *HPV* genotype 18 (322 bp).
7- *HPV* genotype 31 (263 bp).
8- *HPV* genotype 33 (387 bp).
9- *HPV* genotype 16 (457 bp).

Chapter Five

Descriptions of the results

Discussion and Recommendations

5.1 Descriptions of the result:

The table (1.4) is indicating that:

Invasive ductal carcinoma 41 (29%), invasive lobular carcinoma 21 (15%)invasive micropapillary carcinoma12(8%), medullary carcinoma 26 (18%) and inflammatory breast sample 43(30%) of total of sample witch used as control sample, Positive Samples of {Invasive ductal carcinoma 23(16.1%), Invasive lobular carcinoma14(9.8%), invasive micropapillary 9(6.3%), Medullary carcinoma 11(7.7), inflammatory breast Sample10(7%).

There is strong relation between breast cancer sample and (HPV) (PCR) positive sample when compare with inflammatory breast sample chi-square 13.65 and P.value 0.00022

Significant result of (HPV) with invasive ductal carcinoma when compare with inflammatory breast chi-squares 9.38 and P.value 0.00022

It is found that high statistically significant result of (HPV) with invasive lobular carcinoma when compared with inflammatory breast sample chi-squares 18.6 and P.value 0.000016.

There is significant result of (HPV) with invasive micropapillary carcinoma when compared with inflammatory breast sample chi-squares 10.91 and P.value 0.000095.

No significant result of (HPV) with medullary carcinoma when compared with inflammatory breast sample and P.value 0.098.

The table (2.4) shows that: 22 (33%), (HPV) genotype 16, 21(31%) (HPV) genotype 18, 10(15%) (HPV) genotype 31, 14(21%) HPV genotypes 33 were found.

The table (3.4) shows the relation between HPV genotypes and the types of breast cancer:
in invasive ductal carcinoma 13(45%) genotype 16 were found, 9(45%) genotype 18, 0(0.0%) genotype 31 and 2(14%) genotype33.

In invasive lobular carcinoma, 5(25%) genotype 16 was found, 6 HPV genotype 18 was found, no genotype 31, 2(30%) genotype 33(14%) was found. HPV genotype 16, 18 are more common followed by (33, 31 respectively).

In invasive micropapillary carcinoma, no genotype16, 18 were found, 9(65%) genotype 33 was found.

In medullary carcinoma, 5(25%) HPV genotype 16 was found, 5(25%) HPV genotype 18 was found, no genotype 31 was found, 1(7%) genotype 33.able.

In inflammatory breast sample no genotype16, 18, 33 was found, 10(100%) genotype 31 was found.

Table (4.4) shows that the age of the patients is affected by the HPV infection, the age group (41-50) years was the highest affected.
There is statistically significant relationship between age and HPV infection (P = 0.0012).
Table (5.4) stated the relation between stages of breast cancer and HPV: stage0, 10(17.5%) were positively HPV infected.
Stage I, 18(31.6%) were positively HPV infected.
Stage II, 29(50.9%) were positively HPV infected. HPV infection prevails that the

infection appears in stage II intensely followed by stage1 and stage 0 respectively; there is no statistically significant relation between the stages of the breast cancer and HPV infection, P=0.5864.

Table (6.4) shows the relation between menstrual period and HPV infection, in early menstrual period, 7(12.3%) HPV positive cases were performed. In normal menstrual period, 21(36.8%) positive HPV infection cases.
In late menstrual period, 29(50.9%) positive HPV infection cases
There is no statistically significant relation between menstrual period and HPV infection, P value= 0.1697.

Early (9-12) years, normal (13-17), late age (above 20)

With respect to the relation between practicing of exercises and HPV infection in breast cancer patients in **table (7.4);**

In patients practicing exercise, 3(5.5%) positive HPV cases were found. In patients not practicing exercises, 54(54.7%) positive HPV cases were reported. HPV increases among patients not practicing exercises.

There is no statistically significant relation between exercise and HPV infection, P=07223.

According to relation between duration of illness and HPV infection in **table (8.4);**
At (1-2) years of illness, 23(40.4%) positive cases were found,

At (3-4) years of illness, 23(40.4%) positive cases were detected,

At (5-6) years of illness, 11(19.2%) positive cases were shown.

HPV infection was found to be higher at (1-2, 3-4) durations of illness followed by (5-6) years of illness.

There is statistically significant relation between the duration of illness and HPV infection, P=0.034.

Table (9.4) reflects the relation between lymphoid involvement and HPV infection; in lymphoid involvement, 57(100%) positive cases were found. There is no significant relation between HPV infection and lymphoid involvement. P=0.2496.

With regard to family history relation and HPV infection **table (10.4)** is describing the condition;

In patients with family history, 23(60.5%) HPV positive cases were found,

In patients with no family history, 34(54.8%) positive cases were detected; HPV infection appeared higher in patients with no family history of breast cancer. There is no statistically significant relation between family history and HPV in this study P =0.57900.

Table (11.4) explaining the relation between marital status and HPV infection;

In married status, 6(10.5%) have positive HPV infection cases,

In single status, 31(54.4%) positive cases were found,

In divorce status, 20(35.1%) considered to be positive. HPV is increasing in single, divorce and married patients respectively.

There is no statistically significant relation between marital status and HPV infection in this study P=0.4663.

With respect to the relation between the socio-economic status and HPV infection, **table (12.4)** is indicating the matter; it is found that 25(43.9%) house-wives are positive; 2(3.5%) girl student are positive; employed14 (24.6%) are positive, other socio-economic statuses 16 (28.1%) are positive. House wives were found to be most infected by HPV than others, there is no statistically significant relation between HPV infection and socio-economic status in this study (P = 0.8900).

According to relation between the types of breast tissues and HPV infection table (13.4) is prevailing the case;

In fatty breast tissue, 25(43.9%) are HPV positive cases,

In fibrous breast tissue, 7(12.3%) are positively infected,

In granular breast tissue, 25(43.9%) are positive infected cases.

Fatty breast tissue was found to be the most infected by HPV than others.

There is no statistically significant relation between the dens of breast tissue and HPV infection in this study, P =0.5099 **Table (13.4).**

With regard to the relation between body weight and HPV infection table**, (14.4)**

Is showing the linkage;

In obese patients, 9(15.8%) HPV positive cases were found.

In normal weight patients, 47(73.7%) positive cases were detected.

In underweight patients, 6(10.5%) positive cases were known.

Obese patients were found to be most infected by HPV than others.

There is no significant relation between HPV and body weight in this study, P = 0.6963.

With respect to the relation between the types of family planning and HPV infection, the results are presented as ;

Contraceptive pill users, 22(39.0%) positive cases were found to be positively infected,

With natural control, 5 (8%) were positively detected as infected,

With family planning, 30 (52.6%) shown to be HPV infected.

There is no statistically significant relation between family planning and HPV in this study. P=0.6235 **table (15.4).**

According to the relation between previous exposure to radiations and HPV infection, table (16.4) indicating the results as follows; 13(22.8%), HPV positive cases were found in patients exposed to radiations previously, 44(77.2%) has no previous exposure to radiation.

There is no statistically significant relation between HPV and previous radiation in this study P=0.2793 table (16.4).

Table (17.4) shows the association between metastasis breast cancer and HPV infection. In metastasis patients 39(22.2%) positive cases were found,

With no metastasis there were 18(31.5%) positive cases, there is no statistically significant relation between HPV infection and metastasis breast cancer in this study; P=0.72924.2.

The above table shows the association between the type of breast cancer and age group. At mean age (3.09), (41) Patients were found to have invasive ductal carcinoma, At mean age (3.00), (21) patients were found to have Invasive lobular carcinoma. At mean age (2.9), (12) patients were found to have Invasive micropapillary carcinoma,

At mean age (2.4615), (26) patients were found to have Medullary carcinoma, there is statistically significant relationship between breast cancer and age; p=0.0012.

NB: OBS means observation.

According to the relation between the stages of breast cancer and lymphoid involvement, table (19.4) is expressing the following data;

In Stage (0), 21(21%) patients were found to have lymphoid involvement.
In Stage (I), 29(29 %) have lymphoid involvement.
In Stage (II), 21(49%) have lymphoid involvement.

There is no statistically significant relation between the stages of breast cancer and lymphoid involvements; P=0.3451.

The relation between breast feeding and breast cancer types is presented in table (20.4). In the first criteria (less than 1) year of breast feeding, 26(37.7%) patients were found to have invasive ductal carcinoma, 11(15.9%) invasive lobular carcinoma, 9(13.04%) invasive micro papillary carcinoma and 23(33.3%) medullary carcinoma.

In the second criteria (one) year breast feeding, 12(46.2%) invasive ductal carcinoma 8(30.8%) invasive lobular carcinoma, 2(7.7%) invasive micropapillary carcinoma and 3 medullary carcinoma.

In the third criteria (more than one) year, there is 3(11.5%) invasive ductal carcinoma (60%), 1(20%) invasive lobular carcinoma1 (20%) invasive micro papillary carcinoma and no medullary carcinoma was found, there is significant relation between breast feeding and each type of breast cancer, P= 0.0430.

NB: the criteria of breast feeding were extracted from RICK records.

The relation between menstrual cycle and breast cancer types is explained in table (21.4):

In early menstrual period, 1(9.1%) patients were found to have invasive ductal carcinoma, 3(27.3%) have invasive lobular carcinoma, 2(18.2%) invasive micro papillary carcinoma, 5(45.5%) medullary carcinoma.

In normal menstrual period, 21(46.7%) invasive ductal carcinoma, 7(15.6%) invasive lobular carcinoma 5(11.1%) invasive micro papillary carcinoma 12 medullary carcinoma were found (26.7%).

In late menstrual period, 19(43.18%) invasive ductal carcinoma, 10(22.7%) invasive lobular carcinoma, 5(11.4%) invasive micro papillary, 9(20.5%) medullary carcinoma.

There was no significant relation between menstrual period and each type of breast cancer in this study. P=0.4629.

NB:
early menstrual period, menarche (first menstruation) occurs at age of 13 years, normal range (10 – 16) years.
Normal menstrual period (17 – 43) years.
Late menstrual period (cessation of menstruation) occurs by age of 50 years, but normal range (45 – 55) years (Diaa Mwafi, simplified gynecology page (38), the menstrual cycle.

The association between the breast cancer types and lymphoid involvement is mentioned in table (22.4):

In lymphoid involvement patients, 41(41.4%) were found to have invasive ductal carcinoma, 21(21.2%) invasive lobular carcinoma, 12(12.1%) invasive micro papillary carcinoma, 25(25.3%) medullary carcinoma. invasive ductal carcinoma was found the most common among the other types.

there is no statistically significant relation between any type of breast cancer and lymphoid involvement in this result. P=0.5839.

With respect to the relation between breast cancer stages and practicing of exercises is shown in table (23.4);

in Stage 0, 6(28.6%) patients were practicing exercises, but, 15(71.4%) patients did not practice any exercises.

In Stage I, 2(10%) patients were practicing exercises, whereas, 18(90%) patients did not practice any exercises.

In Stage II, 1(1.7%) patient was practicing exercises, on the other hand, 58(98.3%) patients did not practice any exercises.

There was statistically significant relation between the stage of breast cancer and practicing exercises; P = 0.0024.

The table describes the association between the duration of illness and breast cancer types.

At (1-2) years duration of illness, 11(28.9%) patients were found to have invasive ductal carcinoma, 9(23.9%) invasive lobular carcinoma. 2(21.1%) invasive micro papillary carcinoma and 16(42.1%) medullary carcinoma.

At (3-4) years duration of illness, 23(50%) patient were found to have invasive ductal carcinoma, 8(17.4%) invasive lobular, 7(15.2%) invasive micro papillary carcinoma and 8(17.4%) where have medullary carcinoma.

At (5-6) years duration of illness, 7(43.8%) patients were found to have invasive ductal carcinoma, 4(25%) invasive lobular, 3(18.8%), invasive micro papillary carcinoma, 2(12.5%) medullary carcinoma.

There is statistically significant relation between the duration of illness and the types of breast cancer; P=0.0285.

NB: the duration of illness is described in the RICK records.

The relations between family history and breast cancer types are found in table (25.4);

In patients with family history, 10(26.3%) patients were found to have Invasive ductal carcinoma, 9(23.7%) Invasive lobular carcinoma, 4(10.5%) invasive micro papillary carcinoma 15(39.5%) medullary carcinoma. Medullary carcinoma was found to be the most common among the rest

In patients with no family history, 31(50%) where found to have invasive ductal carcinoma, 12(19.4%) invasive lobular carcinoma, 8(12.9%) invasive micro papillary carcinoma and 11(2%) where found to have medullary carcinoma.

There is statistically significant relation between family history and each type of breast cancer in this study. P=0.0432.

With respect to the association between marital status and breast cancer types, the above table explains the following results:
In single women, 21(38.9%) patient were found to have invasive ductal carcinoma, 10(14.3%)10invasive lobular carcinoma,7(13.0%) invasive micro papillary carcinoma(13.0%) and 16(29.6%) patient were found to have medullary carcinoma.
In married women 2(14.3%) patient were found to have invasive ductal carcinoma, 4(28.6%) invasive lobular carcinoma, 8(57.1%) medullary carcinoma.
In divorce women 18(56.3%) patient were found to have invasive ductal carcinoma, 7(21.9%) invasive lobular carcinoma, 5(15.6%) micropapillary carcinoma and 2(6.3%) patients were found to have medullary carcinoma were found (6.3%).

There is significant statistical relation between marital status and breast cancer in this study P=0.0016.

According to the relation between socio-economic status and types of breast cancer in table (27.4)

In house wife women 17(41.5%) patients were found to have invasive ductal carcinoma 6(14.5%) invasive lobular carcinoma, 6(14.5) invasive micropapillary carcinoma 12(29.3%) medullary carcinoma.

In Employee 11(42.3%) patient were found to have invasive ductal carcinoma 6(23.1%) invasive lobular carcinoma, 2(7.7%) invasive micro papillary carcinoma, 7(26.9%) medullary carcinoma.

In student 1(33.3%) patient were found to have invasive ductal carcinoma, 1(33.3%) invasive lobular carcinoma, no invasive micro papillary carcinoma was found, 1(33.3%) medullary carcinoma.

In others, 12(40%) patient were found to have invasive ductal carcinoma, 8(26.7%) invasive lobular carcinoma, 4(13.3%) micro papillary carcinoma, 6(20%) medullary carcinoma there was no significant statistical relation between occupation and breast cancer in this study; P=0.5661.

Table (28.4) describes the relation between the dense of breast tissue and breast cancer types.

In fatty breast tissue, 24(51.1%) patients were found to have invasive ductal carcinoma, 9(19.1%) patients were found to have invasive lobular carcinoma 1(2.1%) patient were found to have invasive micro papillary carcinoma and 13(27.7%) patients were found to have medullary carcinoma, invasive ductal carcinoma is prevails followed by medullary carcinoma, invasive lobular carcinoma, invasive micropapillary carcinoma respectively.

In fibrous breast tissue, 4(28.6%) invasive ductal carcinoma we, 1(7.1%) invasive lobular carcinoma 3(21.4%) invasive micro papillary carcinoma were found, 6(17.9%) medullary carcinoma.

In granular breast tissue, 13(33.3%) invasive ductal carcinoma, this account, 11(28.2%) invasive lobular carcinoma 8(20.5%) micro papillary carcinoma, 7(17.9%) medullary carcinoma (17.9%), there is statistically significant relation between dense of breast tissue and breast cancer in this study; P=0.0293.

The above mentioned table is linking between the body weight and breast cancer types.

In obese patients 4(26.7%) patient were found to have invasive ductal carcinoma, 3(20.0%) invasive lobular carcinoma, 2(13.3%) invasive micro papillary carcinoma and 6(40.0%) patients were found to have medullary carcinoma.

In normal weight, 33(45.8%) patients were found to have invasive ductal carcinoma, 11(15.3%) invasive lobular carcinoma, 8(11.1%) invasive micro papillary carcinoma and no patient was found to have medullary carcinoma.

In underweight patient, 4(30.8%) patients were found to have invasive ductal carcinoma, 7(53.9%) invasive lobular carcinoma, 2(15.4%) invasive micro papillary carcinoma and no patient was found to have medullary carcinoma cases this account ,there is no significant relation between body weight and each type of breast cancer in this study P=0.1122.

The relation between family planning and breast cancer types is explained in table (30.4);

Contraceptive pills users, 17(46.0%) patients were found to have invasive ductal carcinoma, 4(10.8%) invasive lobular carcinoma, 2(5.4%) invasive micro papillary carcinoma and 14(37.8%) medullary carcinoma.

In Natural control, 3 (42.9%) patients were found to have invasive ductal carcinoma, 2(28.8%) have invasive lobular carcinoma, 2(28.8%) invasive micro papillary carcinoma and no patient was found to have medullary carcinoma.

In no family planning, 21(37.5%) patients were found to have invasive ductal carcinoma, 15(26.8%) invasive lobular carcinoma, 8(14.0%) invasive micro papillary carcinoma and 12(21.4%) patients were found to have medullary carcinoma,

There is no significant statistical relation between family planning and each type of breast cancer in this study, P =0.1878.

In respect to the relation between previous radiation exposure and breast cancer types in table (31.4)

Patients exposed to radiation, 10(37.0%) patients were found to have invasive ductal carcinoma, 7(26.0%) invasive lobular carcinoma, 1(3.7%) invasive micro papillary carcinoma, 9(33.3%) patients were found to have medullary carcinoma.

Patients not exposed to radiation, 31(42.5%) patients were found to have invasive ductal carcinoma, 14(19.2%) invasive lobular carcinoma, 11(15.1%) invasive micro papillary carcinoma and 17(23.3%) patients were found to have medullary carcinoma.

There is no significant relation between previous radiation and breast cancer in this study. P=0.2174.

Table (32.4) is describing the relation between metastasis and breast cancer types. In metastasis patients (Yes): 35(52.2%) patients were found to have invasive ductal carcinoma 17(25.3%) invasive lobular carcinoma, 11(16.6%) invasive micro papillary carcinoma, 4(6.0%) were found to have medullary carcinoma, invasive ductal carcinoma is prevails followed by invasive lobular carcinoma, invasive micro papillary carcinoma, and medullary carcinoma respectively.

Not metastasized patients (No): 6(18.2%) patients were found to have invasive ductal carcinoma, 4(12.1%) invasive lobular carcinoma, 1(3.0%) invasive micro papillary carcinoma and 22(66.7%) patients were found to have medullary carcinoma.

There is strong significant statistical relation between metastasis breast cancer and any type of breast cancer in this study. P =0.0000.

Table (32.4) is describing the relation between metastasis and breast cancer types.

In metastasis patients (Yes): 35(52.2%) patients were found to have invasive ductal carcinoma 17(25.3%) invasive lobular carcinoma, 11(16.6%) invasive micro papillary carcinoma, 4(6.0%) were found to have medullary carcinoma, invasive ductal carcinoma is prevails followed by invasive lobular carcinoma, invasive micro papillary carcinoma, and medullary carcinoma respectively.

Not metastasized patients (No): 6(18.2%) patients were found to have invasive ductal carcinoma, 4(12.1%) invasive lobular carcinoma, 1(3.0%) invasive micro papillary carcinoma and 22(66.7%) patients were found to have medullary carcinoma.

There is strong significant statistical relation between metastasis breast cancer and any type of breast cancer in this study. P =0.0000.

5.2 Discussion:

Breast cancer is the most common among females and comprises about (18%) of all cancers affecting them. About (1.7) million new cases are reported in the world each year. Based on the most global recent data, approximately (12.3%) of women are diagnosed with *BC* at a point of time during their life.[12, 15] However, so far and to the best of knowledge there are no records regarding the prevalence and incidence of breast cancer cases in the Sudan.

In this study, (143) cases were screened for breast pathology, (100) were diagnosed to have breast cancer, the remaining (43) diagnosed as having inflammation of the breast (control).

Of the (100) cases, (41) patients (29%) were diagnosed with invasive ductal carcinoma, 21(15%) with invasive lobular carcinoma, 12(8%) with invasive micropapillary carcinoma and finally, 26(18%) with medullary carcinoma **table (1.4).**

Other studies reported similar findings. In one study, invasive ductal carcinoma represented more than (17.1%) of all breast cancers. [25, 26] In another, invasive ductal carcinoma comprised (16.3%) of all breast cancers. [45, 46]

However, a third study reported a higher percentage (92.2% of 102 breast cancer cases) of invasive ductal carcinoma than that reported in this study. [56]

The incidence of breast cancers in the Sudan in this study was higher (26%) than those reported from other regional countries such as Egypt (16.4%), Ethiopia (11.6%) and Chad (17.7%).

Moreover, the incidence of breast cancers from other countries was much lower. In the *U.S.A*. it was (13.1%) and in the *UK*, (6.6%) according to the *IARC*, 2013. However, the reason for the variations in these rates of incidence may be due to the different etiological risk factors and to the life style.

The study results are, therefore, in general agreement with those from other studies regarding the role of *HPV* as the main associative agent of breast cancer. In this study, the presence of *HPV* in biopsies from the breast (malignant and inflammatory breast samples) was investigated. *HPV* was detected from four different types of breast cancer in those biopsies. These were, invasive ductal carcinoma, 23(16.1); invasive lobular carcinoma, 14(9.8%); invasive micropapillary 9(6.3%) and medullary carcinoma, 11(7.7%). Control inflammatory breast samples were positive only for 10 (7%) *HPV*.

A strong relationship was established between *PCR-positive HPV* and *BC* types as compared with *PCR-positive HPV* inflammatory breast samples, (P = 0.00022).

As regards the association of *HPV* with the different types of breast cancer, there was a significant relationship between *HPV* and invasive ductal carcinoma when compared with inflammatory breast (P = 0.00022) **table (1.4).**

Another significant relationship was found between *HPV* and invasive lobular carcinoma with respect to inflammatory breast sample (P = 0.000016, chi-squares 18.6). A third significant association between *HPV* was also found between invasive micropapillary carcinoma and inflammatory breast sample (P = 0.000095).

No statistically significant relationship was seen between *HPV* in medullary carcinoma and inflammation of the breast (P = 0.098) **table (1.4)**.

The study results were similar to those from other studies in Africa. There was a strong relationship between *HPV* and breast cancer in a study from Morocco where 62(34%) of the cases were *HPV-associated breast car*cinomas [56, 57, 81].

Furthermore, studies from other parts of the world reported generally similar results. In Mexico 17/53(32%) cases were positive for *HPV infection* [45, 46].This slightly higher percentage may be attributed to their relatively smaller sample size.

Also the presence of *HPV* in *BC* was investigated by [55, 56] which they assessed (102) cases of breast cancer and identified *HPV* in 33/102 (32.4%) of the cases.

Another study by [51, 52, and 53] screened 237 *BC*, were *HPV infection* was revealed in (38%) of the cases, on the other hand some studies report that *HPV* is not present in *BC* potential explanations include difficulties in detection due to low viral load and low frequency of *HPV* in *BC*s in some populations, other study by [57, 67] where found that a definitive relationship between human breast cancer and *HPV infection* has not been determined such as the study in Hong Kong *BC patients*.

Whoever some study like [45, 57,78] were they found that the association of human papilloma virus *HPV* with the involvement of the virus in *BC* is more controversial, this is considered to be because of the fact that a considerable proportion of *BC* specimens is non-cancerous and that the levels of virus are low in breast cancer. Also the controversy surrounding the role of *HPV* in *BC* may be because of the difficulty that has been encountered in detecting the virus in breast specimens, in contrast to the relative ease of detection in cervical cancer [45, 46]. Indeed, in a previous study from other group, they demonstrated that it was necessary to use *SYBR Green* I, for polymerase chain reaction *PCR* detection of virus in breast cancer in *DNA* extracted from breast tissue [45, 46].

Genotyping of the infected *PCR-positive HPV* specimens was performed. Out of the (143) specimens (*BC* and breast inflammation) the following genotypes were determined and mentioned in a descending order. Twenty two (33%) of the *HPVs* were of the genotype 16, 21(31%) genotype 18, 14(21%) genotype 33 and 10(15%) genotype 31 **table (2.4).**

The study findings are similar to those reported in a study from Nigeria[78, 90] in which *HPV genotype*16 was the most common genotype in breast cancers and [76, 77] reported that, *HPV*16, 18, 33 respectively are frequently detectable in high grade of breast carcinoma,

These results are different from a study conducted in Australia which reported genotype (18) to be the most prevalent and had an affinity or tropism to glandular

as compared with squamous epithelial cells. [33, 35] This difference may be due to the variation in the environment and the social habits.

The other oncogenic *HPV types* (18 and 33) are also detected in invasive cervical cancer biopsies were also detected in breast biopsies. [85, 68] Another study conducted in France by [56, 57] they assessed *HPV genotypes* distribution in breast cancers and they found (*HPV genotypes* 16) was the most prevalent types it was founded in (89.7%) of *HPV-positive* breast cancer cases which was similar to our findings the study by. ([56, 34] in Sweden also showed to the presence of *HR-HPV* in breast cancers particularly type (35).

Also this study finding is similar to other study by. [67.77] where they determine the role of *high-risk HPV infections* in human *BC* in Middle Eastern women, they investigated the presence of *high-risk HPV types* 16, 18, 31, 33 and 35 in a cohort of (113) breast cancer samples from Syrian women by *PCR* , they found that 69 (61.06%) of the (113) samples are *HPV positive* and 24 (34.78%) of these specimens are co-infected with more than one *HPV genotypes* and they found that *HPV types* 16, 18 and 31 are present in only 10, 11 and 8 cancer tissues respectively.

several studies failed to detect HPV in breast carcinoma such as the study by[45, 56] were they studied (HPV genotypes 11, 13, 16, 18, 30, 31, 32, 33, 45, 51) in (95) women with breast cancer without detecting any of these subtypes this failed is me by due to the sample size were they used.

Another study by [45, 67] were they founded *HPV genotypes* 16, 18 in 13 *(IDC),* 15 micropapillary carcinomas cases, furthermore the [45.46] used six different primers, including the following genotypes 16, 18, 31, 33, 45 in (81) Swiss women cases with breast cancer they founded no *positivity of HPV* was detected in other study such as the study by. [67,56] where they studied the prevalence of *HPV genotypes 16, 18, 33, and 45* and low risks (6, 11) in (50) breast cancers in women in France

by other studies have demonstrated the presence of *HPV high-risk types 16, 18* and 33 in breast cancer specimens from diverse populations around the world: Italy, Norway, China, Japan, USA, Austria, Brazil, Australia, Taiwan, Turkey, Greece, Korea, Mexico, Hungary and Syria [45, 46, 68, 78, 79, and 82] the prevalence of *HPV* positive breast cancer in these studies was reported to vary from (4%) in Mexican to (86%) in American women.

PCR technique used in this study was the most reliable tool to diagnose *HPV* from breast tissues. Significant relationship was established between *BC* types and *PCR-positive HPV* as compared with inflammatory breast samples, (chi-squares 13.65 and P = 0.00022) **table (1.4).**

The study results are similar to those reported where a *standard PCR* was used on extracted *DNA* viral sequences from each sample. [23, 33]

Other studies were in general agreement with our results [34, 56] in which the presence of *HPV* was demonstrated using the *PCR* in *BC* cell lines.

As regards the age of the patients that is most affected with HPV infection, the age group (41-50) years was the highest, 36(63.16 %); followed by the (31-40) years age group, 13(8.81%); and the least affected was the over 50-years age group at age group 8(14.04%) (P = 0.0012) **table (4.4).**

Other studies [34, 44, 45, and 46] reported that the incidence of breast cancers is progressively increasing particularly among old age groups, other study by [23, 34] found that there is no evidence of significant relationship between the age group and *HPV*. Similarly finding was established by [45, 56] were they reported that there was no significant difference in breast cancer between levels of the different age groups of the patients. In contrast [34, 57] was founded statistically significant differences between the age group and *HPV* in *BC* patient were the find (23) *HPV* positive cases in less than (45) years age group (28.0), and 12 *HPV positive* cases in

more than (45) years age group respectively, moreover, breast invasive ductal carcinoma (*BCIDC*) was founded in elder people, an increasing number of old patients are being affected worldwide, with up to (5.5% <40) these are predominantly. Some patients have heavy exposure to the usual risk factors, but an increasing number do not Part of this trend appears to be due to rising numbers of *HPV associated invasive lobular carcinoma*, particularly in age group (40-50) this finding is adopted by [88, 89] third studies which have shown that women under the age of (25) years have a higher prevalence of *HPV positivity* detection with a linear decrease rate as age increases [45, 46]

With respect to the relationship between the socio-economic status and *HPV* infection, the study found that 25(43.9%) house-wives were positive for *HPV*; 2(3.5%) girl student; employed14 (24.6%) others occupation 16 (28.1%). *HPV* is found prevails in house wife followed by other occupation employee, and student respectively, there is no significant relation between *HPV* and occupation in this study. (P = 0.8900) **table (12.4)**.the finding of this study was similar to other study by (45,46) were found that In regard to occupation, the prevalence of *HPV* is widely found in housewives and employees, but statistically no significant association between *HPV* detection and occupation (P= 0.435). Up to now there is no available information correlate between *HPV* infection in *BC* and specific occupation.

According to relation between menstrual period and *HPV* this study found that at early menstrual period 7(12.3%) HPV positive cases were found, Normal menstrual period 21(36.8%); late menstrual period 29(50.9%). *HPV* is prevails at late menstrual period followed by normal and early respectively , there is no significant relation between the time of menstrual period and *PCR* result of *HPV* (P = 0.1697) **table (6.4).**These findings is supported by other studies conducted in many countries which they detected many *HPV genotypes* detection fluctuated

periodically and depended on sampling times within a menstrual cycle and they found no statistical associations between *HPV* and menstrual cycle.

In respect to the relation between body weight and *HPV* this study found in obese patient 9(15.8%) *HPV positive* cases were found, normal weight 47(73.7%), underweight patient 6(10.5%) *HPV* is highest in normal weight followed by obese and underweight, there is no significant relation between *HPV* and body weight in this study (P = 0.6963) **table (14.4).**

These findings is similar to the studies by [56,67] were they found that obesity was not associated with prevalent *HPV* (adjusted odds ratio for body mass index after adjusting for age, race/ ethnicity, and contraceptive use, premenopausal status as well as lifetime and recent sex relationships.

According to relation between the types of family planning and *HPV*. 22(39.0%) positive cases were found in contraceptive pill user's patient, 5(8 %) in normal birth control, 30(52.6%).in no birth control, there was no significant relationship between birth control and *HPV* determined in this study (P=0.6235).

Similar finding were found in study conducted by [45, 47] were they find that contraceptive Pills used did not influence the prevalence *HPV* **table (15.4).**

In regard to the association between the type of *BC* and age. at mean age (3.1) (41) patients were found to have invasive ductal carcinoma , (21) invasive lobular carcinoma at mean age (3.0), (12) patients invasive micropapillary carcinoma, at mean age (2.9), (26) patients medullary carcinoma, at mean age (2.5) invasive ductal carcinoma is prevails followed by medullary carcinoma, invasive lobular carcinoma, invasive micropapillary carcinoma respectively, there is significant relationship between breast cancer and age (P=0.0012) **table (18.4).**similar finding is obtain by some authors which they found the risk of developing breast cancer increased in elder patient by (1 out of 8) invasive

breast cancers are found in women younger than (45) while about (2 of 3) invasive *BC* are found in women age (55) or older some study found there is relation between *HPV* and age of patient. [96, 97]

In regard to association between body weight and *BC* types this study found there is no significant relation between body weight and each type of *BC* in this study (P=0.1122) **table (29.4).** This result is supported by other result which found that gaining weight after menopause can increase a woman's risk factor. [83] Gaining (10) kg after menopause increased the risk of developing breast cancer by (18%). Lack of exercise has been linked to breast cancer. [84] Physical activity after breast cancer diagnosis has shown some associations with reducing *BC* recurrence and mortality independent of weight loss. [85]

Other study in *USA* [45, 67] found that weight change and obesity are risk factors for *BC* in women.

In association between breast feeding and *BC* types, this study found that at less than (1) year of breast feeding 26(37.7%) patients were found to have invasive ductal carcinoma as type of breast cancer, at one year breast feeding 12(46.2%) patients have invasive ductal carcinoma at more than one year there is 3 patients were found to have invasive ductal carcinoma, there is significant relationship between breast feeding and each type of breast cancer was determined in this study (P= 0.0430) **table (20.4).**

Similar finding were found ([34, 35] in their study which involving (13,907) breast cancer cases there study found that the duration of breast feeding was associated with the risk of breast cancer.

According to the relation between the dense of the breast tissue and *BC* types this study found in fatty breast tissue24(51.1%), patient were found to have invasive

ductal carcinoma 9(19.1%) invasive lobular carcinoma, 1(2.1%) invasive micropapillary carcinoma 13(27.7%) medullary carcinoma. invasive ductal carcinoma is highest in this study. In fibrous breast tissue4 (28.6%) invasive ductal carcinoma were found, 1(7.1%) invasive lobular carcinoma, 3(21.4%), invasive micropapillary carcinoma, and 6(17.9%) medullary carcinoma, in granular breast tissue13 (33.3%) invasive ductal carcinoma, 11(28.2%) invasive lobular carcinoma, 8(20.5%) micropapillary carcinoma and 7(17.9%) medullary carcinoma. There is significant relation between dense of breast tissue and *BC* was investigated (P=0.0293) **table (13.4).** [98, 99] They found the type of breast tissues is considered as essential part in risk factor of breast cancer.

In regard to the relation between metastasis and *BC* types this study found in metastasis patient 35(52.2%) patients were found to have invasive ductal carcinoma 17(25.3%) have invasive lobular carcinoma 11(16.4%) have invasive micropapillary carcinoma and 4(6.0%) patients have medullary carcinoma. Invasive ductal carcinoma is more common. In no metastasis patient 6(18.2%) patients were found to have invasive ductal carcinoma, 4(12.1%) patients have invasive lobular carcinoma, 1(3.0%) patients have invasive micropapillary carcinoma and 22(66.7%) patients have medullary carcinoma there is strong significant relationship between metastasis breast cancer and any type of breast cancer in this study (P=0.0000) **table (17.4).**

Other study such as [67, 77] which found that there was association between the grade of cancers and the types.

5.3 Recommendations

1- Further advanced studies, in general, are required to understand the pathology of *HPV* as very few have been performed in both the Arab and African worlds.

2- To develop new methodology for the diagnosis and identification of *HPV* in *HPV-affected tissues* and in particular those from the breast.

3- More studies are strongly needed to shed light on the mode(s) of infection of *HPV* as no clear information is available regarding this.

4- To nip in the bud any development of *BC*, primary and secondary school girls are to be trained for self-investigation of any breast tumors.

5- Research for the possible development of a vaccine against *HPV* is long-awaited.

6- To advance the frontiers of treatment, funds are urgently needed for the provision of the materials and equipment necessary to serve both levels of routine diagnosis as well as more research on *HPV*.

7- The isolation of *HPV* from affected tissue(s) in order to understand its behavior and its pathogenesis.

8- The establishment of authenticated data base for *BC* and the percentage of virus-induced cases with special regard to *HPV*.

9- More studies may be needed to understand the role of heredity in *BC*-affected patients and *HPV*-associated *BC*.

10- Additional studies are also required to determine the percentage of *HPV*-affected male breast.

11- More support - in terms of medical, psychological and social care - is needed for the patients with *HPV* in the Sudan.

12- The advent of both stationary centers and mobile units for the early diagnosis of breast cancer in all states of the Sudan.

13- To establish the relationship between *BC*, in general, and that caused by *HPV*.

14- To exchange information between local, regional and international research centers with respect to *HPV*-associated breast cancers.

15- To introduce educational programs for the enhancement of the patient's cellular and/or cellular/humoral immunity that may participate in the combat or stop the dissemination of the *HPV*.

16- To avoid, whenever possible, the risk factors which may accelerate the development of *BCs* such as smoking, use of contraceptive drugs, exposure to irradiation, etc.

17- The foundation of a "specialized national centre" for the investigation of virus-associated diseases and in particular HPV which reached endemicity in some localities.

18- To provide healthy atmospheres for the hospitalized cancerous patients to relieve their stress in addition to isolating them from infection with other viruses.

Chapter six

References& Appendices

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6.2 Appendices:

Gantt chart

Task	Responsible person	Month 1-2	Month 3 to 6	Month 7 to 8	Month 9-10	Month 11-13	Month 15 to 24	Month 25-30
Meeting faculty authorities and present the study proposal	Researchers							
Data collection	Patients and controls attending the study areas							
Biochemical Analysis	Researchers							
Data analysis	Researchers							
Writing of the results	Researchers							
Finalizing the study	Researchers							
Submission of the final project	Researchers							

بسم الله الرحمن الرحيم

جامعه شندي

كليه الدراسات العليا

استمارة مشاركه في بحث مقدم لنيل درجه الدكتوراه في الأحياء الدقيقة بعنوان:- معرفة إمكانية أن يكون لفيروس الورم الحليمي البشري دور في الإصابة بمرض سرطان الثدي وتشخيصه عن طريق اختبار تفاعل البلمرة التسلسلي

السيدة .....

أنني أقوم بدراسة لمعرفه إمكانية أن يكون لفيروس الورم الحليمي البشري دور في الإصابة بمرض سرطان الثدي وتشخيصه عن طريق اختبار تفاعل البلمره التسلسلي وهي دراسة تفيد في الكشف ومكافحه هذا الفيروس في المستقبل إن شاء الله . لذا آمل منكم المشاركة معي في هذا البحث وذلك بالسماح لي بأخذ قطعه من عينات الثدي المأخوذة للفحص وسوف تكون بياناتكم 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 ( )

Shandi University

Faculty of postgraduate study

PhD degree in clinical microbiology

Molecular Diagnosis of *Human Papilloma Virus (HPV)* Isolated from Breast Cancer in Radiation and Isotopes Center Khartoum (RICK) - Sudan

A. Basic information and Others Risk Factors:-

1. Age (in yrs) 20-30 ( ) 31-40 ( ) 41-50 ( ) over 50 ( )
2. Birth place ……
3. Duration of the illness/ years 1-2 ( ) 3-4 ( ) 5-6 ( ) 6-7 ( ) over 7( )
4. Family History :- Yes ( ) No ( )
5. Married statues :- Single ( ) Married ( ) Divorce ( )
6. Occupation:- House wife ( ) Student ( ) Employee ( ) others ( )
7. Race :- white ( ) black ( )
8. Breast feeding :- less than 1 year ( ) one year ( ) more than one year ( )
9. Dense of breast tissue :- Fatty ( ) Fibrous ( ) Granular ( )
10. Menstrual period :- Early ( ) Normal ( ) Late ( )
11. Wight :- Obese ( ) Normal ( ) Underweight ( )
12. Birth control :- contraceptive ( ) Normal control ( ) No control ( )
13. Smoking :- yes ( ) No ( )
14. Physical activity :- yes ( ) No ( )
15. Radiation :- yes ( ) No ( )

B. Cancer characteristics:

- Lymph node involvement: yes ( ) No ( )
- Metastasis: yes ( ) No ( )
- Stage: primary ( ) Moderate ( ) Chronic ( )
- Type of breast cancer (Histopathology): ……
- Grade: A( ) B( ) C ( )

C. Result of PCR:

Virus	HPV Type	Positive	Negative
HPV			

بسم الله الرحمن الرحيم
جامعة شندي
كلية الدراسات العليا
بحث مقدم لنيل درجة الدكتوراه في الاحياء الدقيقة

العنوان :- معرفة ان يكون لفيروس الورم الحليمي البشري دور في الاصابة بسرطان الثدي وتشخيصه عن طريق التفاعل التبلمر التسلسلي

أ. المعلومات الاساسيه وعوامل الضراوة الاخري

1. العمر بالسنوت:- 20-30( ) 31-40( ) 41-50 ( ) اكبر من 50 ( )
2. مدة المرض بالسنوات:- 1-2 ( ) 3-4 ( ) 5-6 ( ) 7-8( ) اكثر من 8 ( )
3. وجود المرض في الاسرة :- يوج د ( ) لا يوجد ( )
4. الحالة الاجتماعيه :- عازب ( ) متزوجه ( ) مطلقه ( )
5. الوظيفه :- ربة منزل ( ) طالبه ( ) موظفة ( ) اخري ( )
6. لون البشره :- ابيض ( ) اسود ( )
7. الرضاعه الطبيعيه :- اقل من سنه ( ) سنه واحده ( ) اكثر من سنة ( )
8. نوع نسيج الجسم :- دهني ( ) ليفي ( ) حبيبي ( )
9. الدورة الشهريه :- مبكره ( ) طبيعيه ( ) متاخره ( )
10. الوزن :- سمينه ( ) طبيعيه ( ) ضعيفه ( )
11. تنظيم الولاده :- حبوب منع الحمل ( ) تنظيم طبيعي ( ) ليس هنالك تنظيم ( )
12. التدخين :- مدخنه ( ) غير مدخنه ( )
13. الرياضه :- امارس رياضه ( ) لا امارس رياضه ( )
14. التعرض للاشعاع :- نعم ( ) لا ( )

ب. خصائص السرطان :-

1. تورط العقد الليمفاويه :- نعم ( ) لا ( )
2. الانتشار :- نعم ( ) لا ( )
3. طور الورم :- اولي ( ) متوسط ( ) مزمن ( )

نوع السرطان :- .....

درجه السرطان :- أ( ) ب( ) ج( )

C. Result of PCR:

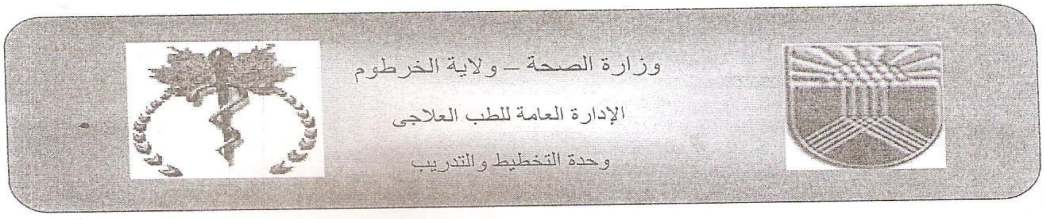
Virus	HPV Type	Positive	Negative
HPV			

D. Result of PCR:

Sample number	Positive	Negative	HPV Type

Figure (1.6) Radiation and Isotopes Center Khartoum *(RICK)*

Figure (2.6) PCR machine



التاريخ: 6/7/ 2015م

النمرة: وص / و خ/ ا ع ط ع/44/ا

السـيد /مدير عام مستشفى الطب النووي والعلاج بالإشعاع

المحترم

السلام عليكم ورحمة الله تعالى وبركاته

الموضوع/الموافقة على تنفيذ بحث

بالاشاره الى الموضوع اعلاه ، نفيد سيادتكم بأن الباحث /الباحثة / هشام عبد الله الطيب النور

بصدد أجراء بحث بعنوان : Molecular diagnosis of Human Papilloma virus (HPV) From breast cancer - Nuclear medicine and Isotope Center - Khartoum

وعلية نرجو التكرم بمساعدة الباحث وتسهيل مهمة جمع البيانات .

وجزاكـم الله خـراً ،،،،،،

ع/د/ نجـر علي عباس

مدير إدارة التدريب والبحوث

Ethical clearance