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Nourhan Mohamed Attia Mohamed

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List Of Abbreviations

Abbreviation	**Meaning**
BC	Breast cancer
LCIS	lobular carcinoma in situ
BRCA1	Breast cancer antigen 1
BRCA2	Breast cancer antigen 2
AJCC	The American Joint Committee on Cancer
TNM	tumor size (T), lymph node status (N), and distant metastasis (M)
T	Primary tumor
N	Regional lymph nodes
M	Distant metastasis
HER2	Human epidermal growth factor receptor 2
ER	Estrogen receptor
PR	Progesterone receptor
DCIS	Ductal carcinoma in situ
NST	Invasive Breast Carcinoma of No Special Type
NOS	Invasive Ductal Carcinoma
ILC	Invasive lobular carcinoma
MRI	Magnetic Resonance Imaging
US	Ultrasound
NICE	The National Institute for Health and Clinical Excellence
SIGN	Scottish Intercollegiate Guidelines Network
UK	United Kingdom
BCS	Breast conservation surgery
CT	Chemotherapy
NACT	Neoadjuvant chemotherapy
ACT	Adjuvant chemotherapy
IV	Intravenous
AET	Adjuvant endocrine therapy
GM-CSF	Granulocyte–macrophage colony-stimulating factor
ncRNAs	non-coding RNAs
lncRNAs	long non-coding RNAs
IGF2	Insulin-like growth factor 2
DMRs	differentially methylated regions
GO	Gene Ontology
SNPs	Single nucleotide polymorphisms
UTRs	Untranslated regions

GWAS	Genome-wide association studies
HCC	hepatocellular carcinoma
DNMTs	DNA methyltransferases
E2F1	E2F transcription factor 1
SAH	S-adenosylhomocysteine
PTX	Paclitaxel
CBC	Complete blood picture
HB	Hemoglobin
WBC	White blood cell
LFTs	Liver function tests
ALT	Serum alanine aminotransferase
AST	Serum aspartate aminotransferase
KFT	Kidney function tests
EDTA	Ethylene diamine tetra-acetic acid
OD	Optical density
RT	Reverse transcription
cDNA	Complementary DNA
BA	Benign adenoma
HC	Healthy controls
PCR-based RFLP	Polymerase chain reaction – based restriction fragment length polymorphism
OD	Optical density
SPSS	Statistics Package for Social Sciences
N	Numbers
χ2	Chi-square
FET	fisher exact test
SD	Standard deviation
TSG	Tumor suppressor gene
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
miRNAs	Micro RNA
DNMT1	DNA methyltransferase
E2F1	E2F transcription factor 1
AUS	Axillary ultrasound
BS	Bone scintigraphy
PET-CT	Positron-emission tomography- Computed tomography
PET-MRI	Positron-emission tomography- Magnetic Resonance Imaging
WBMRI	Whole body Magnetic Resonance Imaging

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Introduction

Breast cancer (BC) is one of the most frequently diagnosed malignancies and the leading cause of death from cancer in women worldwide. For the year 2016, it was estimated that in the US approximately 246,660 female patients would be diagnosed with BC and 40,450 would die from it ***(Siegel et al., 2016).***

Worldwide, BC is the most common cancer affecting women, and its incidence and mortality rates are expected to increase significantly the next 5–10 years ***(Greaney et al., 2015).***

In Egypt, the incidence rates continue to increase. It has been reported that BC is the most common cancer among females. It constitutes about 38.8% of all malignant tumors among Egyptian female individuals ***(Elsisi et al., 2020).***

The development of BC is a complex multistep process involving both environmental factors and genetic variations. It is well established that age, obesity, previous benign breast disease, positive family history of BC, and female menstrual and reproductive status are associated with the development of BC ***(Lin et al., 2017).***

For genetic factors, numerous single-nucleotide polymorphisms (SNPs) have been identified to be associated with an elevated risk of BC, suggesting a significant contribution of inherited factors in BC susceptibility. Therefore, the identification of additional potential SNPs could have a great impact on risk estimation for BC and provide earlier application of proper therapeutic strategies to decrease its mortality rate ***(Fejerman et al., 2014).***

The total of SNPs has been identified in cancers; nearly 10 percent were associated with a change in the amino acid sequence, while a large proportion occurred in the coding or noncoding regions. ***(Haemmerle and Gutschner, 2015***).

In recent years, long noncoding RNAs (lncRNAs), a novel kind of RNA, have attracted extensive attention for their wide range and complex regulatory functions in human diseases. lncRNAs are defined as transcribed RNA molecules that are longer than 200 nucleotides and not translated into proteins ***(Ponting et al., 2009).***

Although their functions were not originally clear, lncRNAs are now known to play critical roles in carcinogenesis, including transcriptional, posttranscriptional, and epigenetic regulation of cancer-related genes, thereby resulting in the cell-cycle progression, apoptosis, invasion, and migration ***(Zhao et al., 2015).***

The H19 lncRNA is located on human chromosome 11p15.5, encoding a 2.3 kb long, spliced, and polyadenylated non-coding RNA (ncRNA) that plays important roles in embryonic development and growth control. It acts as an imprinted gene expressed from the maternal chromosome ***(Gabory et al., 2010)***.

Accumulating evidence has demonstrated that H19 lncRNA is abnormally expressed and promotes cancer-cell proliferation in many tumors, such as hepatocellular, esophageal, and bladder cancers**,** suggesting an oncogenic function. SNPs locating on lncRNA *H19* have also been identified to regulate its expression and function ***(Gao et al., 2014).***

The aim of the work

The aim of this study aims to:

- Evaluate the efficacy of H19 lncRNA expression as potential molecular noninvasive tumor markers in diagnosis and prognosis of BC in Egyptian females.
- Evaluate the rs217727 polymorphism as possible prognostic biomarker for BC.
- Study the associations between H19 SNP (rs217727) and BC & its effect on the expression of H19 lncRNA.

Review of Literature

A. Breast Cancer Overview

❖ Epidemiology of breast cancer:

Breast cancer (BC) is the most frequently diagnosed malignant tumor and the first leading cause of cancer death among women ***(Fan et al., 2014)***.

For the year 2016, it was estimated that in the US approximately 246,660 female patients would be diagnosed with BC and 40,450 would die from it ***(Siegel et al., 2016)***. BC is the most common malignancy in women in the United States and is second only to lung cancer as a cause of cancer death ***(Gradishar et al., 2016).*** In China, the incidence of BC has increased rapidly in recent years and become the most common cancer for women in major cities ***(Chen et al., 2016)***. In China, an increasing trend in mortality is observed for 3 of the 10 most common cancers (breast, cervix, and ovary), while it tends to be stable for others such as colorectal, lung, uterine, and thyroid cancers ***(Chen et al., 2015).*** BC alone is expected to account for 29% of all new cancer cases diagnosed in American women; also it represents about 14% of all estimated cancer death in American women (Fig. 1). BC is the most common malignancy in young women aged 15-39 years, and young age is an independent risk factor for death from breast cancer ***(Livi et al., 2010).***

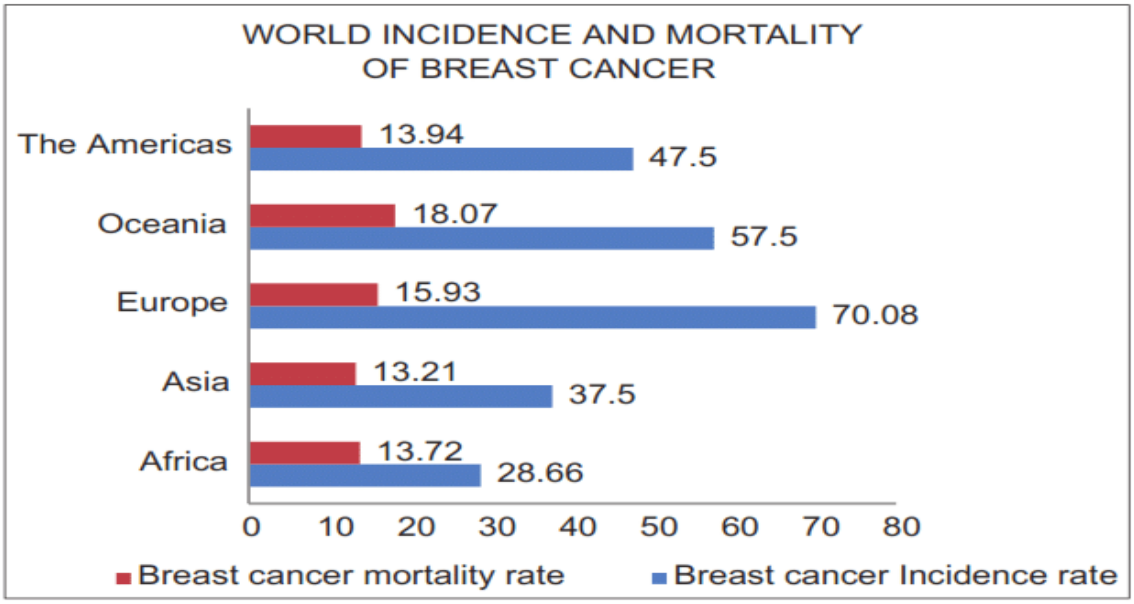

Fig. (1): Rate of BC incidence and mortality worldwide according to 2012 world cancer report. Quoted from ***Donepudi et al. (2014)***.

Despite important advances in research, BC remains a major health problem and represents a top biomedical research priority. The incidence of this aggressive disease with approximately 1,7000,000 new cases each year remains alarmingly high; these rates are suggestive of slow progress made in the prevention setting ***(DeSantis et al., 2014***). Nevertheless, for women with already established diagnosis mortality rates have been improved, but unfortunately the median survival in the metastatic setting is dramatically low (*24 months). Worldwide, BC is the most common cancer affecting women, and its incidence and mortality rates are expected to increase significantly the next 5–10 years ***(Greaney et al., 2015).*** Early diagnosis and more effective treatment strategies have diminished the mortality rates in recent years ***(Filipova et al., 2014).***

BC, one of the most common diagnosed invasive malignancies with the highest cancer incidence rate, remains a leading cause of cancer death in

females worldwide and has led to 522,000 death since 2008 ***(Sabatier et al.,2014).*** Although great advancement has been made in the diagnosis and therapy, such as surgery, radiation, and chemotherapy, the mortality rate of breast cancer has not dramatically changed. ***(Tilli et al., 2016).*** BC accounts for approximately one million new cases and leads to more than 400,000 deaths per year in the world ***(Chen et al., 2017).***

- ***Incidence in Egypt:***

In Egypt, BC is the most frequent cancer among Egyptian females (fig. 2). It represents about 38% of all reported cancer cases in Egyptian females, with an average age of 49.6 per 100,000 populations, with higher incidence in urban areas compared to rural areas ***(Dey et al., 2010)***. It is also the leading cause of cancer- related mortality accounting for 29.1% of their total with 6546 deaths. These estimates are confirmed in many regional Egyptian cancer registries as well as in hospital-based frequencies ***(Zeeneldin et al., 2013).***

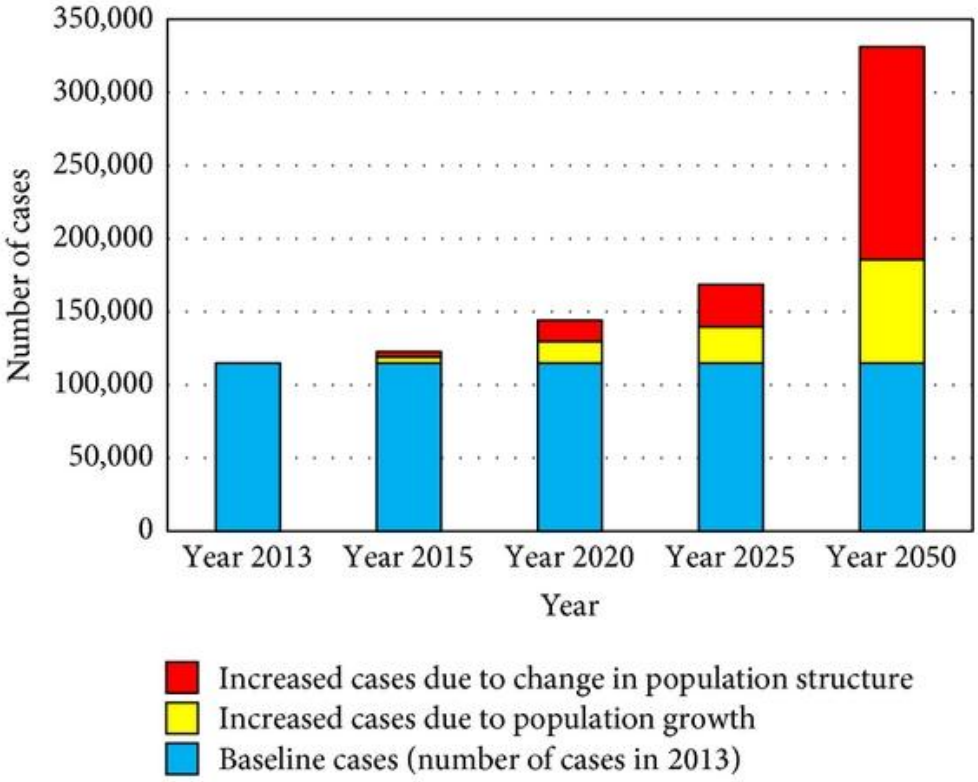

Fig. (2): Estimated number of cases in Egypt (2013–2050) and causes of the increase in cases. Quoted from ***Ibrahim et al. (2014).***

❖ ***Risk factors of breast cancer:***

There're numerous risk factors such as sex, aging, estrogen, family history, gene mutations and unhealthy lifestyle, which can increase the possibility of developing BC ***(Majeed et al., 2014).***

Risk factors for BC can be divided into 7 broad categories ***(Doren et al., 2018)*** showed in fig. (3):

- ***Age***: The age-adjusted incidence of breast cancer continues to increase with advancing age of the female population.

- ***Gender:*** Most breast cancers occur in women.

- ***Personal history of breast cancer***: A history of cancer in one breast increases the likelihood of a second primary cancer in the contralateral breast.

- ***Histologic risk factors***: Histologic abnormalities diagnosed by breast biopsy constitute an important category of breast cancer risk factors. These abnormalities include lobular carcinoma in situ (LCIS) and proliferative changes with atypia.

- ***The family history of breast cancer and genetic risk factors***: First-degree relatives of patients with breast cancer have a 2-fold to 3-fold excess risk for development of the disease. Five percent to 10% of all BC cases are due to genetic factors, but they may account for 25% of cases in women younger than 30 years. BRCA1 and BRCA2 are the 2 most important genes responsible for increased BC susceptibility.

- ***Reproductive risk factors:*** Reproductive milestones that increase a woman's lifetime estrogen exposure are thought to increase her BC risk. These include the onset of menarche before 12 years of age, first live childbirth after age 30 years, nulliparity, and menopause after age 55 years.

- ***Exogenous hormone use:*** Therapeutic or supplemental estrogen and progesterone are taken for various conditions, with the two most common scenarios being contraception in premenopausal women and hormone replacement therapy in postmenopausal women.

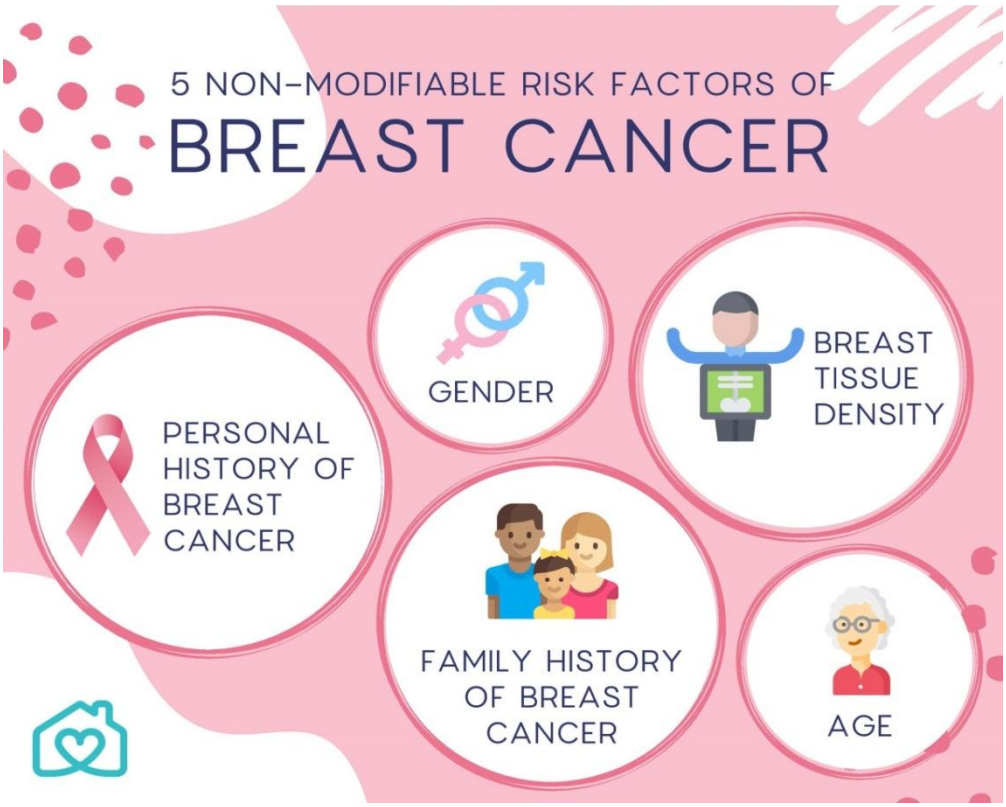

Fig. (3): BC risk factors. Quoted from ***Doren et al. (2018).***

❖ ***BC classification:***

BC can be classified by the different schemata:

(1) Clinical classification

The American Joint Committee on Cancer (AJCC) provides two principal groups for BC staging: anatomic, which is based on extent of cancer as defined by tumor size (T), lymph node status (N), and distant metastasis (M); and prognostic, which includes anatomic TNM plus tumor grade and the status of the biomarkers (human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR)). The prognostic stage group is preferred for patient care and is to be used for reporting of all cancer patients in the United States ***(Amin et al., 2017).***

Table 1: TNM Classification for Breast Cancer; American Joint Committee on Cancer ***(Amin et al., 2017):***

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
T1	Tumor ≤ 20 mm in greatest dimension
T1mi	Tumor ≤ 1 mm in greatest dimension
T1a	Tumor > 1 mm but ≤ 5 mm in greatest dimension
T1b	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	Tumor > 50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules), not including invasion of dermis alone
T4a	Extension to chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema

T4c	Both T4a and T4b
T4d	Inflammatory carcinoma
Regional lymph nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis (on imaging or clinical examination)
N1	Metastasis to movable ipsilateral level I, II axillary lymph node(s)
N1mi	Micrometastases (approximately 200 cells, larger than 0.2 mm, but none larger than 2.0 mm)
N2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
N2a	Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastases only in ipsilateral internal mammary nodes and in the absence of axillary lymph node metastases
N3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s), with or without level I, II axillary node involvement, *or* in ipsilateral internal mammary lymph node(s) with level I, II axillary lymph node metastasis; *or* metastases in ipsilateral supraclavicular lymph node(s), with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

Distant metastasis (M)	
M0	No clinical or radiographic evidence of distant metastasis
cM1	Distant metastases detected by clinical and radiographic means
pM1	Any histologically proven metastases in distant organs; or if in non-regional nodes, metastases > 0.2 mm

Table 2: Anatomic stage/prognostic groups; American Joint Committee on Cancer ***(Amin et al., 2017):***

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T0	N1mi	M0
	T1	N1mi	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

(2) WHO classification (histo-pathological classification)

WHO tumor classification of the breast covers not only invasive breast cancers, but also precursor lesions, lesions of low malignant potential, benign epithelial proliferations, fibroepithelial, myoepithelial and mesenchymal neoplasms, among others ***(Lakhani et al., 2012).***

Invasive Breast Carcinoma of No Special Type (NST), Previously Known as Invasive Ductal Carcinoma (NOS): The terminology for the most common type of BC has changed from invasive ductal carcinoma, not otherwise specified (NOS) (2003) to invasive carcinoma of no special type (NST) (2012). This group of BC comprises all tumors without the specific differentiating features that characterize the other categories of breast cancers. The 2012 definition of invasive carcinomas (NST) is identical to the 2003 definition of invasive ductal carcinoma (NOS), except that the name 'ductal' has been omitted in the new terminology. The rationale for this is that the term 'ductal' conveys unproven histogenetic assumptions (derivation of the tumors from the ductal system) and that invasive ductal carcinoma (NOS) does not comprise a uniform group of carcinomas. ***(Lakhani et al., 2012)***

Carcinomas of mixed type have a specialized pattern in at least 50% of the tumor and a non-specialized pattern in between 10% and 49%. These tumors are designated as mixed invasive NST and special type or mixed invasive NST and lobular carcinoma ***(Sinn and Kreipe, 2013).***

Special Subtypes of Invasive Breast Carcinoma

The most common specific subtypes include invasive lobular, tubular, cribriform, metaplastic, apocrine, mucinous, papillary, and micropapillary carcinoma, as well as carcinoma with medullary, neuroendocrine, and salivary gland/skin adnexal type features. These specific tumor types are defined by their morphology, but are also linked to particular clinical, epidemiological, and molecular features, With invasive lobular carcinoma (ILC), variants such as solid, alveolar, pleomorphic, tubulolobular, and

mixed variants are recognized and related to differences in prognosis when compared to ILC of classic type ***(Lakhani et al., 2012).***

Tubular carcinoma and invasive cribriform carcinoma are carcinomas with a particularly favorable prognosis and similar low-grade tumor nuclear features. The new WHO classification stresses the strict diagnostic requirements for these tumor types with their characteristic features being present in > 90% of the tumor ***(Huo et al., 2011).***

Carcinomas with medullary features are an overlapping group of tumors with more or less 'medullary' appearance, and are described in a separate chapter of the new WHO classification. The authors advocate abandoning the terms medullary carcinoma, atypical medullary carcinoma and invasive carcinoma (NST) with medullary features, and recommend using the term carcinoma with medullary features for this group of tumors because of the overlapping morphological and immunohistological features and low interobserver reproducibility ***(Niemeier et al., 2010).***

Metaplastic carcinoma represents a group of unrelated invasive breast cancers displaying differentiation of the tumor cells into squamous or mesenchymal-looking elements. This includes, but is not limited to, spindle, chondroid, osseous, and rhabdomyoid cells, and these elements may be mixed with carcinoma of usual type. Depending on their cellular features, metaplastic carcinomas may be either low-grade tumors (e.g. low-grade adenosquamous carcinoma or low-grade spindle cell carcinoma), or high-grade tumors (e.g. high-grade squamous cell carcinoma, or high-grade spindle cell carcinoma ***(Lee et al., 2012).***

Adenoid cystic carcinoma is the most frequently encountered salivary-type tumor of the breast and is, in the great majority of cases, a low-grade malignant tumor ***(Foschini et al., 2010).***

Mucinous carcinomas and carcinomas with signet-ring cell differentiation are described together in the new WHO classification. Carcinomas with signet-ring cell differentiation are listed among the invasive breast carcinomas, but are not regarded to represent a tumor type of its own. Rather, signet-ring cell differentiation may be observed either with invasive lobular carcinomas (mostly), where it is pattern seen with the pleomorphic variant or with invasive carcinomas NST, and, rarely, also with high-grade mucinous carcinomas that otherwise show predominantly extracellular mucin production. No specific prognosis is believed to be associated with signet-ring cell differentiation ***(Lacroix-Triki et al., 2010).***

Invasive papillary carcinoma of the breast is regarded as a specially differentiated adenocarcinoma of the breast with papillary morphology, but otherwise no distinguishing clinical, genetic, or prognostic features ***(Sinn and Kreipe, 2013).***

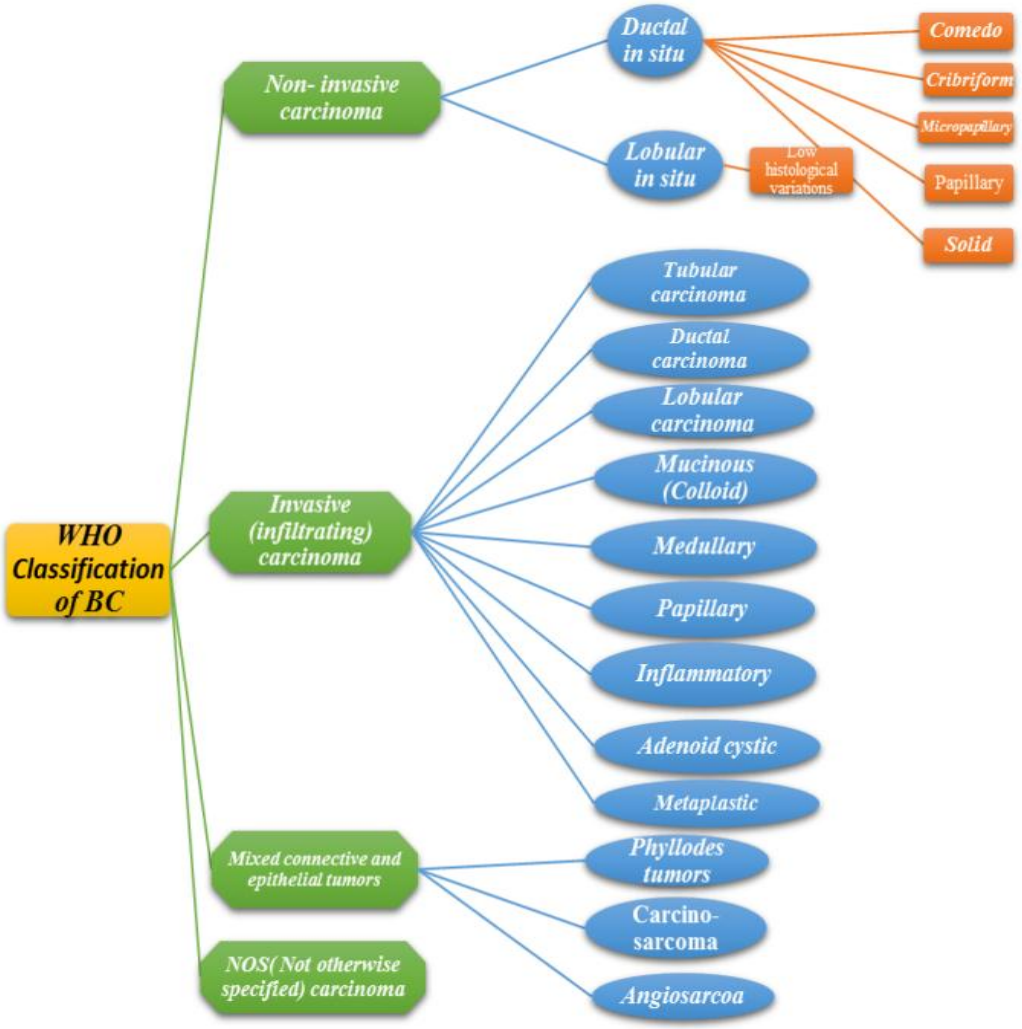

Figure (4): Histopathological classification of BC. Quoted from ***Makki (2015).***

(3) Molecular classification

Perou and Sorlie proposed "Molecular Classification" terminology in BC for the first time with a comprehensive study showing the differences in gene expression in 2000 ***(Perou et al., 2000).*** In this study, BC was divided into different sub-groups according to various gene expression: "Luminal" (often differentiated in two or three subgroups; reflecting ER, ER regulatory genes and the expression of genes expressed in normal luminal epithelial cells), "HER-2 positive "(reflecting HER-2 amplification and

overexpression)," basal "(reflecting ER, PR, and HER-2 negative and the expression of genes expressed in normal breast basal and / myoepitelial cells). A normal-like subgroup has been described, but the importance of identifying this subgroup and its consequences are not clear, because it seems to represent samples with low tumor cell content and more normal tissue components ***(Perou et al., 2000).***

Detection of difference in response to treatment and metastatic pattern according to molecular subtypes further increased the value of molecular classification. Ultimately, the idea that a patient with BC can be classified according to the molecular subtype of the tumor and thus directed to appropriate, specific, targeted therapies has become very attractive. Nowadays the search for specific, targeted, personalized treatment programs are ongoing in all types of cancer ***(Kennecke et al., 2010).***

Table (3): Major molecular subtypes of BC ***(Eliyatkın et al., 2015):***

	Molecular Subtype			
	Luminal A	**Luminal B**	**HER2/neu**	**Basal like[a]**
Gene expression pattern	Expression of luminal (low molecular weight) cytokeratins, high expression of hormone receptors and related genes	Expression of luminal (low molecular weight) cytokeratins, moderate-low expression of hormone receptors and related genes	High expression of HER2/*neu*, low expression of ER and related genes	High expression of basal epithelial genes and basal cytokeratins, low expression of ER and related genes, low expression of HER2/*neu*
Clinical and biologic properties	50% of invasive bresat cancer, ER/PR positive, HER2/neu negative	20% of invasive breast cancer, ER/PR positive, HER2/neu expression variable, higher proliferation than Luminal A, higher histologic grade than Luminal A	15% of invasive breast cancer, ER/PR negative, HER2/neu positive, high proliferation, diffuse TP53 mutation, high histologic grade and nodal positivity	~15% of invasive breast cancer, most ER/PR/HER2/*neu* negative (triple negative), high proliferation, diffuse TP53 mutation, BRCA1 dysfunction (germline, sporadic)
Histologic correlation	Tubular carcinoma, Cribriform carcinoma, Low grade invasive ductal carcinoma, NOS, Classic lobular carcinoma[b]	Invasive ductal carcinoma, NOS Micropapillary carcinoma	High grade invasive ductal carcinoma, NOS	High grade invasive ductal carcinoma, NOS Metaplastic carcinoma, Medullary carcinoma
Response to treatment and prognosis	Response to endocrine therapy	Response to endocrine therapy (tamoxifene and aromatase inhibitors) not as good as Luminal A	Response to trastuzumab (Herceptin)	No response to endocrine therapy or trastuzumab
	Variable response to chemotherapy	Variable response to chemotherapy (better than Luminal A)	Response to chemotherapy with antracyclins	Sensitive to platinum group chemotherapy and PARP inhibitors
	Good prognosis	Prognosis not as good as Luminal A	Usually unfavorable prognosis	Not all, but usually worse prognosis

❖ *BC Diagnosis:*

1- Screening:

BC is generally diagnosed through either screening or a symptom (e.g., pain or a palpable mass) that prompts a diagnostic examination. Screening of healthy women is associated with the detection of tumors that are smaller,

have lower odds of metastasis, are more amenable to breast-conserving and limited axillary surgery, and are less likely to require chemotherapy ***(Fuller et al., 2015)***.

The only screening modality proven to reduce breast cancer–specific mortality is mammography. As a result, screening mammography is recommended by the American Cancer Society beginning at age 45, or sooner depending on individual preference ***(Pace and Keating, 2014).***

Supplementing mammography with other imaging modalities for higher-risk patients leads to the additional detection of mammographically occult cancers. A meta-analysis of 14 studies of high-risk women found that MRI had a higher sensitivity for malignancy (84.6%) than mammography (38.6%) or ultrasound (US) (39.6%). Further, the use of MRI as an adjunct to mammography had a higher sensitivity for malignancy (92.7%) than the use of US as an adjunct to mammography (52%), US is a viable option for the screening of high-risk women who cannot have breast MRI or women with intermediate risk, such as those with dense breasts ***(Lehman, 2012).***

2- Pathologic Evaluation and Specimen Processing and Evaluation:

In clinical practice, diseased tissue is usually obtained by fine-needle aspiration, core biopsy, or surgical excision. A diagnostic challenge for pathologists is the distinction of closely related diseases, such as atypical ductal hyperplasia and in situ disease, in situ disease and micro invasion, or ductal cancer and lobular cancer. The size of the tumor is determined by careful clinical and pathologic correlation. When a breast cancer forms a distinct mass outward from a point of origin, the size can be easily assessed

by imaging and gross pathologic examination, accurate sizing can be challenging ***(Brem et al., 2015)***.

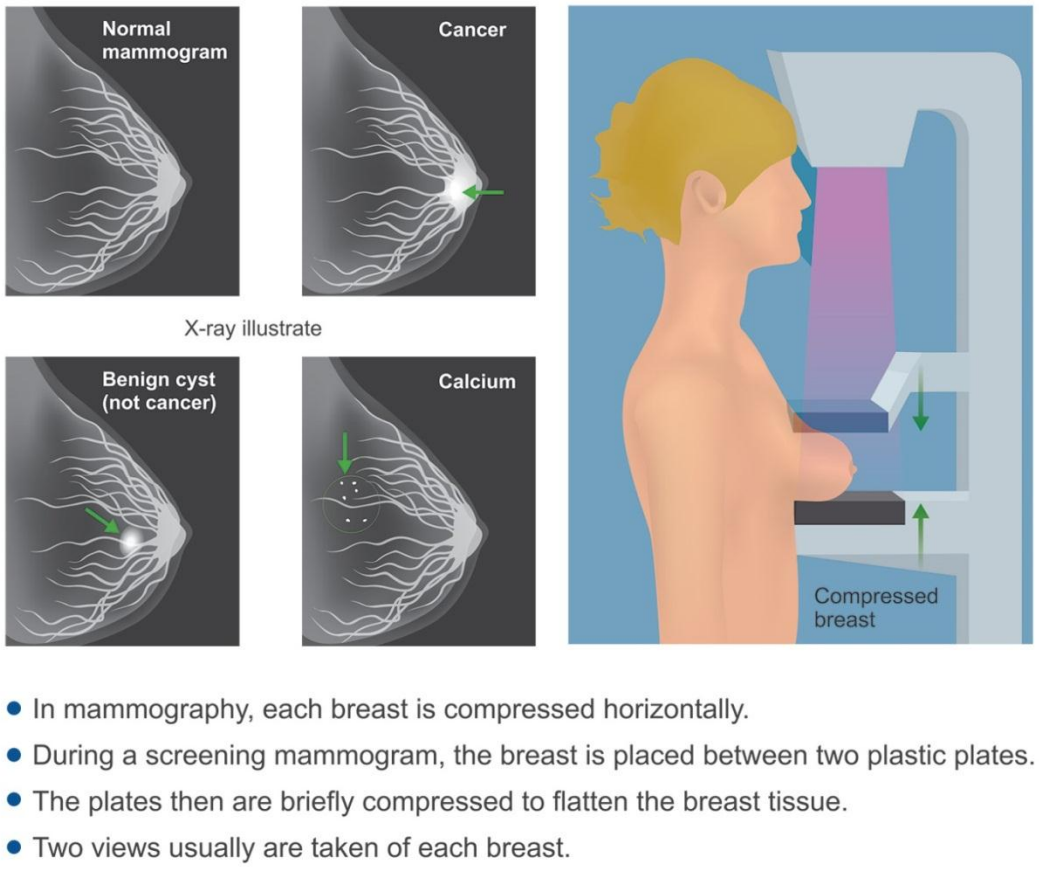

Fig. (5): Mammography. Quoted from ***Brem et al. (2015).***

3- Predictive Tumor Markers:

Critical treatment decisions are made on the basis of protein expression assays that are independent of tumor morphologic characteristics. IHC analysis of paraffin sections is routinely performed for the evaluation of ER, PR and HER2 status. Although widely used to predict responses to targeted agents, histologic tumor markers are limited by significant

intratumoral variation, even within a single biopsy specimen ***(Bennett and Farah, 2014).***

4- Imaging and Staging:

Physical examination, mammography, or US for the diagnostic work-up of a patient with newly diagnosed BC is usually sufficient for local–regional staging. MRI is sometimes recommended, especially when a patient is younger, a genetic mutation or multifocal disease is suspected, or a mammogram or US yields indeterminate findings. Further, it is possible that small additional cancers detected by MRI would never be clinically significant or responsible for a local recurrence because of adjuvant systemic or whole-breast radiation treatments ***(Dorn et al., 2013).***

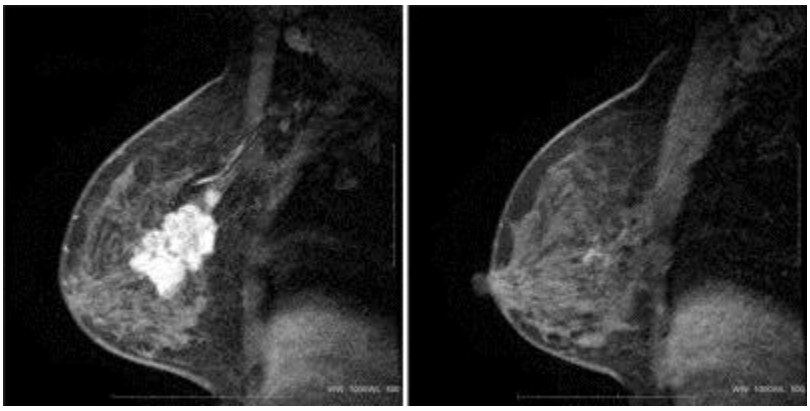

Fig. (6): MRI showing Breast mass. Quoted from ***Li et al. (2015).***

A chest radiograph and routine laboratory blood tests are sufficient for staging in a patient with clinical stage I or II breast cancer and no specific symptoms of metastatic disease. For suspected advanced (stage IIIB/C or IV) disease, National Comprehensive Cancer Network guidelines (version 1.2015) recommend either chest, abdomen, and pelvis CT or chest CT with

abdomen and pelvis MRI as well as bone scan or sodium fluoride PET/CT ***(Xu et al., 2012).***

❖ ***BC Treatment:***

The National Institute for Health and Clinical Excellence (NICE) and Scottish Intercollegiate Guidelines Network (SIGN) have both published guidelines to improve and standardize breast cancer treatment in the UK ***(SIGN, 2014).***

There are several treatment regimens available for BC. The choice of treatment is dependent on certain factors such as the type of BC, the size of the breast tumor, the stage and grade of the tumor, the menstrual status of the patient, expression of certain proteins and endocrine receptors and general health of the patient. Five treatment options available in clinics include surgical resection, radiotherapy, immunotherapy, molecular based therapy (endocrine and biological as targeted anti-HER2 treatment therapy) and chemotherapy ***(Goldhirsch et al., 2013).***

1- Surgery:

The primary means of local and regional BC treatment remains surgical intervention. During the first half of the 20th century, women diagnosed with BC were commonly treated by radical mastectomy, as first described by William Stewart Halsted in 1894. Breast conservation surgery (BCS) was pioneered by Fischer et al. ***(Fischer et al., 2014***), who reported that survival with lumpectomy and radiation was equivalent to that with mastectomy in the treatment of early breast cancer. It considered the primary treatment for BC, either mastectomy, in which the whole breast is removed, or removal of

the tumor only with a safety margin (lumpectomy). If the regional lymph nodes are affected, they are also removed ***(EBCTCG et al., 2011)***.

2- Radio therapy:

In women with early BC prescribed radiotherapy after tumor excision or mastectomy, the effective dose of radiation is adjusted to balance the risk of local cancer recurrence against the risk of harmful effects on healthy tissues. Radiotherapy reduces the risk of local relapse by about 70% and reduces BC mortality ***(Clarke et al., 2005)***.

3- Chemotherapy (CT):

It is a systemic treatment that involves the use of a drug or a combination of drugs that are cytotoxic to cancer cells. Rapidly dividing normal cells can also be affected by these cytotoxic drugs but are more likely to undergo repair ***(Petit et al., 2011)***.

There are three major types of chemotherapy:

- Neoadjuvant chemotherapy (NACT): Defined as the administration of systemic therapy prior to surgical removal of a breast tumor, neoadjuvant chemotherapy was originally designed to be used in patients with locally advanced disease in order to convert inoperable tumors into operable tumors. Since the introduction of this concept, the significance of neoadjuvant chemotherapy in increasing the rate of conservation therapy and the associated reduced morbidity and better self-image has been fully acknowledged ***(Shin et al., 2013).***

- Adjuvant chemotherapy (ACT): given in addition to surgery and/or radiotherapy to eliminate micro-metastasis and improve risk of disease-free survival and decrease the risk of recurrence of cancer cells ***(Petit et al., 2011).***

- Palliative chemotherapy: is an interdisciplinary medical caregiving approach aimed at optimizing quality of life and mitigating suffering among people with serious, complex illness (***Zhukovsky et al., 2019)***.

Table (4): The most effective drugs for treating early and locally advanced BC ***(Tuffery et al., 2018)***:

Drug (abbreviation)	Brand name	Pill or Intravenous (IV) drug
1- Capecitabine	Xeloda	Pill
2- Carboplatin (C)	Paraplatin	IV drug
3- Cyclophosphamide (C)	Cytoxan	Pill or IV drug
4- Docetaxel (T)	Taxotere	IV drug
5- Doxorubicin (A)	Adriamycin	IV drug
6- Epirubicin (E)	Ellence	IV drug
7- Methotrexate (M)	Various brand names	Pill or IV drug
8- Paclitaxel (T)	Taxol	IV drug

The benefit of CT is more pronounced in ER-negative BC. CT is recommended in the majority of TNBC, in HER2-positive BC, and in high-risk luminal tumors. The current CT standards in early BC are anthracyclines and taxanes, given as a combination or in sequence over a period of 18–24 weeks. Generally, recommended regimens do not differ between neoadjuvant and adjuvant settings. The EBCTCG meta-analysis

suggested that anthracycline and taxane-containing CT reduced 10-year BC mortality by about one-third ***(Lancet et al., 2012).***

4- ***Endocrine (hormonal) therapy:***

Adjuvant endocrine therapy (AET) (including tamoxifen and aromatase inhibitors [AIs]) is widely recognized as a critical component of BC treatment for women with hormone receptor–positive disease (***Chlebowski et al., 2014)***. Clinical guidelines have historically recommended AET to women with hormone receptor–positive disease for five years following primary treatment (***Burstein et al., 2010).*** Updated guidelines now recommend as many as 10 years of continuous therapy in light of emerging data demonstrating increased survival benefits for a longer period of treatment (***Regan, 2015).***

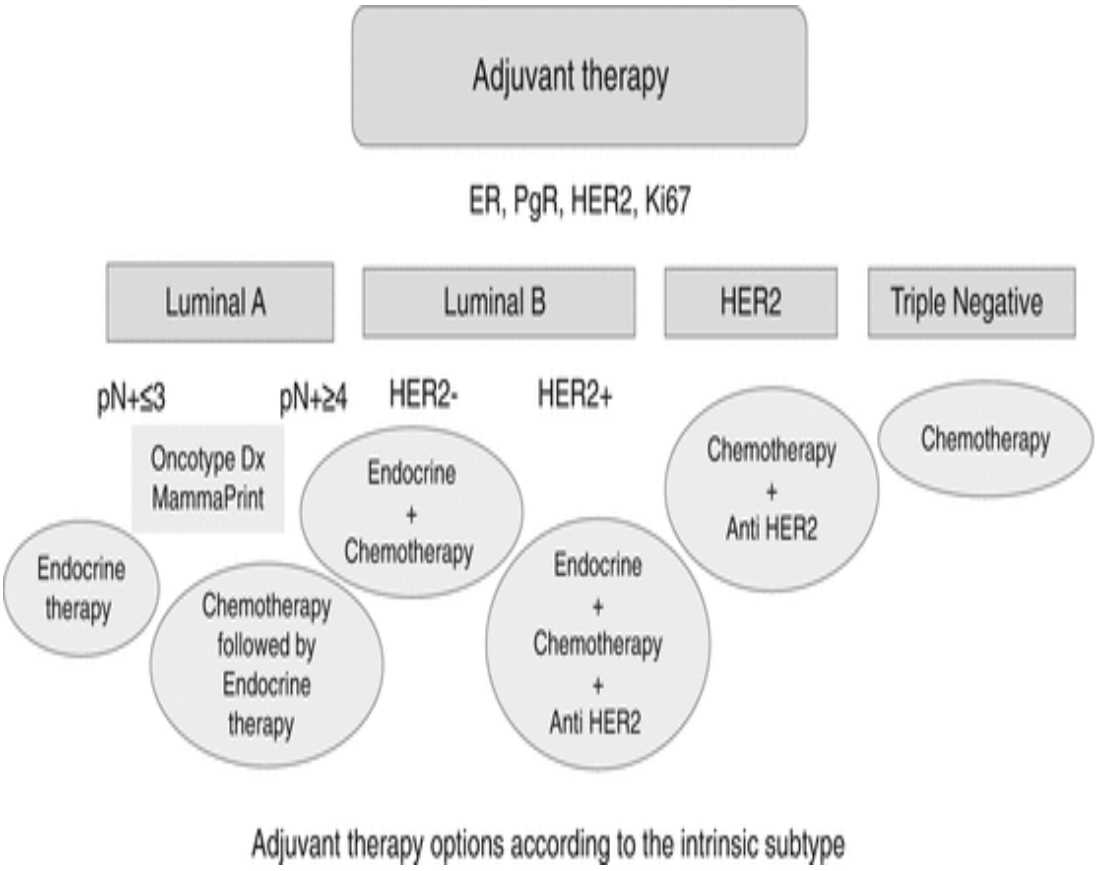

Fig. (7): In patients with luminal tumors, several multigene assays like MammaPrint and Oncotype DX assess long-term relapse risk, duration of adjuvant ET, and adoption of CT. Quoted from ***Tangoku et al. (2018).***

5- Immunotherapy:

It is one of the important options in the treatment of cancer as it can directly target the tumor and its microenvironment. Thus, it is possible to have individualized therapy with less toxicity and less side effects ***(Seledtsov et al., 2015).*** The main purpose of cancer immunotherapy is re-activating the immune system which is silenced by the tumor cells in various ways and making the tumor cells become glands ***(Visage and Joubert, 2010).*** The mainly used treatment methods in cancer immunotherapy are cancer vaccines, adoptive cell therapy, cytokines and monoclonal antibodies

(Karlitepe et al., 2015). Cancer vaccines try to influence the immune system cells by creating an attack against the cancer cells. Cancer vaccines are designed to induce tumor-specific or tumor-reactive immunoreactivity in vivo ***(Özlük et al., 2017).*** Cytokines are chemicals produced by some immune system cells. Cytokines play an important role in the production and activity of the immune system cells and blood cells. Although there are many different types, the most commonly used are interleukins, interferons, and granulocyte–macrophage colony-stimulating factor (GM-CSF) ***(Barbaros and Dikmen, 2015).*** Monoclonal antibody *therapy*: HER2 positive tumors can be effectively treated with the systemic HER2 protein-targeting monoclonal antibody trastuzumab, which considerably improves patient survival and decreases the tumor size e.g transtuzamab ***(Stern, 2012)***.

B- Overview on long non coding RNA H19 gene

❖ Introduction:

Approximately 93% of human genome DNA is transcribed into RNAs, but <2% of these nucleotide sequences can code for proteins, while the other 98% are non-coding RNAs (ncRNAs) that partially or completely lack the ability to be coded into proteins. The majority of these ncRNAs are known as long non-coding RNAs (lncRNAs) whose length exceeds 200 nucleotides ***(Kapranov et al., 2007)***. LncRNAs were at first regarded as the 'noise' of gene transcription. According to the position where they are relative to the protein-coding genes, lncRNAs can be roughly divided into antisense lncRNAs, enhancer lncRNAs, large intergenic non-coding RNAs, bidirectional lncRNAs and intronic transcript lncRNAs ***(Dahariya et al., 2019).***

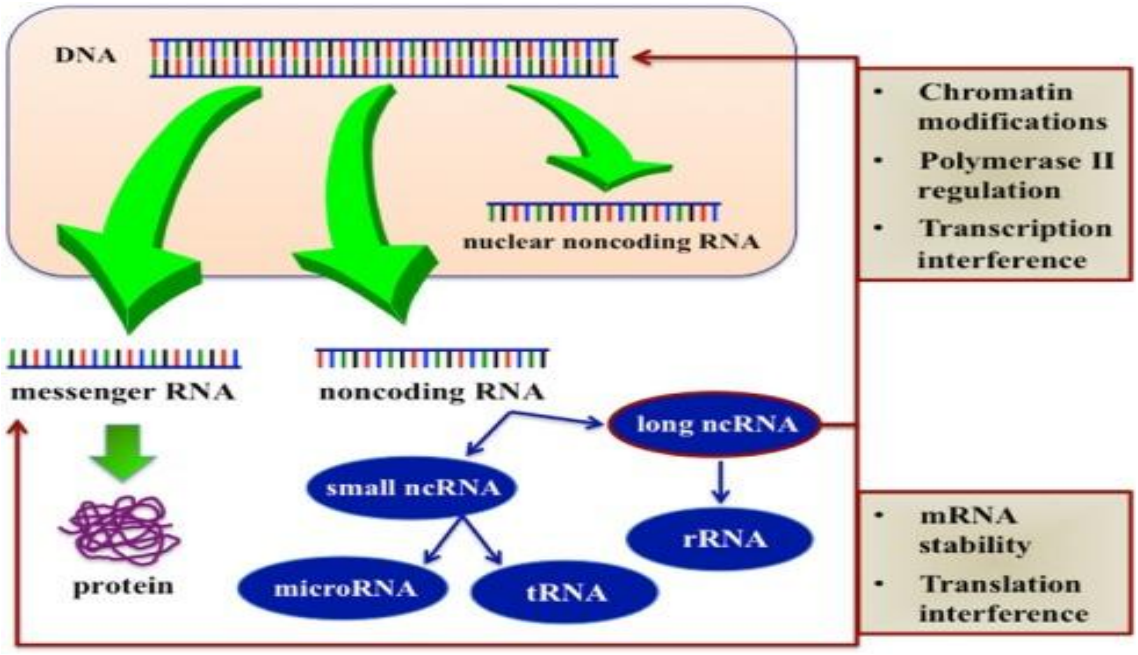

Fig. (8): Non coding RNA and its types. Quoted from ***Giuseppe Palmieri et al. (2017).***

❖ **Structure of H19 gene:**

LncRNAs resemble mRNAs as they are generally transcribed by RNA polymerase II, 5′ capped, 3′ polyadenylated, and often undergo splicing of multiple exons via canonical genomic splice motifs ***(Rutenberg-Schoenberg et al., 2016).*** There are four main locations in which lncRNAs can originate that further aid in their classification. LncRNAs can be genomically located between two protein coding genes (intergenic lncRNA), transcribed from a promoter of a protein-coding gene, yet in the opposite direction (bidirectional lncRNA), originate from the antisense RNA strand of a protein coding gene (antisense lncRNA), or overlap with one or more introns/exons of different protein-coding genes in the sense RNA strand (sense-overlapping lncRNAs) ***(Ma et al., 2013***).

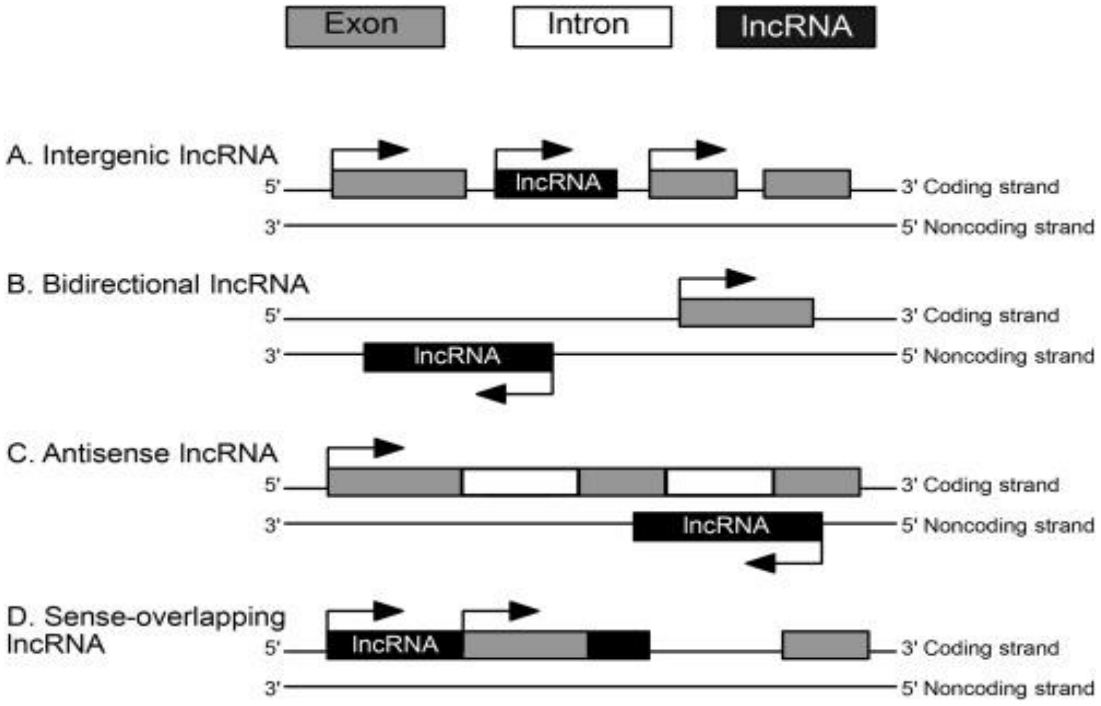

Fig. (9):LncRNA classification based on genomic location. Quoted from ***Rutenberg-Schoenberg et al. (2016).***

One of the *LncRNA* found in humans is H19. This RNA which is transcribed by RNA polymerase II, spliced and polyadenylated, seems to have a role in some forms of cancer. H19 lncRNA is expressed from both parental alleles in the early placentae (6–8 weeks gestation), it is expressed exclusively from the maternal allele on chromosome 11p15.5 after 10 weeks gestation. This is due to a differentially methylated region which is also an imprinting control region. The paternal allele of the H19 gene is methylated and silent as well. On the other hand, the maternal allele is unmethylated and expressed ***(Gabory et al., 2010***).

Being adjacent to the insulin-like growth factor 2 (IGF2) gene, and is expressed only from the maternally inherited chromosome, while IGF2 is expressed only from the paternally inherited chromosome. H19, a lncRNA, is the transcription product of the H19 gene, and diversified transcript variants exist due to alternative splicing. Although H19 RNA molecules can be detected in both the cytoplasm and nucleus, H19 RNA primarily exists in cytoplasm ***(Raveh et al., 2015)***. Moreover, differentially methylated regions (DMRs), which lie upstream of H19, were found to be critical in the regulation of H19 gene expression ***(Park et al., 2014)***. DMRs are commonly considered CpG-rich and frequently meet the criteria for CpG islands. Therefore, it is likely that some DMRs are related to genetic or epigenetic modifications of tissue-specific imprinted genes ***(Reik et al., 2001).***

- **Expression and function of the H19 gene:**

Tumorigenesis is a multistep process that involves both the neoplastic tissue and its surroundings. In order to survive and flourish, cancer cells acquire a unique genetic background, proliferate rapidly, evade growth

suppressors, cell death pathways and immune system attacks, and resist multiple drug treatments. In many aspects, a cancer cell resembles an embryonic cell, they share extraordinary plasticity, proliferation, motility and invasiveness capabilities, as well as the ability to make metabolic adjustments and other attributes, all orchestrated by common molecular pathways and epigenetic patterns, one of the pivotal players in both embryonic development and tumorigenesis is the oncofetal lncRNA gene H19 ***(Hanahan and Weinberg, 2011).***

H19 is highly expressed in the developing embryo, mainly in mesoderm- and endoderm-derived tissues. Its expression is strongly down-regulated after birth, except in cardiac and skeletal muscle, suggesting that it may play a role during muscle differentiation and explaining why it was found in the MyoD screening ***(Poirier et al., 1991).***

H19 functions in the form of regulatory RNA or ribosome regulators. H19 promotes biological processes such as apoptosis, angiogenesis, inflammation and cell death ***(Yoshimura et al., 2018).*** Furthermore, Gene Ontology (GO) analyses predicted that H19 is connected with neurogenesis, angiogenesis and inflammation through DNA transcription, RNA folding, methylation and gene imprinting. The aberrant expression of H19 is associated with multiple diseases, including carcinoma, sarcoma, type 2 diabetes and hypertrophic cardiomyopathy ***(Prasanth and Spector, 2007***).

In recent years, lncRNAs have been implicated in a variety of regulatory processes, ranging from X chromosome inactivation, genomic imprinting and chromatin modification to transcriptional activation, transcriptional interference and nuclear trafficking ***(Gomez et al., 2018***). The

exact mechanisms by which these lncRNAs exert their effects remain unclear. Nevertheless, it has become apparent that lncRNAs can act both in cis and in trans, and that some function as precursors for short ncRNAs, while others act independently as long transcripts ***(Zeng et al., 2019).*** The transcriptions of most lncRNAs are cell type and disease-specific expression pattern and was found to be differentially expressed in breast cancer tissues compared with normal breast tissues.

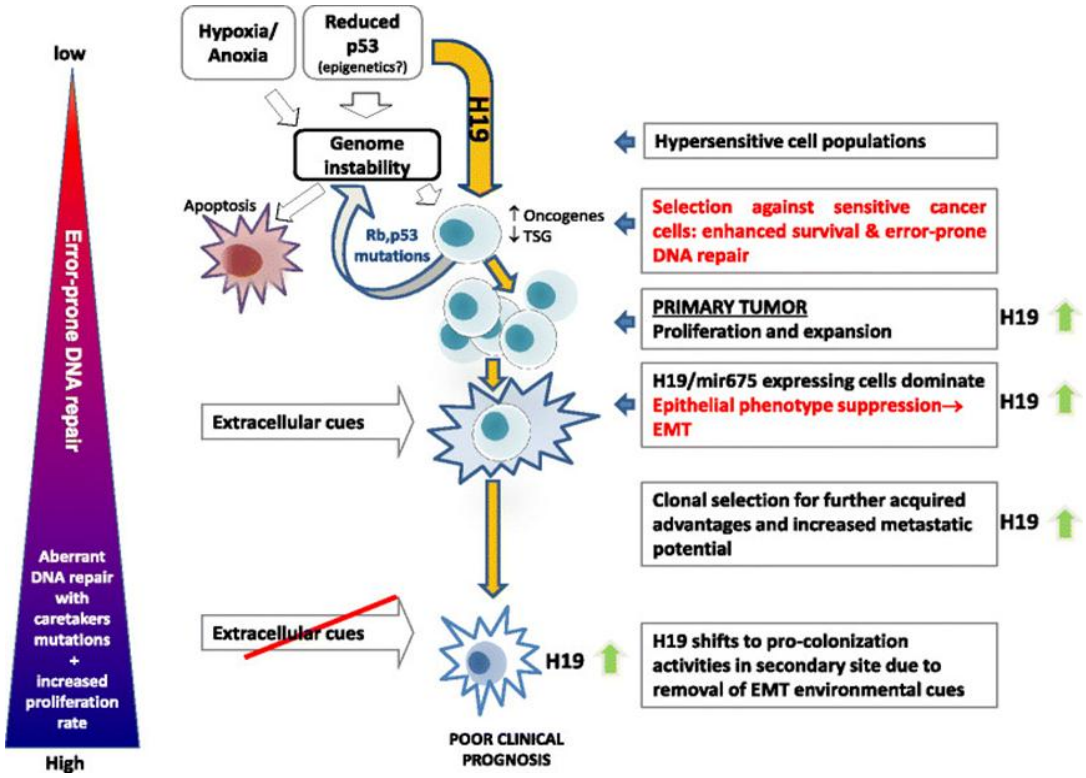

Fig. (10): Outlines for H19 functions during tumor progression. Quoted from ***Raveh et al. (2015).***

Accumulating evidence has demonstrated that H19 lncRNA is abnormally expressed and promotes cancer-cell proliferation in many tumors, such as BC and hepatocellular, esophageal, and bladder cancers suggesting an oncogenic function ***(Gao et al., 2014).***

The function of the vast majority of lncRNAs is currently a mystery despite this recent progress. Indeed, doubts have been raised as to whether these remaining transcripts are functional at all. Certainly, lncRNAs lack discernable features to facilitate categorization and functional prediction. And yet, there are several reasons to believe that many of these lncRNAs are likely to be functional, their expression is often tissue- and/or cell-specific and localized to specific subcellular compartments, which suggests they are regulated and biologically significant ***(Dinger et al., 2008***).

Second, as mentioned earlier, there are already numerous precedents of lncRNAs having function, and the number of examples will continue to grow as research in this fledgling area continues. Finally, Willingham and colleagues recently screened several hundred novels lncRNAs for function in a limited battery of cell-based assays and successfully identified multiple functional ncRNAs, which highlights the untapped functional potential of these transcripts ***(Lein et al., 2007).***

Table (5): Expression and functional mechanisms of H19 in different types of cancer**:**

Cancer type	Expression level	Mechanisms	(Refs.)
Lung cancer	Increased	i) H19 depresses miR-196b to elevate LIN28B; ii) H19 attaches miR-17 to modulate STAT3 expression; iii) H19 promotes EMT by downregulating miR-484; iv) H19 cisplatin resistance in patients.	***(Yu et al., 2018)***
Gastric cancer	Increased	i) H19 regulate HER2 expression by suppressing let-7c expression; ii) H19-PEG10/IGF2BP3 axis promotes EMT in gastric cancer; iii) H19/miR-675 axis inhibits the expression of FADD and the downregulation of FADD inhibits the caspase cleavage cascades including caspase 8 and caspase 3	***(Yan et al., 2017)***
Pancreatic cancer	Increased	i) H19/miR-675/E2F-1 regulatory loop affects the cell cycle; ii) H19 increases HMGA2-mediated EMT through antagonizing let-7	***(Ma et al., 2014)***
Liver cancer	Increased	i) H19 targets miR-193a-3p and regulates PSEN1 expression, which influences the survival rates and proliferative abilities of HCC cells; ii) aberrant TGF-β/H19 signaling axis via Sox2 in TICs that regulates hepatocarcinogenesis; iii) abnormal regulation of H19 results in biallelic expression of IGF2, leading to exceptional cell proliferation	***(Ma et al, 2018***)
Colorectal cancer	Increased	i) Overexpression of H19 activates the RAS-MAPK signaling pathway, promoting invasion and metastasis of colorectal cancer; ii) H19 induces the EMT process in colon cancer cells; iii) H19 sponges miR-138 to upregulate the expression of HMGA1, enhancing the invasion and migration of colon cancer; iv) H19 competitively binds to miR-200a and depresses the expression of β-catenin in colorectal cancer	***(Yang et al., 2018)***
Endometrial cancer	Increased	i) Overexpression of H19 regulates the expression of HOXA10 via targeting miR-612, promoting cell proliferation of endometrial cancer; ii) H19 modulates EMT process, reinforcing the aggressiveness of endometrial cancer; iii) H19 acts as a sponge to bind let-7, leading to high expression of IGF1R and therefore promotes endometrial stromal hyperplasia	***(Zhang et al., 2018)***
Bladder cancer	Increased	i) High expression of H19 inhibits E-cadherin expression and strengthens metastasis of bladder cancer; ii) H19 acts as a ceRNA to sponge miR-29b-3p and promotes the expression of DNMT3B, resulting in metastasis and EMT of bladder cancer; iii) H19 increases miR-675 expression, which can inhibit the activation of p53 and reduce the expression of Bax/Bcl-2 and cyclin D1, leading to bladder cancer cell proliferation.	***(Zhu et al., 2018)***

Polymorphism in H19 gene:

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variations in the human genome. SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are associated with genetic susceptibility to cancer (***Ulaganathan et al., 2015).*** Understanding the mechanisms underlying the effects of SNPs that result in cancer susceptibility is critical to understanding the molecular pathogenesis of various cancers. From a clinical perspective, SNPs are potential diagnostic and therapeutic biomarkers in many cancer types.

SNPs are located in different regions of genes such as promoters, exons, introns as well as 5′- and 3′ UTRs. Therefore, alterations in gene expression and their effect on cancer susceptibility vary depending on the location of the SNPs. The promoter region SNPs affect gene expression by altering promoter activity, transcription-factor binding, DNA methylation and histone modifications ***(He et al., 2016).*** The exonal SNPs affect cancer susceptibility by suppressing gene transcription and translation ***(Fang et al., 2014).*** SNPs in intron regions generate splice variants of transcripts and promote or disrupt binding and function of lncRNAs ***(Xiong et al., 2015).***

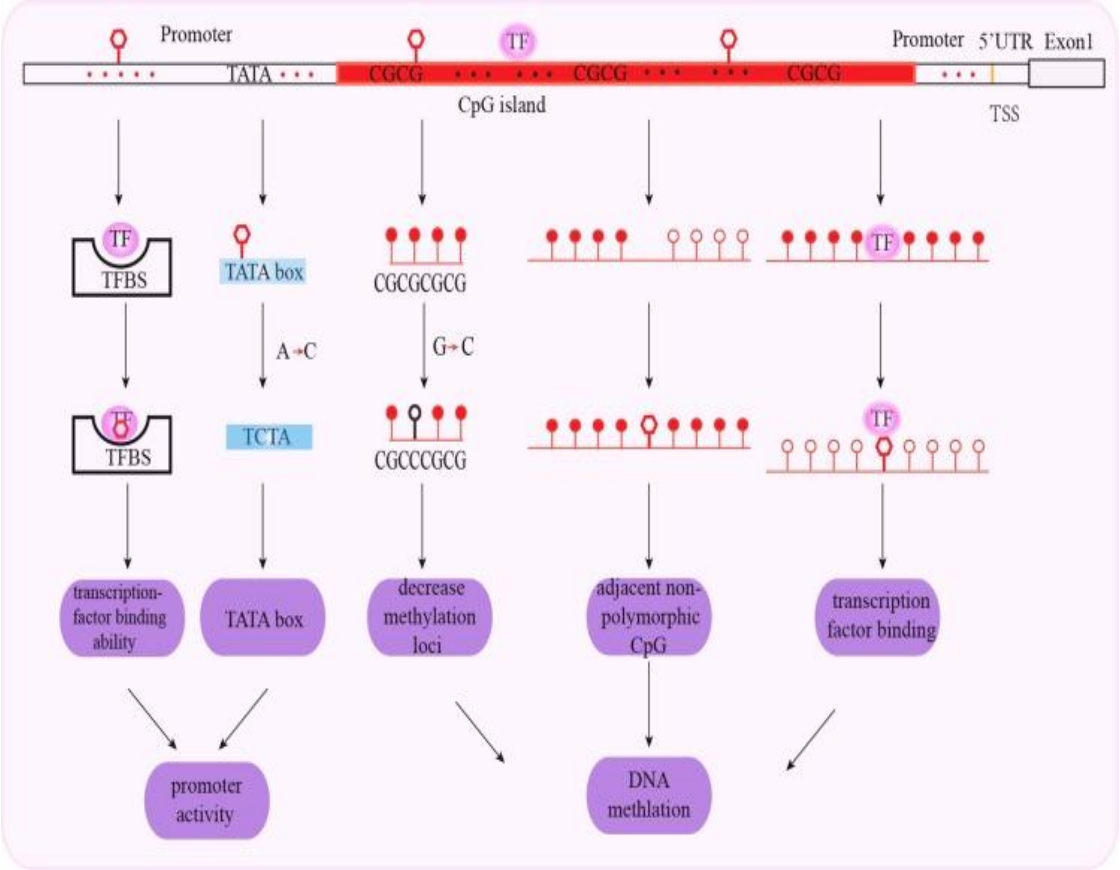

Fig. (11): Schematic representation of mechanisms associated with promoter SNPs and cancer susceptibility. Quoted from ***Deng et al. (2017).***

SNPs have been confirmed to have profound effects on gene expression and function, and participate in carcinogenesis. Recently, studies on the effects of SNPs have extended to functional lncRNAs. For example, HOTAIR has been widely identified to participate in tumor pathogenesis, acting as a promoter in colorectal cancer carcinogenesis ***(Sun et al., 2015)***.

H19 is abnormally expressed in several tumors, and it acts as either a tumor suppressor, or an oncogene ***(Matouk et al., 2007)***.

Molecular studies have shown that lncRNAs play important roles in cell cycle regulation and affect proliferation, differentiation, and apoptosis ***(Xia***

et al., 2016). LncRNAs are also important regulators of tissue pathology and disease processes related to cancer ***(Lin et al., 2017)***.

In addition, the introduction of the genome-wide association studies (GWAS) allowed for identification of an increased number of H19 SNPs that were associated with various types of cancer. Some original studies and previous meta-analyses reported the relationship between H19 rs217727 and cancer risk, but the results were inconsistent. In addition, several recently published studies provide the basis for updating data sets and more accurately evaluating the relationship between H19 rs217727 and cancer risk. Thus, we performed meta-analysis to explore the association between H19 polymorphisms and the risk of cancer ***(Yuan et al., 2019).***

H19 acts as a gene that is up-regulated in hypoxic stress and certain tumors, including lung cancer, and is therefore an indispensable regulator of tumor development ***(Cui et al., 2015)***. The expression of H19 in airway epithelial cells in non-smokers is lower than that in smokers ***(Kaplan et al., 2003).*** Thereby, the up-regulation of airway epithelial H19 expression can be considered as an early marker of epithelial cell development into lung cancer. It have found that the Myc oncogene lead to H19 upregulation by specifically binding to the H19 promoter region, and also observed the strong relationship between H19 and c-MYC expression levels in lung cancer cells ***(Barsyte-Lovejoy et al., 2006).***

Up-regulated level of H19 is involved in proliferation of gastric cancer cells. They found that H19 may inactivate P53 and so can be regarded as a potential therapeutic target for gastric cancer ***(Yang et al., 2012)***. The role of H19 in gastric cancer progression might be due to the direct up-regulation

of ISM1 and the indirect suppression of CALN1 expression via miR-675 ***(Li et al., 2014).***

It seems that the imprinted expression of H19 is usually lost in hepatocellular carcinoma cells. The reports indicate that in hypoxic condition, the H19 expression is up-regulated ***(Matouk et al., 2007***). Lizuka et al. found that dysregulated H19 transcripts are correlated with advanced tumor stage and poor outcome in HCC patients. They suggested that H19 and IGF2 genes have little or no functional contribution to the progression of HCC. They proposed that changes in transcriptional regulation of these genes are involved in the progression and metastatic potential of HCC. They found that HCCs with high H19 expression were at more advanced stages than those without (***Iizuka et al., 2004).*** However, H19 was found to be down-regulated in invasive HCC specimens compared with non-invasive tissues. The reduced expression of H19 induced EMT by regulating the miR-200 family ***(Zhang et al., 2013).***

In endometrial and ovarian tumors, the H19 is highly expressed compared to normal tissues ***(Tanos et al., 2004)***. The loss of imprinting of H19 and IGF2 is also reported to be involved in the development of ovarian cancer ***(Dammann et al., 2010***).

Recently, it has been shown that lncRNA-H19 gene polymorphisms are associated with several disorders. The nucleotide change in rs217727 C to T is associated with an increased risk of coronary artery disease, while the rs2067051 G to A is associated with a reduction in the risk of coronary artery disease ***(Gao et al., 2015)***.

C- Role of long non coding RNA H19 gene in BC

❖ Role of H19 gene variations of in BC:

H19 promotes breast tumor genesis, recent case-control study in China revealed that high expression levels of H19 were associated with an increased risk of breast carcinogenesis in both codominant and dominant models, and the association was more apparent in patients with estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-), and ER+-HER2-negative (HER2-) molecular subtypes. The biological role and the potential molecular mechanism of H19 in breast cancer are still unclear ***(Lin et al., 2019).***

DNA hypermethylation, which leads to insensitivity to growth inhibitory signals and evasion of programmed cell death through inhibiting tumor suppressor genes, is a major epigenetic feature differentiating cancer cells from normal cells. DNA hypermethylation is involved in breast cancer carcinogenesis and cell survival, and initiated by abnormal expression of DNA methyltransferases (DNMTs) including DNMT1, DNMT3a, and DNMT3b ***(Zhang et al., 2016).*** DNMT1, a key maintenance methyltransferase, is most abundantly expressed in dividing cells compared with nondividing cells, becoming a major therapeutic target for methylation inhibition in cancer cells ***(Singh et al., 2013).***

The implication of *H19* in tumor genesis has been reported and H19 is overexpressed in many solid tumors such as prostate, bladder or BC ***(Liu et al., 2016***).

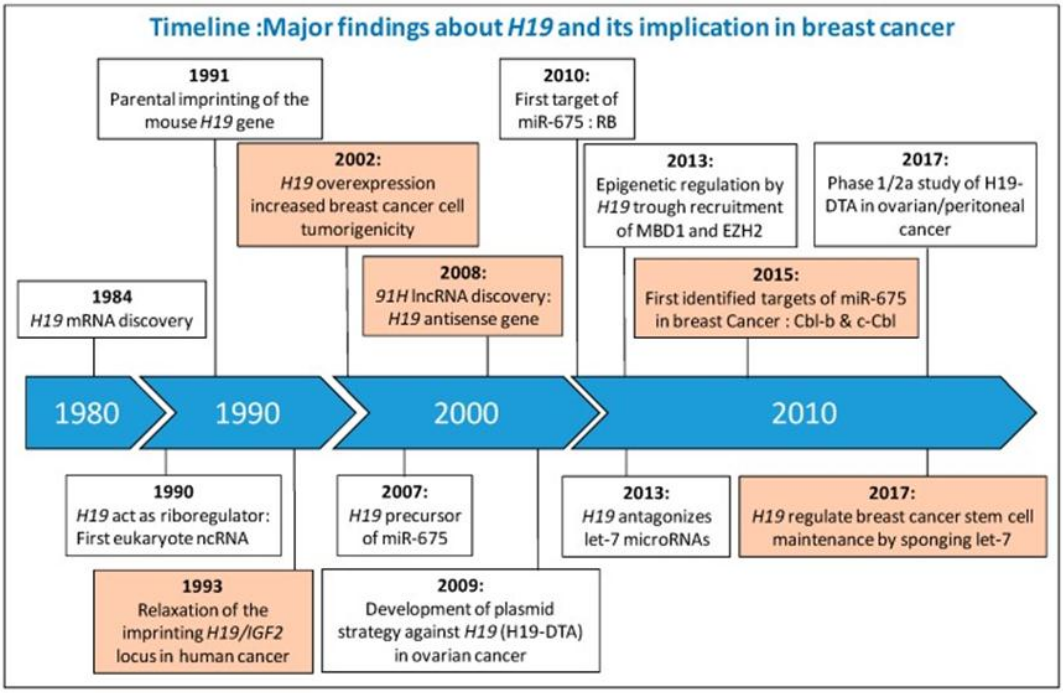

Fig. (12)**:* Major finding about H19 and its implication in breast cancer. Quoted from ***Jordan Collette et al. (2017).

It has been showed that H19 is overexpressed in 73% of BC tissues when compared to healthy tissues. Several studies showed that H19 is controlled by steroid hormones in normal and cancerous mammary gland, uterus and prostate. In BC, the expression of H19 is higher in ER positive cells, but in the ER negative MDA-MB-231 cell line, ectopic overexpression of H19 is associated with increased proliferation ***(Basak et al., 2015***). Furthermore, animal experiments demonstrated that the probability of breast carcinogenesis was increased in severe combined immunodeficiency mice injected with cells overexpressing the H19 gene ***(Lottin et al., 2002).***

The molecular mechanisms underlying H19-associated carcinogenesis may involve several aspects. The H19 promoter was activated by transcription factor 1 (E2F1), which promoted cell cycle progression

(particularly in the S-phase) of MCF-7 cells ***(Berteaux et al., 2005).*** Furthermore, H19 contributed to the epigenetic regulation of gene expression in BC. H19 bound to and inhibited S-adenosylhomocysteine hydrolase, the sole enzyme that can hydrolyze S-adenosylhomocysteine (SAH) in humans. SAH can markedly suppress S-adenosylmethionine-dependent methyltransferases, which can methylate multiple cellular components, including DNA, RNAs and proteins, through a feedback mechanism. H19 knockdown increased the DNMT3B-mediated methylation of Nctc1, a gene encoding lncRNAs, within the Igf2-H19-Nctc1 locus. Thus, H19 altered DNA methylation and led to breast tumorigenesis ***(Zhou et al., 2015).*** A new lncRNA within the H19/IGF2 locus named H19 is an antisense gene to H19. The H19 lncRNA also regulated the expression levels of H19 and IGF2 by epigenetic modifications and increased the tumorigenic properties of MDA-MB-231 cells both *in* vitro and in vivo ***(Vennin et al., 2017).***

H19 serves as a potential biomarker for the diagnosis of breast cancer. The overexpression of H19 is associated with cells exhibiting higher tumorigenic phenotypes, which indicates that H19 expression levels can be used in the clinical diagnosis of BC, and there was a significant correlation between the levels of plasma H19 and ER, PR and lymph node metastasis in BC. Overexpression of H19 increases the drug resistance of BC cells. H19 is a downstream target molecule of ER, and the expression of ER has been demonstrated to alter H19 levels ***(Zhang et al., 2019).***

The aberrant events and increased variation in imprinted gene methylation are more frequent in invasive BC and more associated with

negative ER and PR status. The associations between the H19 SNPs and BC risk were investigated by molecular epidemiology ***(Barrow et al., 2015***).

❖ **Role of H19 SNP in BC:**

Few studies have attempted to reveal the association between lncRNA-H19 gene polymorphism with the risk of BC. However, the relationship between lncRNA-H19 polymorphism and BC remained unclear ***(Lin et al., 2017)***.

The positive relationship between the rs217727 polymorphism and BC susceptibility demonstrating that the presence of rs217727 polymorphism may play crucial roles in the pathogenesis of BC ***(Abdollahzadeh and Ghorbian, 2019)***. A nucleotide variation of lncRNA may be changing the structure and affects miRNA-lncRNA interaction. Increasing evidence suggests that lncRNAs can be directly regulated by miRNAs ***(Yuan et al., 2018)***. The effect of rs3741219 T>C lncRNA-H19 gene polymorphism and interaction of miRNA-lncRNA in BC cells remained unclear, and so further studies are needed to find the mechanism. Xia et al showed that the release of miR-675 with the *lncRNA-H19* prevented estrogen proliferation of ER of cancerous cells. Furthermore, the SNPs in the lncRNA-H19 may have a relationship with the risk of BC ***(Xia et al., 2016).***

SNPs locating on lncRNA H19 have also been identified to regulate its expression and function. For example, the CT + TT genotype of rs217727 and rs2839698 is significantly associated with an increased risk of gastric cancer ***(Yang et al., 2015***). An elevated risk of BC and bladder cancer has also been discovered in the TT carriers for *H19* rs217727 ***(Hua et al., 2016).***

Subjects and Methods

This study was a case-control study which was carried on 200 female subjects who lived in Qualubia governorate.

After approval of the study scheme by the research ethical committee of Benha Faculty of Medicine and obtaining informed consent from the included subjects, the subjects were recruited from:

- General Surgery Department, Faculty of Medicine, Benha University Hospital.
- Oncology unit of General Medicine department, Faculty of Medicine, Benha University Hospital.

Subjects:

Our study subjects were classified into:

A. **Control group:** 50 females who were age matched and free from any breast lesion (benign or malignant) clinically & by US and mammography.

B. **Benign breast lesion group**: 50 females, diagnosed by clinical & radiological examination (breast US and mammography).

C. **Malignant breast lesion group**: 100 females, diagnosed as breast cancer by clinical, radiological, and histopathological examinations.

- Exclusion criteria:

1. Patients diagnosed not subjected to surgery.

2. Females less than 20 years and more than 70 years as they are less risky.

3. Patient had other associated cancers.

Ethical considerations:

A written informed consent was taken from all the subjects of the study groups prior to participation in the study that was approved by the Ethical Committee of the Faculty of Medicine of Benha University.

Methods:

All cases of our study were subjected to:

1. Full history taking including age, number of pregnancy, number of abortion, breast feeding, family history, systemic diseases, smoking, contraception and menses status.

2. Complete clinical examination.

3. Laboratory investigations:

- Cancer Antigen (CA15-3): (normal up to 30 U/mL) **(BIO Tek, ELX 50/8 ELISA reader)** ***(Gion et al., 1991).***

- Alpha fetoprotein**:** to exclude metastasis ***(Abelev G. 1971).***

4. Radiological investigations:
   - Mammography, breast US, abdominal US, isotope bone scan and CT Chest, abdomen & pelvis.

- Conventional MRI ***(Daffner et al., 1986)*** and Advanced imaging as Positron-emission tomography-CT (PET-CT), Positron-emission tomography-MRI (PET-MRI) and Whole body MRI (WBMRI), which detects distant metastases with higher sensitivity than conventional imaging ***(Catalano et al., 2017).***

5. Diagnostic biopsy for histopathology.

6. Molecular study of the gene variations:

- Quantitative reverse transcription polymerase chain reaction (qRT-PCR) for detection of gene expression levels of H19 lncRNA.

- PCR for detection of gene polymorphism (rs217727)

Sampling:

A venous blood sample (2 ml) on ethylene diamine tetra-acetic acid (EDTA) was taken from each subject. The blood sample of each subject was aliquoted in 2 Eppendorf tubes; one Eppendorf for gene expression and the other for genotyping.

I. Genotyping of lncRNA H19 single nucleotide polymorphism (rs217727): (Figures 18 & 19)

Genotyping of rs217727 SNP was detected by polymerase chain reaction – based restriction fragment length polymorphism (PCR-based RFLP) on 3 steps:

1. **DNA extraction:**

DNA was extracted from 100 µl blood sample; using ***Quick-gDNA Miniprep kit,*** Catalog No. D3024 (**Zymo research, USA**) according to manufacturer's instructions. Elution of DNA was done by 50 elution buffers. The extracted DNA concentration was measured by Nanodrop Spectrophotometer 2000 **(Thermo-Fisher Scientific, Wilmington, USA).** Readings were taken at wave lengths 260 and 280 nm ***(Wilfinger et al., 1997).*** The ratio of optical density (OD) at 260 nm and 280 nm provided an estimate of DNA purity. Pure preparations of DNA have OD260/OD280 of 1.7 - 2.0. If contaminated with protein or phenol, the ratio is <1.7, but if contaminated with RNA, the ratio is >2.0.

- The extracted DNA was kept at -80^{o}C for further processing.

2. Genomic DNA amplification:

DNA amplification was done in 25 µl reaction / sample using primers for lncRNA *H19* rs217727 previously reported by ***Abdollahzadeh and Ghorbian (2019);*** 5´-ACTCACGAATCGGCTCTGGAAGGTG-3´ and 5´-ATGTGGTGGCTGGTGGTCAACGGT-3´. Amplification was done in Veriti™ Thermal Cycler **(Applied Biosystems).** The reaction mix contained 12.5 µl ***Easy taq PCR SuperMix*** **(Transgen biotech, China), 1** µl FP, 1 µl RP, 5 µl DNA and completed up to 25 µl by nuclease-free water. The PCR conditions were 5 min at 95^{o}C initial denaturation, 35 cycles (denaturation at 95^{o}C for 30 s, annealing at 60^{o}C for 30 s, extension at 72^{o}C for 1 min) and then final extension at 72^{o}C for 5 min. PCR products (10 µl) and 100 base pair ladder (5 µl) were resolved in 3 % agarose gel stained with 0.3ug/ml ethidium bromide to check the PCR products at 247 bp fragment.

3. Digestion by RsrII restriction enzyme:

Digestion was done for lncRNA *H19* rs217727 by ***Fast-digest RsrII restriction enzyme*** **(New England Biolabs, England)** in 50 µl total volume by mixing: 10 µl of PCR products + 1 µl RsrII restriction enzyme (1 unit) + 5 µl 10X buffer + 34 µl nuclease-free water. The digestion mixtures were incubated at 37◦C for one hour then inactivated by incubation at 65°C for 20 minutes. DNA fragments (10 µl) and 100 bp ladder (5 µl) were separated on 3% agarose gel stained with 0.3ug/ml ethidium bromide. The bands (pre- and post-digestion) were visualized using UV transilluminator (254 nm) and imaged with a digital camera 8 mega pixel. The image was analyzed by computer software **(Alpha InoTech Gel Documentation System).** Pre-digestion bands were visualized at 247 bp. Post-digestion; the T allele (uncut) gave one fragments (247 bp), while the C allele was (cut) gave 2 fragments (221 bp & 26 bp). The small band (26 bp) was lost in the gel. The success rate was 95%. The failed PCR were rerun by the same conditions.

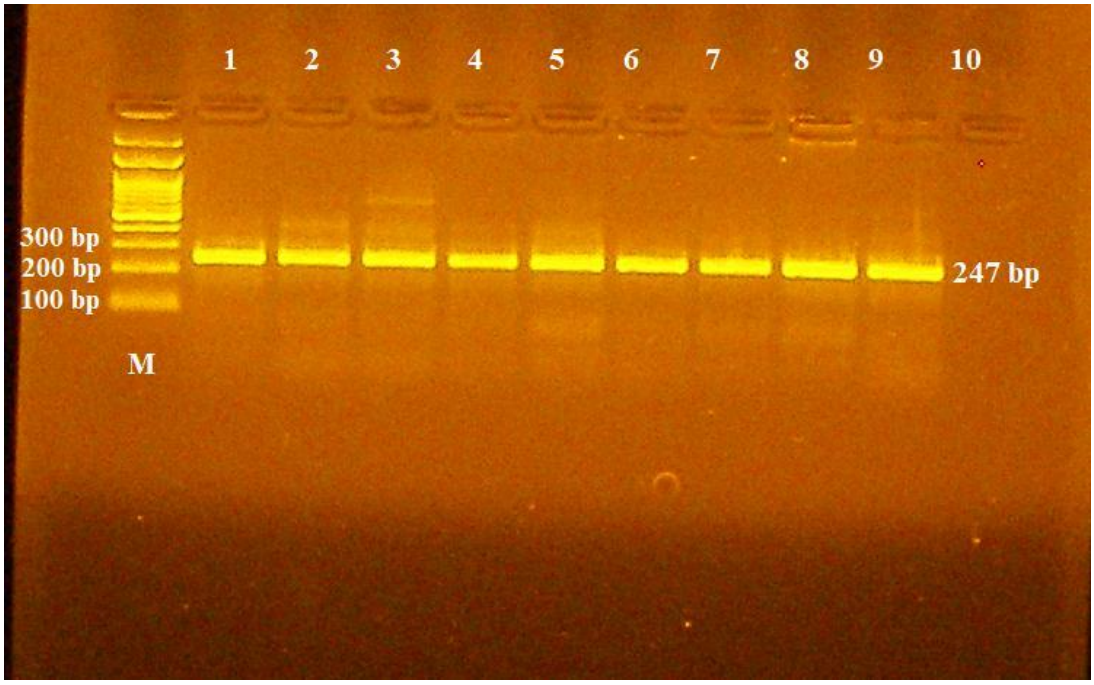

Figure 18: Amplification product of rs217727 before digestion by RsII restriction.

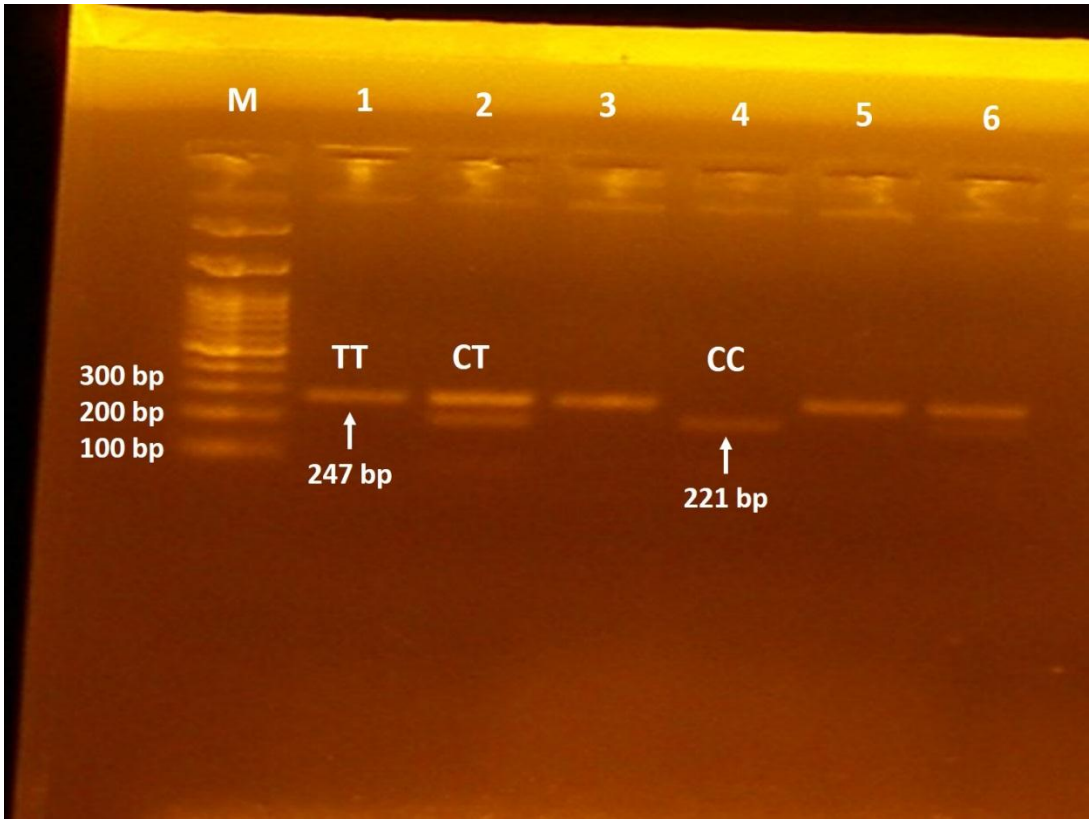

Figure 19: PCR-based RFLP of rs217727 after digestion by RsII restriction enzyme [(TT → 247 bp), (CC → 221 bp & 26 bp) , (CT → 247, 221 & 26 bp)] The smallest 26 bp band is lost so it does not appear on the gel.

II. Gene expression of lncRNA H19: on 3 steps; (Figures 13-17)

A. Total RNA Extraction:

It was performed with 100 µl EDTA whole blood via Total RNA Purification Kit ***Cat No.*** PP-210S, ***(*Jena Bioscience, Germany*)*** according to the manufacturer's instructions. Quantification of RNA was done by Nanodrop 2000 Spectrophotometer **(Thermo Fisher Scientific, Wilmington, USA).** Pure RNA preparations had an optical density (OD) ratio of 1.9-2.3 at 260/280 nm ***(Wilfinger et al., 1997)***.

- The extracted RNA was kept at -80^{o}C for further processing.

B. Reverse transcription (RT) of RNA into complementary DNA (cDNA):

It was done in a Veriti™ Thermal Cycler **(Applied Biosystems),** using ***Maxime RT PreMix*** (random primer) Kit **(Intron Biotechnology, Korea).** To each RT tube supplied; 5 µl RNA template and 15 µl nuclease-free water were added. Thermal conditions were set at 42^{o}C for 1 hour then RTase inactivation at 85^{o}C for 10 minutes.

C. Relative quantitation of lncRNA H19 gene expression:

It was performed using ***Hera Sybr Green qPCR kit*** **(Willowfort, UK).** Human U6 was the endogenous housekeeping gene. The primers for lncRNA H19 were; FP: 5′- ATCGGTGCCTCAGCGTTCGG -3′, RP: 5′- CTGTCCTCGCCGTCACACCG -3′ ***(Zhou et al., 2015).*** U6 primers were; FP: 5′- GTGCTCGCTTCGGCAGCA -3′, RP: 5′- CAAAATATGGAACGCTTC -3′ ***(Li et al., 2017).*** Singleplex reactions were done. Each singleplex reaction mix contained 10 µl Hera Sybr master mix (2X), 1 µl FP, 1 µl RP, 4 µl cDNA and up to 20 µl nuclease-free water. Amplification was run in ***Stepone Real-Time Cycler*** **(Applied Biosystem, Singapore).** An initial holding stage of 95^{o}C / 10 min was performed followed by cycling for 40 cycles (denaturation at 95^{o}C for 15 sec then Annealing / Extension at 58^{o}C for 1 min). Melting curve analysis was done in each run to ensure specificity of the assay.

B. Data analysis:

According to the Stepone software v2.2.2, the data were produced as sigmoid-shaped amplification curves in which the number of cycles was plotted against normalized reporter fluorescence (Rn) **(Figure 13).** lncRNA H19 gene expression levels in the apparent health control group (HC) were set to 1. The relative quantitation of target gene expression was normalized to that of human U6. Gene expression fold changes were calculated using the equation $2^{-\Delta\Delta CT}$ ***(Livak and Schmittgen, 2001)***. ΔCt values were determined by subtracting the threshold cycle (Ct) value of U6 from the Ct value of lncRNA H19. ΔΔCt was determined by subtracting the ΔCt of controls from ΔCt of cases.

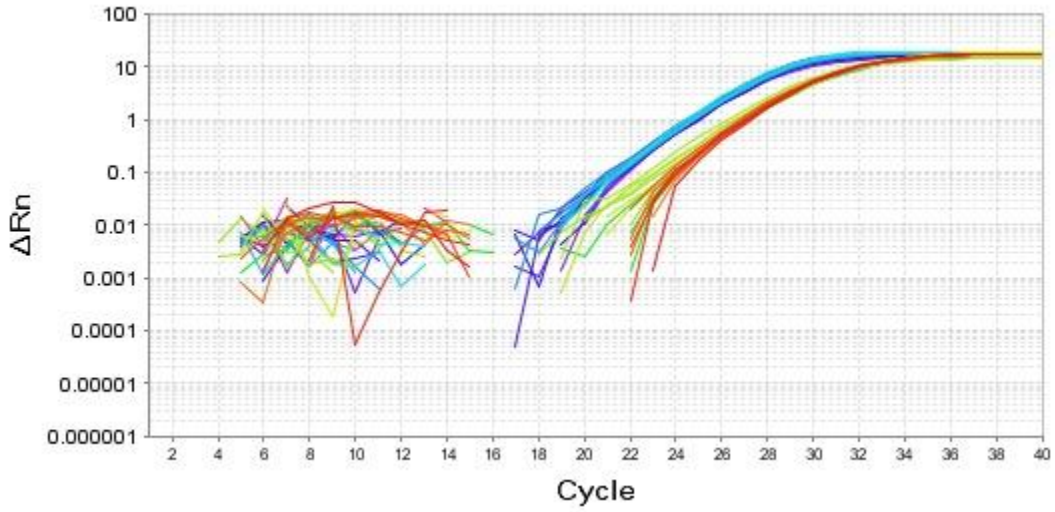

Figure 13: Amplification plot of lncRNA H19 gene

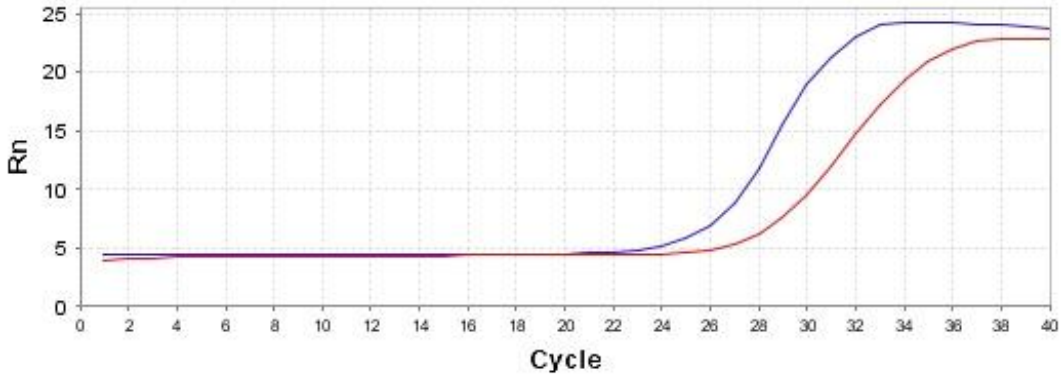

Figure 14: Amplification plot single sample The blue curve (U6) & the red curve (lncRNA H19)

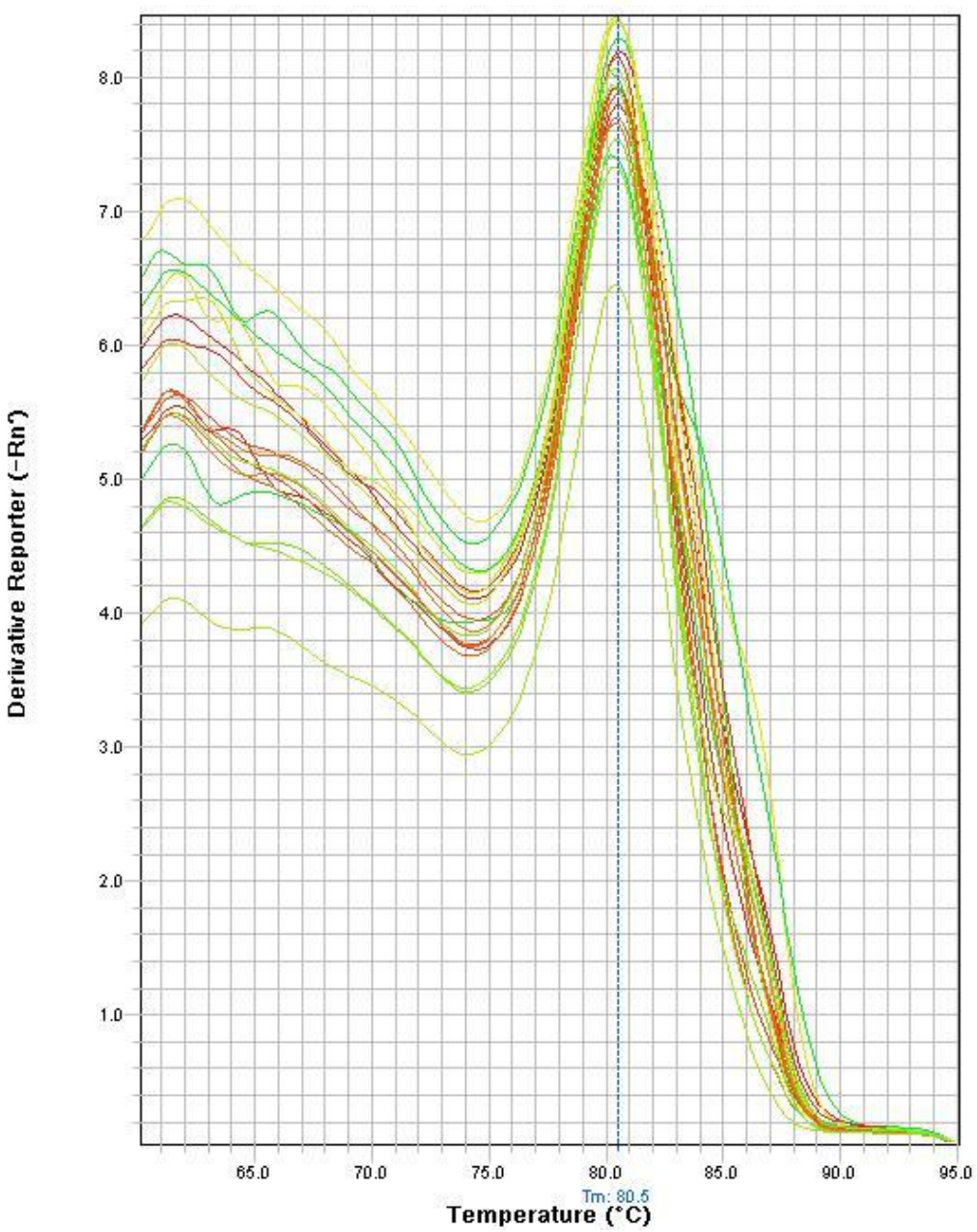

Figure 15: Melt curve of H19 gene expression

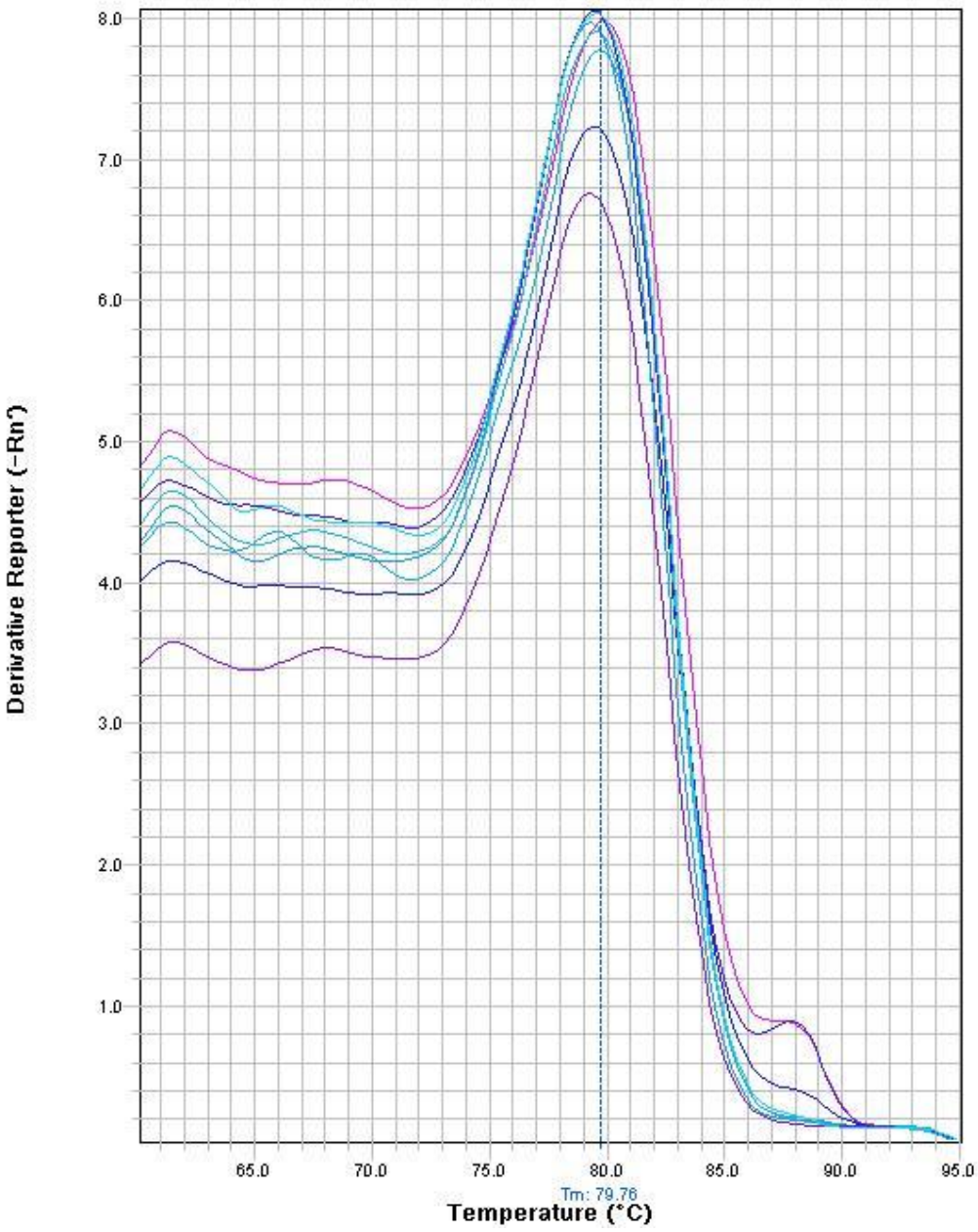

Figure 16: Melt curve of human U6 housekeeping gene

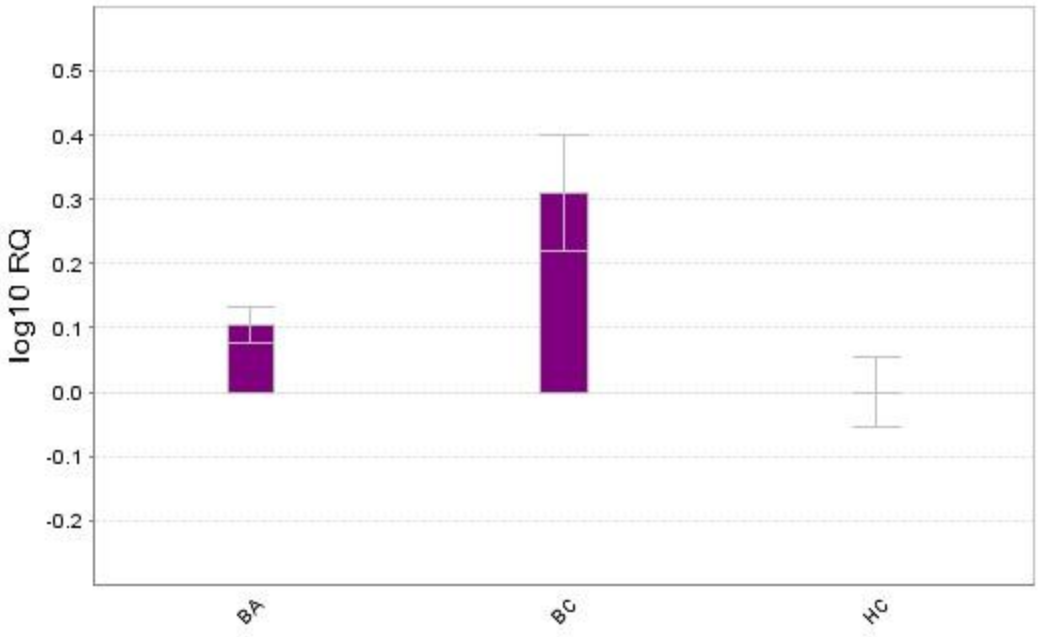

Figure 17: Gene expression plot of lncRNA H19 among the studied subjects BA: benign adenoma, BC: breast cancer, HC: healthy controls

Statistical analysis:

The clinical data were recorded on a report form. These data were tabulated and analysed using the computer program SPSS (Statistical package for social science) version 26 to obtain:

Descriptive data

Descriptive statistics were calculated for the data in the form of:

1. Mean and standard deviation $(\pm SD)$. for quantitative data.
2. Number and Percentage for qualitative data.

Analytical statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests

1- Student's *t*-test:- Used to compare mean of two groups of quantitative data.

2- ANOVA test (F value):-Used to compare mean of more than two groups of quantitative data.

3- Inter-group comparison of categorical data was performed by using chi square test (X^2-value) and fisher exact test (FET).

$$x^2 = \frac{\sum (observed - \exp ected)^2}{Expected}$$

$$Expected = \frac{col.total \ x \ row total}{Grand \ total}$$

4- Correlation coefficient:- to find relationships between variables.

A *P* value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

Kaplan-Meier method was used for survival analysis and defined as the period between diagnosis and local or distant recurrence. The survival curves calculated for groups were compared using the log-rank test.

Results

The current case-control study was conducted at Benha University Hospital. 200 hundreds women were included in the study, one hundred suffering from BC, fifty women suffering from benign breast lesion and fifty apparently healthy females as control group.

Table (6): Demographic characteristics of the breast cancer, benign & control group.

Variable	Healthy control (50)	Patient groups		Statistical test	P value
		Benign group (50)	BC (100)		
Quantitative	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Age	48.32 ± 12.02	45.5 ± 12.28	49.45 ± 10.71	F= 1.99	0.14
Age at menarche	13.02 ± 1.66	12.92 ± 1.35	12.95 ± 1.33	F= 0.07	0.94
Age at menopause (postmenopausal)	48.45 ± 4.69	46.57 ± 10.42	49.86 ± 4.62	F= 2.75	0.068
Qualitative	**No (%)**	**No (%)**	**No (%)**		
Marital status **Single** **Married**	 10 (20%) 40 (80%)	 10 (20%) 40 (80%)	 15 (15%) 85 (85%)	X2= 0.87	0.65
No of pregnancy **Nulli gravida** **Primi gravida** **Multigravida**	 10 (20%) 3 (6%) 37 (74%)	 12 (24%) 5 (10%) 33 (66%)	 19 (19%) 8 (8%) 73 (73%)	FET= 1.3	0.88
No of abortion **0** **1** **2**	 42 (84%) 3 (6%) 5 (10%)	 43 (86%) 3 (6%) 4 (8%)	 82 (82%) 7 (7%) 11 (11%)	FET= 0.46	0.997
Menopausal status **Pre** **Post**		 27 (54%) 23 (46%)	 47 (47%) 53 (53%)	X2= 0.65	0.42
Breast feeding	30 (60%)	30 (60%)	66 (66%)	X2= 0.77	0.68
Family history	12 (24%)	14 (28%)	26 (26%)	X2= 0.21	0.90
Systemic disease **No** **DM** **HTN** **Both**	 18 (36%) 10 (20%) 14 (28%) 8 (16%)	 19 (38%) 13 (26%) 11 (22%) 7 (14%)	 21 (21%) 27 (27%) 36 (36%) 16 (16%)	X2= 7.5	0.28
Smoking	3 (6%)	3 (6%)	7 (7%)	FET= 0.12	1.0
Contraception **No** **Pills** **IUD**	 16 (32%) 13 (26%) 21 (42%)	 17 (34%) 11 (22%) 22 (44%)	 23 (23%) 23 (23%) 54 (54%)	X2= 3.28	0.51

χ^2***:*** Chi-square test*;* ***FET:*** fisher exact test; $p>0.05$: Non-significant difference.

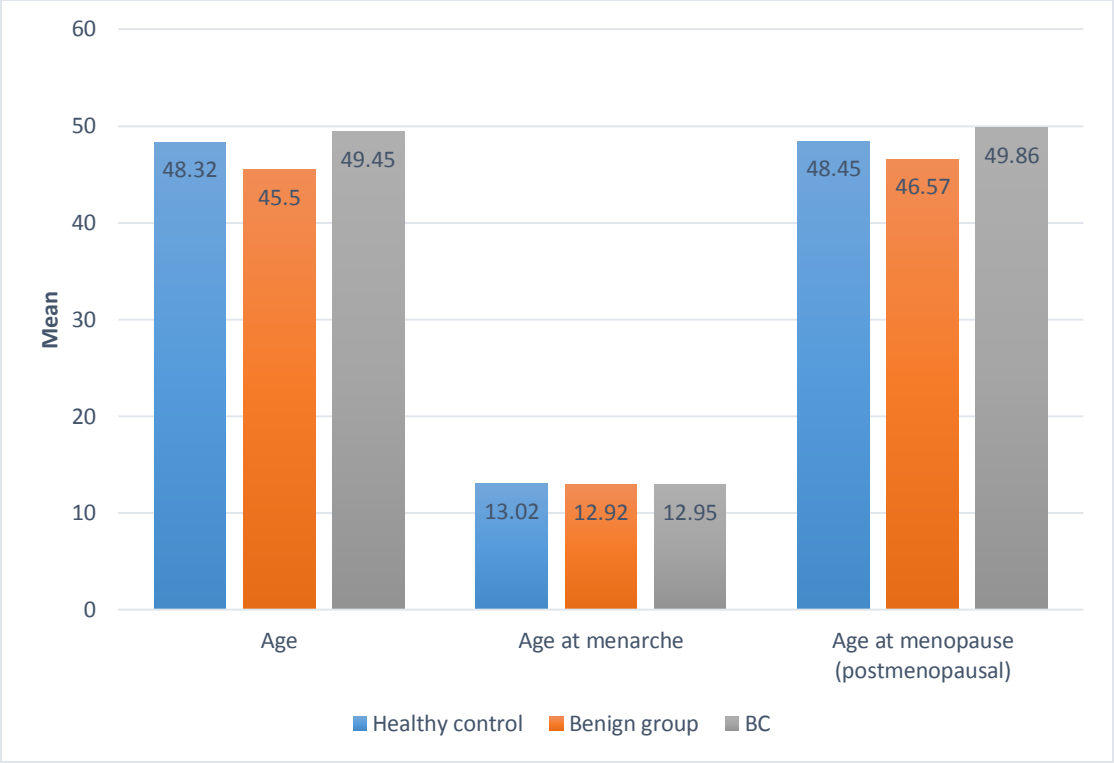

Fig. (20): Quantitative characteristics of the breast cancer, benign & control group.

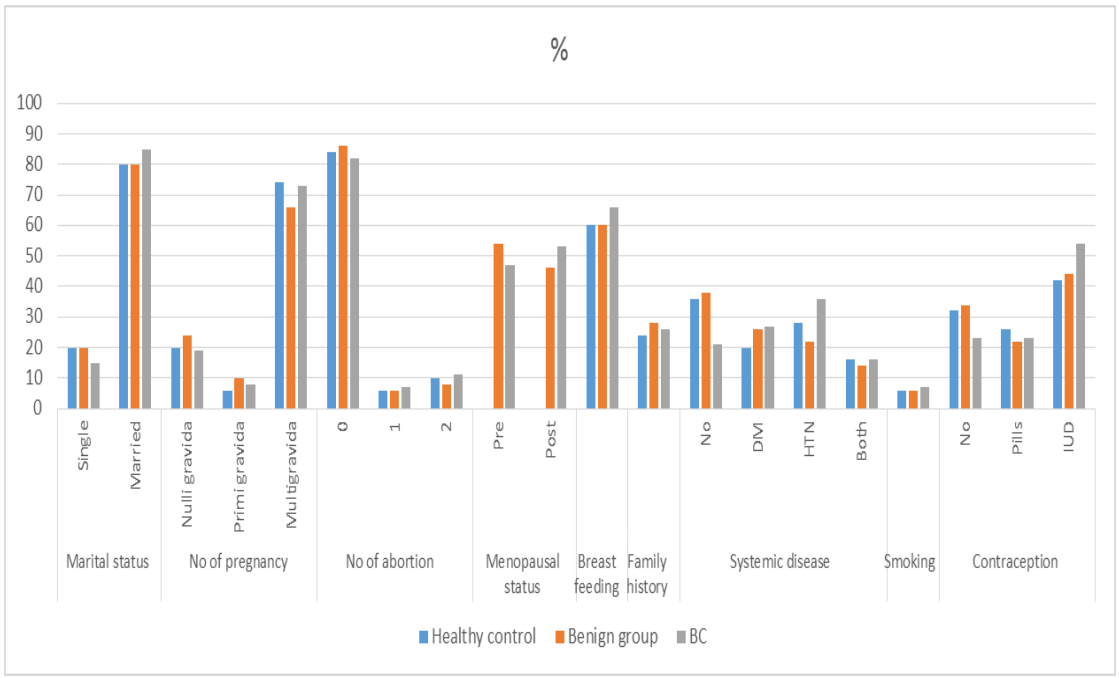

Fig. (21): Qualitative characteristics of the breast cancer, benign & control group.

Table (6) and fig. (20, 21) show description of the demographic data of breast cancer cases, benign group and normal control group. These data include marital status, age, age at menarche, age at menopause, no of pregnancy, no of abortion, menopausal status, breast feeding, family history, systemic diseases, smoking and contraception. We observed no statistical difference in the demographic data among the studied groups.

Table (7): Laterality in the BC &Benign patients.

Laterality	Patient groups		Statistical test	P value
	Benign group (50)	BC (100)		
	No (%)	No (%)		
Rt **Lt** **Both**	29 (58%) 16 (32%) 5 (10%)	57 (57%) 36 (36%) 7 (7%)	X2= 0.54	0.77

χ^2**:** Chi-square test; ***p*>0.05:** Non-significant difference.

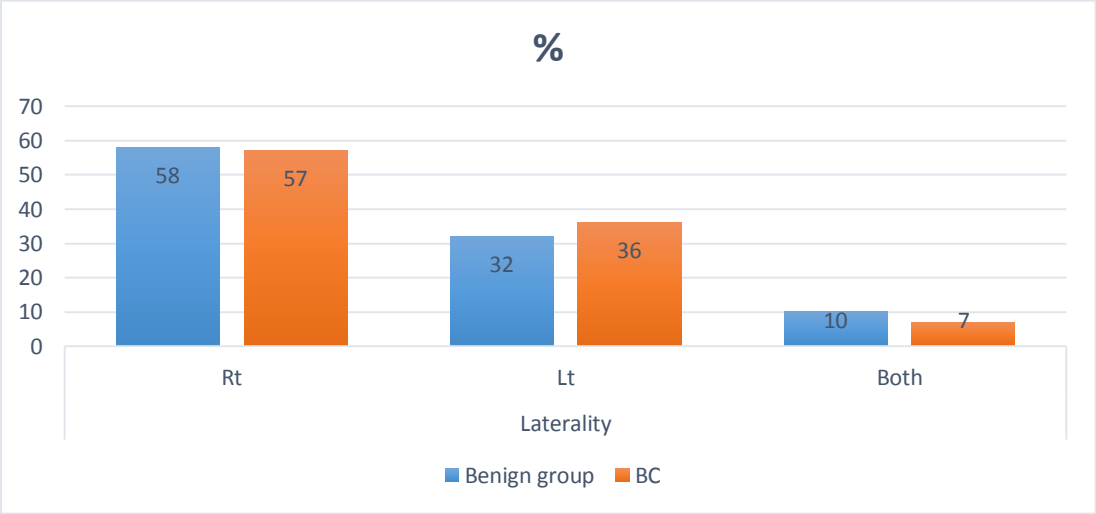

Fig. (22): Laterality in the BC &Benign patients.

Table (7) and fig. (22) show that 57% of patients had breast cancer in the right side while 36% of them had their cancer in the left side, only 7%

had breast cancer in both sides. While benign group showed that 58% had breast mass in the right side, 32% had their mass in the left side and 10% had the mass on both sides. We observed no statistical difference in laterality among BC group and benign group.

Table (8): Tumor grade in the BC group.

BC group	No (100)	%
Grade		
I	21	21.0
II	53	53.0
III	26	26.0

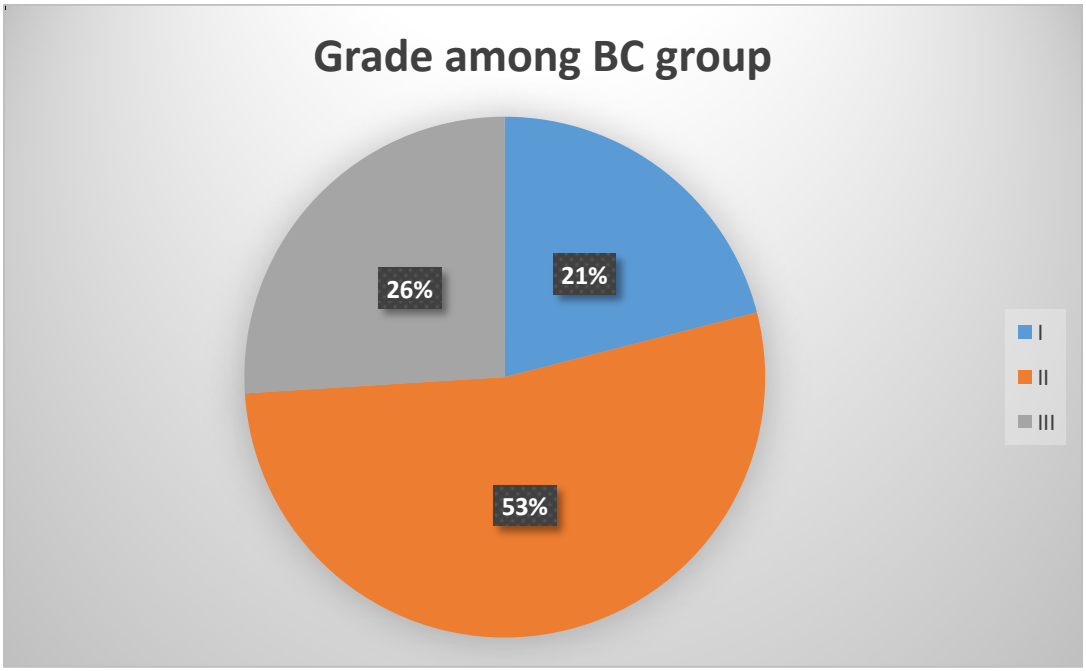

Fig. (23): Tumor grade in the BC group.

Table (8) and fig. (23) show that most of cases were of grade II representing 53% and the rest of cases were grade III and representing 26% followed by grade I (21%).

Table (9) Tumor stage in the BC group.

BC group	No (100)	%
Stage		
I	14	14.0
II	41	41.0
III	26	26.0
IV	19	19.0

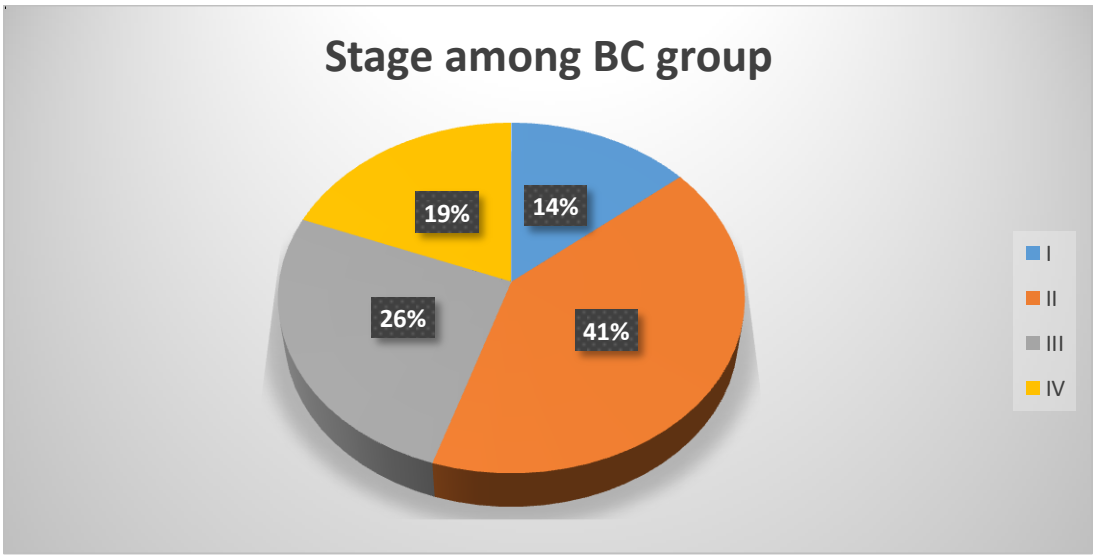

Fig. (24): Tumor stage in the BC group.

Table (9) and Fig. (24) show that most of cases were of stage II representing 41%, followed by stage III representing 26%, then stage IV representing 19% and stage I representing 14%.

Table (10): Lymph nodes status in the BC group.

BC group	No (100)	%
LN		
0	13	13.0
1	21	21.0
2	35	35.0
3	31	31.0

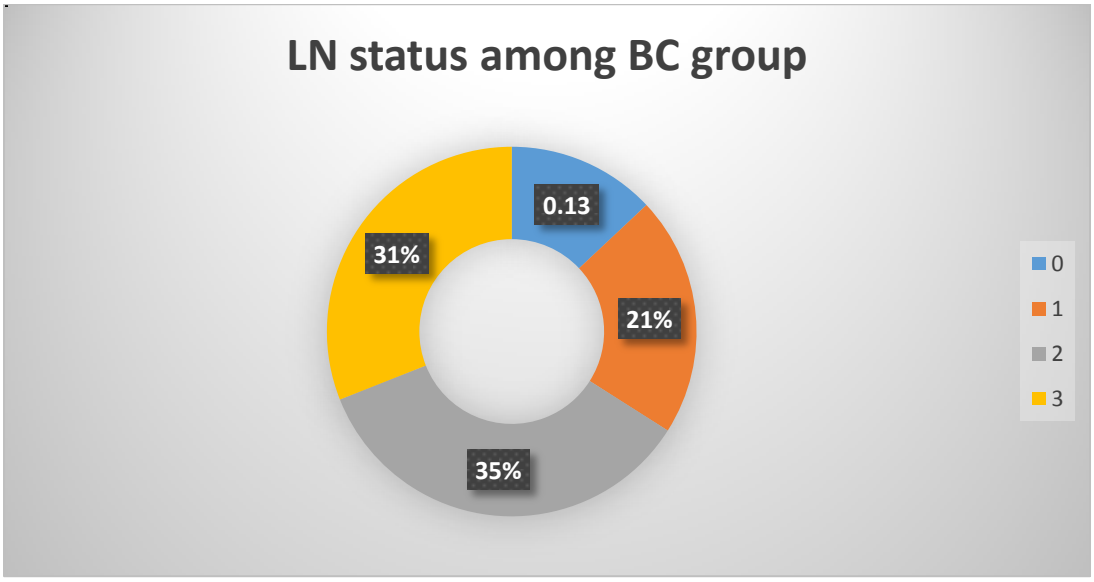

Fig. (25): Lymph nodes status in the BC group.

Table (10) and fig. (25) illustrate that 13% of BC cases were negative, while lymph nodes positive cases were 87%. Nodes positive cases were categorized according to the number of involved nodes according to TNM ***(Frederick, 2002)*** into N1 (1 - 3), N2 (4 - 9) and N3 (≥10). Most of our cases were N2 category constituting 35% of all studied cases, followed by N3 (31%) and N1 (21%).

Table (11): Hormonal receptors status.

BC group	No (100)	%
ER status		
Yes	74	74.0
No	26	26.0
PR status		
Yes	72	72.0
No	28	28.0
HER2 status		
Yes	75	75.0
No	25	25.0

ER: estrogen receptors, PR: progesterone receptors, Her2/neo: human epidermal growth factor receptor 2, +ve: positive, -ve: negative

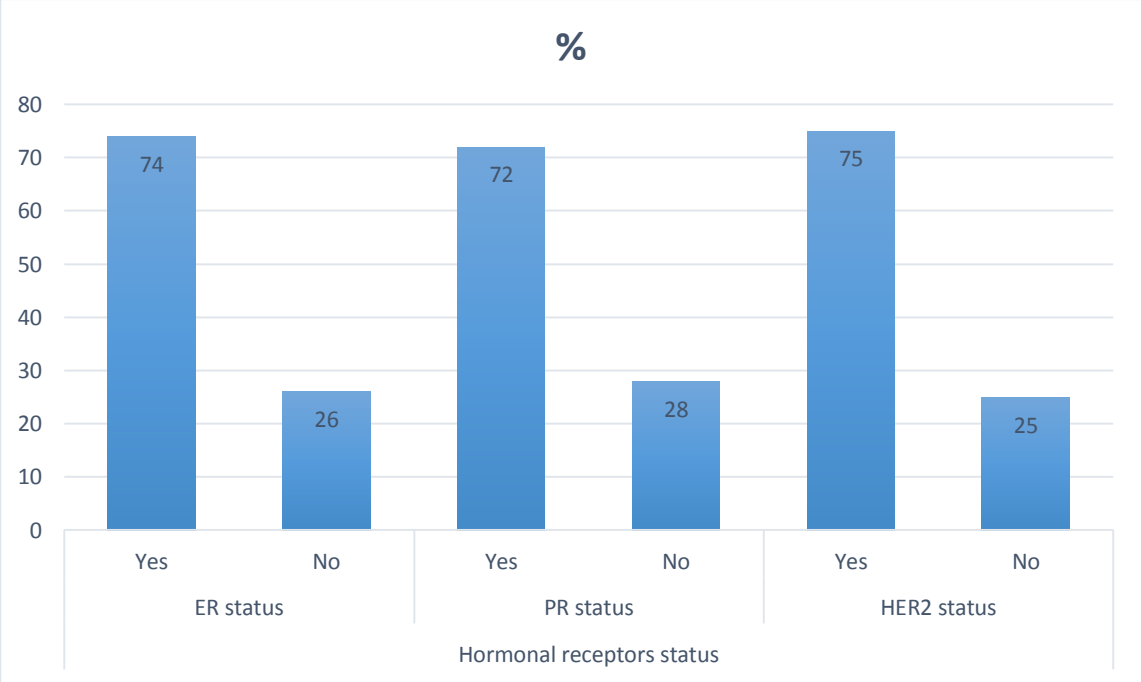

Fig. (26): Hormonal receptors status.

Table (11) and fig. (26) demonstrate that expression of estrogen receptors was observed in 74% of cases and progesterone receptors was observed in 72% of cases. Human epidermal growth factor receptor 2 (HER2) was expressed in 75% of cases.

Table (12): Progression events in the cancer patients.

BC group	No (100)	%
Distant metastasis		
Yes	25	25.0
No	75	75.0

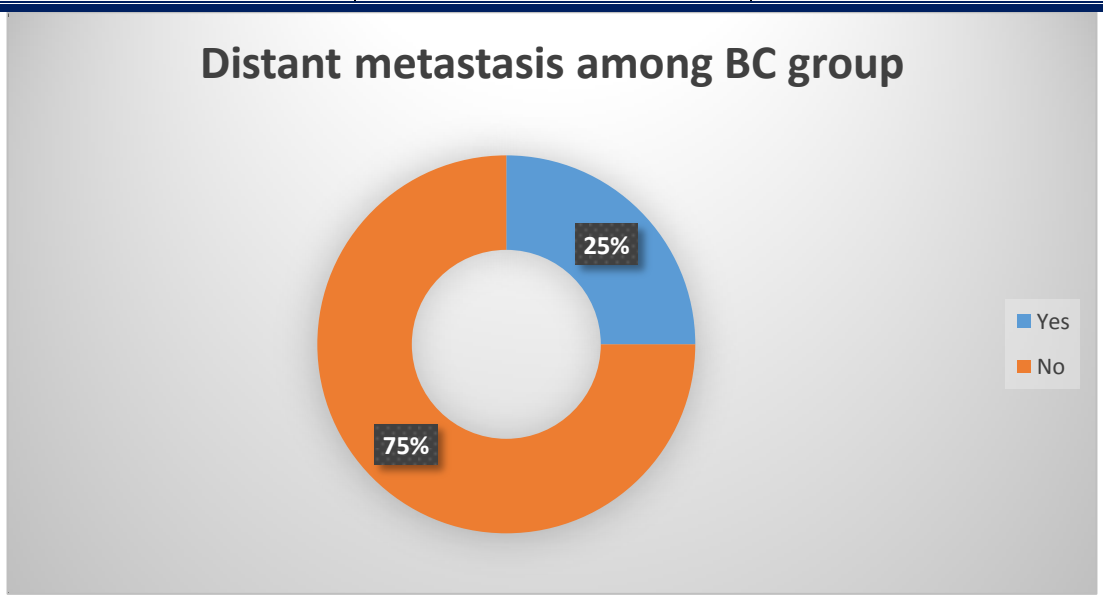

Fig. (27): Progression events in the cancer patients.

Table (12) and fig. (27) show that 25% of cases developed distant metastasis while 75% show no metastasis.

Table (13): rs217727 lncRNA-H19 genotypic frequencies in the study groups.

Genotype	Healthy control		Patient groups				P value	OR(95% CI)
			Benign group		BC group			
	No	%	No	%	No	%		
n (%) TT	3	6.0	4	8.0	17	17.0	P1=0.062 P2=0.134 P3=0.03*	3.21(0.89-11.52) 2.36 (0.75-7.42) 2.72 (1.08-6.89)
CT	19	38.0	20	40.0	46	46.0	P1=<0.001** P2=0.49 P3=0.004**	1.39 (0.70-2.78) 1.28 (0.64-2.55) 1.33 (0.76-2.34)
CC	28	56.0	26	52.0	37	37.0	P1=0.027* P2=0.32 P3=0.016*	0.46 (0.23-0.92) 0.54 (0.27-1.08) 0.50 (0.28-0.88)

P1: BC compared to healthy control group, **P2:** BC compared to benign group, **P3:** BC compared to non-malignant females; χ^2**:** *Chi-square;* ***OR****:odd ratio;* ***CI****: confidence interval;* ***OR(CI)****>1:risk &<1: protective;* ***p*** >0.05: Non-significant difference.

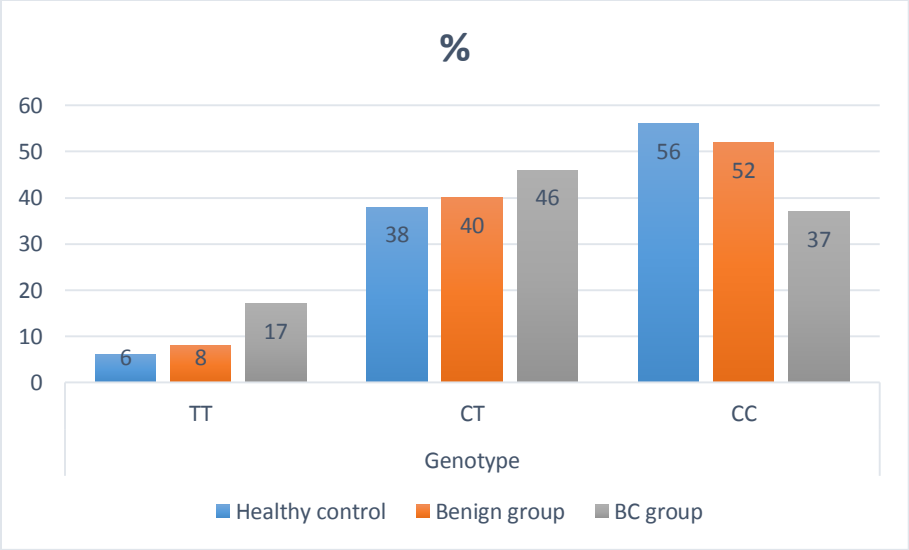

Fig.(28): rs217727 lncRNA-H19 genotypic frequencies in the study group.

Table (13) and fig. (28) show that the CC genotype was observed in 37% (37/100) of BC patients compared with 52% (26/50) of benign group and 56% (28/50) of the controls. The heterozygous genotype (CT) was observed in 46% (46/100) of BC patients, 40% (20/50) of benign and 38% (19/50) of the controls.

Only 17 patients (17%) had the homozygous mutant TT genotype, 4 women in benign group (8%) and 3 women in controls (6%).

There is a significant increase in the frequency of the heterozygous variant CT genotype was observed in BC patients compared with the controls (p1 <0.001) and significant increase in frequency in BC compared with non-malignant group (P3=0.004). The polymorphic genotype (TT) was likely to be significantly increased in BC patients as compared to non-malignant group (p3=.0.003).The CC genotype was observed to be significant higher in BC compared to control group (P1=0.027) and significant higher in BC to non-malignant group (P3=0.016).

Table (14): rs217727 lncRNA-H19 allelic frequencies of the study group.

Variable	Healthy control (50)	Patient groups		Statistical test	P value			OR (95%CI)
		Benign group(50)	BC (100)					
	No (%)	No (%)	No (%)					
Allele				X2= 8.41	0.014*	0.015*	P1=0.01*	2.0 (1.17-3.41)
T	25 (25%)	28 (28%)	80 (40%)				P2=0.04	1.71 (1.02- 2.88)
							P3=0.004**	1.85 (1.21-2.82)
C	75 (75%)	72 (72%)	120 (60%)					

P1: BC compared to healthy control group, **P2:** BC compared to benign group, **P3:** BC compared to non-malignant females; χ^2**:** *Chi-squaretest;* ***OR****:odd ratio;* ***CI****: confidence interval;* ***OR(CI)****>1;risk &<1: protective; p>0.05: Non-significant difference.*

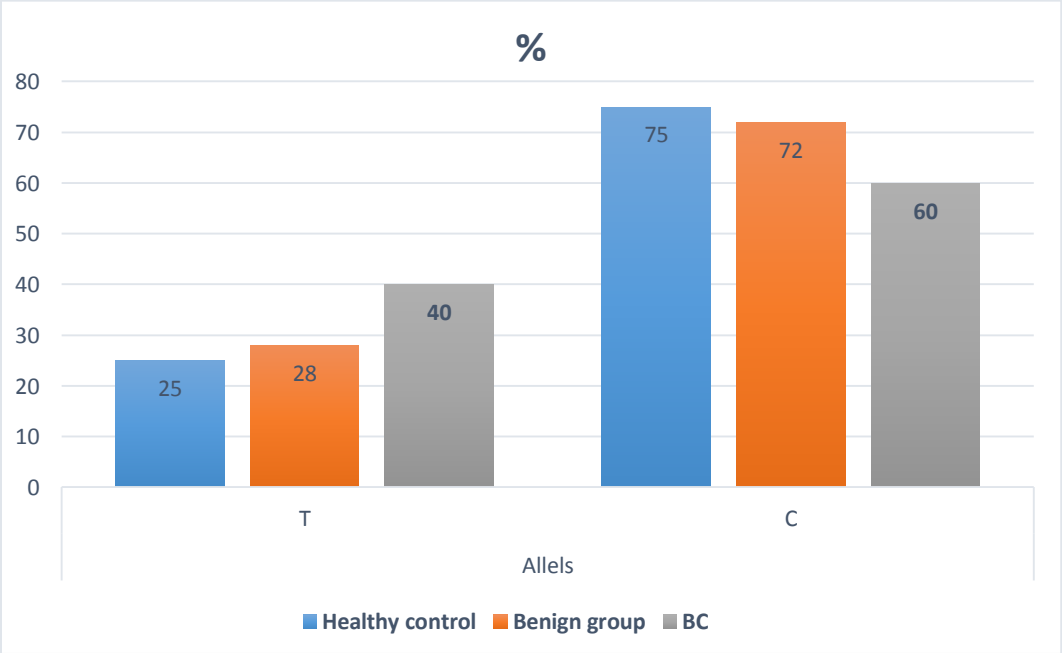

Fig. (29): rs217727 lncRNA-H19 allelic frequencies of the study group.

Table (14) and fig. (29) show that the T allele was high (40%) in BC patients and low in benign group (28%) and controls (25%). While the C allele was low (60%) in BC patients and higher in benign group (72%) and

controls (75%). we found a significant correlation between increase T allele, decrease C allele and increase risk of breast cancer (*P* = 0.015), T&C alleles showed significant differences when BC compared to control group (P1=0.01; OR=2.0; CI=1.17-3.41), when compared to benign group (P2=0.04; OR=1.71; CI=1.02- 2.88) and when compared to non-malignant group (P3=0.004; OR=1.85; CI=1.21-2.82).

Table (15): rs217727 lncRNA-H19 genotypic frequencies between metastasis and non- metastasis groups.

BC group (100)	Distant metastasis (25)	No distant metastasis	Statistical test	P value	OR(95% CI)
	No (%)	No (%)			
TT	2 (8%)	15 (20%)	X2= 2.06	0.36	0.35 (0.07-1.64)
CT	12 (48%)	34 (45.3%)			1.11(0.45-2.76)
CC	11 (44%)	26 (34.7%)			1.48 (0.59-3.72

χ^2***:*** *Chi-square;* ***OR****:odd ratio;* ***CI****: confidence interval;* ***OR(CI)****>1:risk &<1: protective; p*>0.05: Non-significant difference.

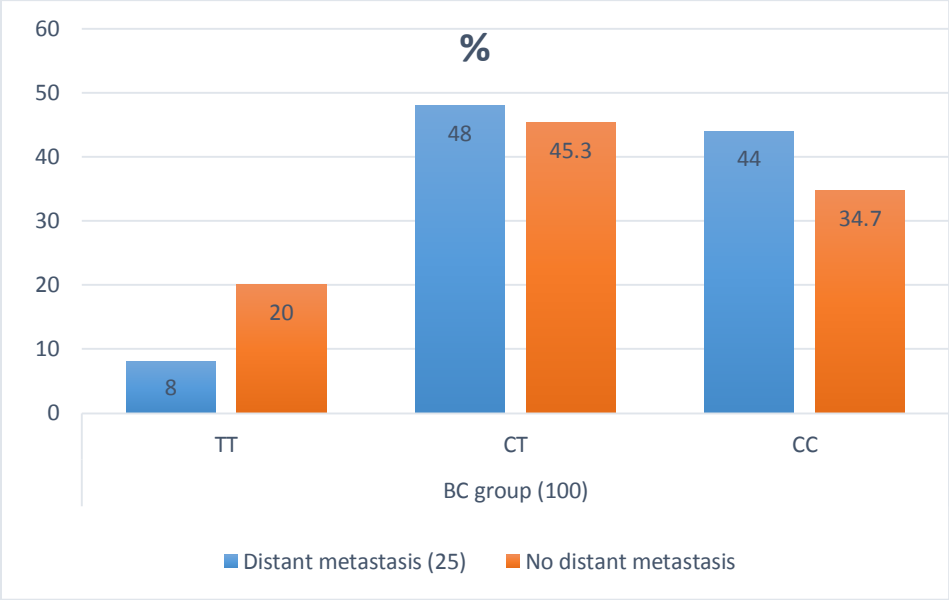

Fig. (30): rs217727 lncRNA-H19 genotypic frequencies between metastasis and non-metastasis groups.

Table (15) and fig. (30) show that there was no significant difference between genotype frequency and metastasis.

Table (16): Demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of control group.

Control group (50)	TT (3)	CT (19)	CC (28)	Statistical test	P value
Quantitative	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Age	44.67 ± 8.96	52.21 ± 12.10	46.07 ± 11.89	F= 1.67	0.20
Age at menarche	12.33 ± 0.58	12.42 ± 1.54	13.5 ± 1.69	F= 2.87	0.07
Age at menopause (postmenopausal)	47.0 ± 4.24	48.56 ± 5.43	48.53 ± 4.14	F= 0.097	0.91
Qualitative	**No (%)**	**No (%)**	**No (%)**		
Marital status **Single** **Married**	 0 (0%) 3 (100%)	 3 (15.8%) 16 (84.2%)	 7 (25%) 21 (75%)	FET= 0.91	0.86
No of pregnancy **Nulli gravida** **Primi gravida** **Multigravida**	 0 (0%) 0 (0%) 3 (100%)	 4 (21.1%) 0 (0%) 15 (78.9%)	 6 (21.4%) 3 (10.7%) 19 (67.9%)	FET= 2.79	0.62
No of abortion **0** **1** **2**	 3 (100%) 0 (0%) 0 (0%)	 15 (78.9%) 2 (10.5%) 2 (10.5%)	 24 (85.7%) 1 (3.6%) 3 (10.7%)	FET= 1.82	0.84
Breast feeding	1 (33.3%)	12 (63.2%)	17 (60.7%)	FET= 1.07	0.72
Family history	1 (33.3%)	3 (15.8%)	8 (28.6%)	FET= 1.46	0.48
Systemic disease **No** **DM** **HTN** **Both**	 2 (66.7%) 0 (0%) 1 (33.3%) 0 (0%)	 4 (21.1%) 4 (21.1%) 7 (36.8%) 4 (21.1%)	 12 (42.9%) 6 (21.4%) 6 (21.4%) 4 (14.3%)	FET= 4.53	0.61
Smoking	0 (0%)	1 (5.3%)	2 (7.1%)	FET= 0.62	1.0
Contraception **No** **Pills** **IUD**	 1 (33.3%) 1 (33.3%) 1 (33.3%)	 4 (21.1%) 5 (26.3%) 10 (52.6%)	 11 (39.3%) 7 (25%) 10 (35.7%)	FET= 2.54	0.70

χ^2*: Chi-square;* ***FET:*** *fisher exact test; p*>0.05: Non-significant difference.

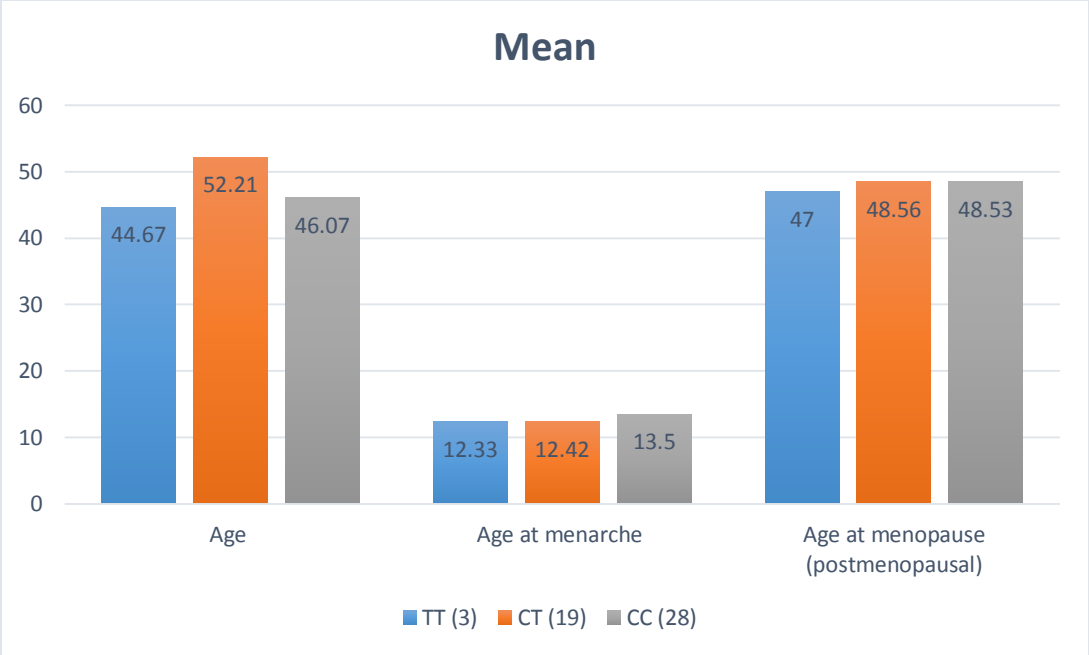

Fig. (31): Quantitative demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of control group.

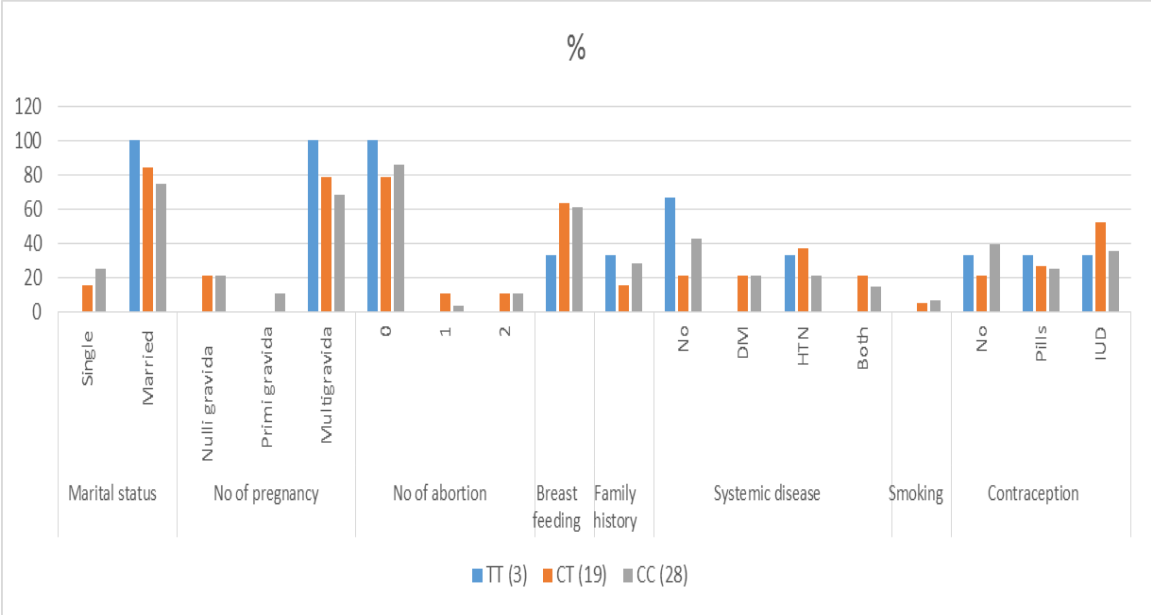

Fig. (32): Qualitative demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of control group.

Table (16) and fig. (31, 32) show non-significant statistical differences regarding marital status, age , age at menarche, age at menopause, no of

pregnancy, no of abortion, menopausal status, breast feeding , family history, systemic diseases, smoking and contraception between different lncRNA-H19 rs217727 genotypes of control group (*p* values > 0.05).

Table (17): Demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of the benign group.

Benign group (50)	**TT (4)**	**CT (20)**	**CC (26)**	**Statistical test**	**P value**
Quantitative	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Age	50.5 ± 11.82	42.0 ± 10.84	47.42 ± 13.11	F= 1.49	0.24
Age at menarche	13.25 ± 0.96	13.2 ± 1.54	12.65 ± 1.23	F= 1.05	0.36
Age at menopause (postmenopausal)	51.5± 7.78	47.38 ± 5.61	45.67 ± 12.32	F= 0.30	0.74
Qualitative	**No (%)**	**No (%)**	**No (%)**		
Marital status **Single** **Married**	 1 (25%) 3 (75%)	 5 (25%) 15 (75%)	 4 (15.4%) 22 (84.6%)	FET= 1.07	0.57
No of pregnancy **Nulli gravida** **Primi gravida** **Multigravida**	 2 (50%) 0 (0%) 2 (50%)	 6 (30%) 1 (5%) 13 (65%)	 4 (15.4%) 4 (15.4%) 18 (69.2%)	FET= 3.77	0.41
No of abortion **0** **1** **2**	 3 (75%) 1 (25%) 0 (0%)	 17 (85%) 1 (5%) 2 (10%)	 23 (88.5%) 1 (3.8%) 2 (7.7%)	FET= 3.15	0.60
Menopausal status **Pre** **Post**	 2 (50%) 2 (50%)	 13 (65%) 7 (35%)	 12 (46.2%) 14 (53.8%)	FET= 1.74	0.39
Breast feeding	2 (50%)	11 (55%)	17 (65.4%)	FET= 0.86	0.68
Family history	2 (50%)	6 (30%)	6 (23.1%)	FET= 1.53	0.52
Systemic disease **No** **DM** **HTN** **Both**	 2 (50%) 2 (50%) 0 (0%) 0 (0%)	 9 (45%) 4 (20%) 5 (25%) 2 (10%)	 8 (30.8%) 7 (26.9%) 6 (23.1%) 5 (19.2%)	FET= 3.63	0.77
Smoking	0 (0%)	2 (10%)	1 (3.8%)	FET= 1.1	0.67
Contraception **No** **Pills** **IUD**	 1 (25%) 1 (25%) 2 (50%)	 8 (40%) 5 (25%) 7 (35%)	 8 (30.8%) 5 (19.2%) 13 (50%)	FET= 1.58	0.87

χ^2*: Chi-square;* ***FET:*** *fisher exact test; p*>0.05: Non-significant difference.

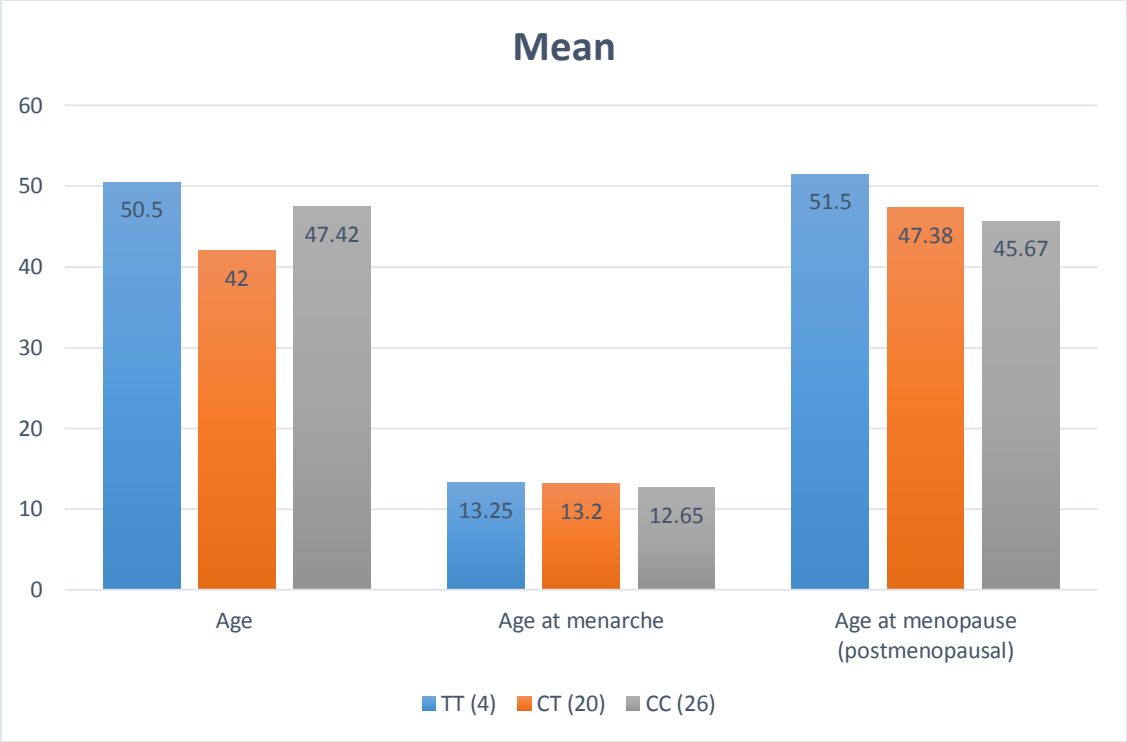

Fig. (33): Quantitative demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of the benign group.

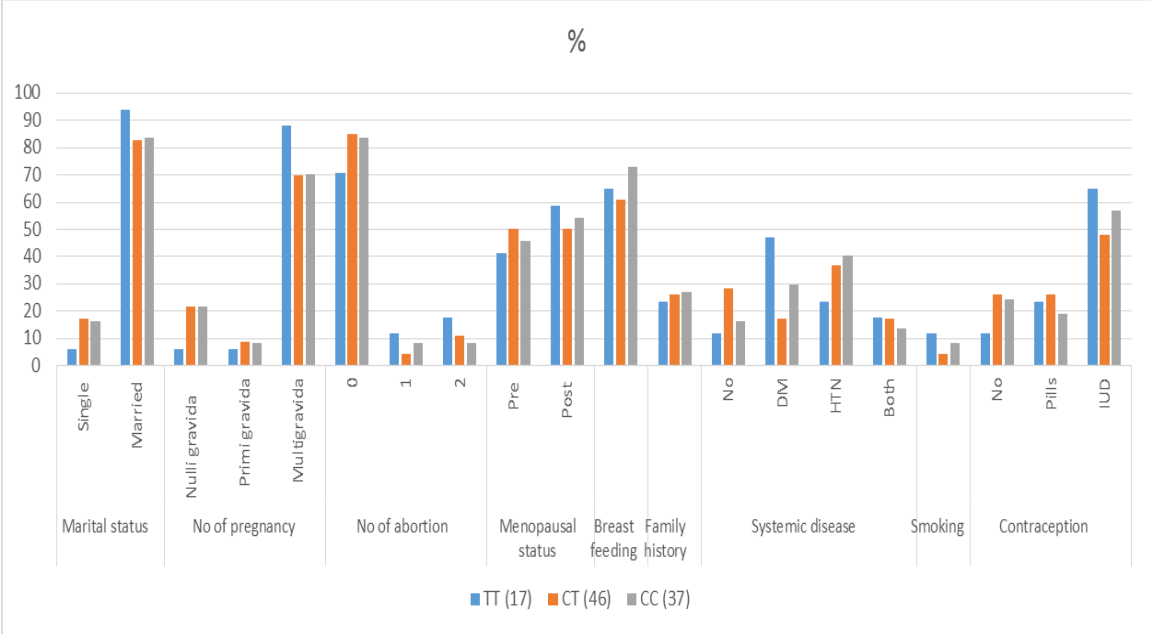

Fig. (34): Qualitative demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of the benign group**.**

Table (17) and fig. (33, 34) show non-significant statistical differences regarding marital status, age, age at menarche, age at menopause, no of pregnancy, no of abortion, menopausal status, breast feeding , family history, systemic diseases, smoking and contraception between different lncRNA-H19 rs217727 genotypes of benign group (*p* values > 0.05).

Table (18): Demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of BC group.

BC group (100)	TT (17)	CT (46)	CC (37)	Statistical test	P value
Quantitative	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
Age	47.94 ± 7.9	49.3 ± 11.15	50.32 ± 11.42	F= 0.29	0.75
Age at menarche	12.59 ± 1.37	12.96 ± 1.30	13.11 ± 1.35	F= 0.89	0.41
Age at menopause ,(postmenopausal)	48.75 ± 5.01	50.13 ± 4.19	50.04 ± 5.05	F= 0.41	0.67
Qualitative	**No (%)**	**No (%)**	**No (%)**		
Marital status **Single** **Married**	 1 (5.9%) 16 (94.1%)	 8 (17.4%) 38 (82.6%)	 6 (16.2%) 31 (83.8%)	X2= 1.36	0.51
No of pregnancy **Nulli gravida** **Primi gravida** **Multigravida**	 1 (5.9%) 1 (5.9%) 15 (88.2%)	 10 (21.7%) 4 (8.7%) 32 (69.6%)	 8 (21.6%) 3 (8.1%) 26 (70.3%)	FET= 2.59	0.67
No of abortion **0** **1** **2**	 12 (70.6%) 2 (11.8%) 3 (17.6%)	 39 (84.8%) 2 (4.3%) 5 (10.9%)	 31 (83.8%) 3 (8.1%) 3 (8.1%)	FET= 2.86	0.58
Menopausal status **Pre** **Post**	 7 (41.2%) 10 (58.8%)	 23 (50%) 23 (50%)	 17 (45.9%) 20 (54.1%)	X2= 0.41	0.81
Breast feeding	11 (64.7%)	28 (60.9%)	27 (73%)	X2= 1.35	0.51
Family history	4 (23.5%)	12 (26.1%)	10 (27%)	X2= 0.07	0.96
Systemic disease **No** **DM** **HTN** **Both**	 2 (11.8%) 8 (47.1%) 4 (23.5%) 3 (17.6%)	 13 (28.3%) 8 (17.4%) 17 (37%) 8 (17.4%)	 6 (16.2%) 11 (29.7%) 15 (40.5%) 51 (3.5%)	FET= 7.27	0.29
Smoking	2 (11.8%)	2 (4.3%)	3 (8.1%)	FET= 1.49	0.52
Contraception **No** **Pills** **IUD**	 2 (11.8%) 4 (23.5%) 11 (64.7%)	 12 (26.1%) 12 (26.1%) 22 (47.8%)	 9 (24.3%) 7 (18.9%) 21 (56.8%)	FET= 2.31	0.69

χ^2**:** *Chi-square test;* ***FET:*** *fisher exact test; p*>0.05: Non-significant difference.

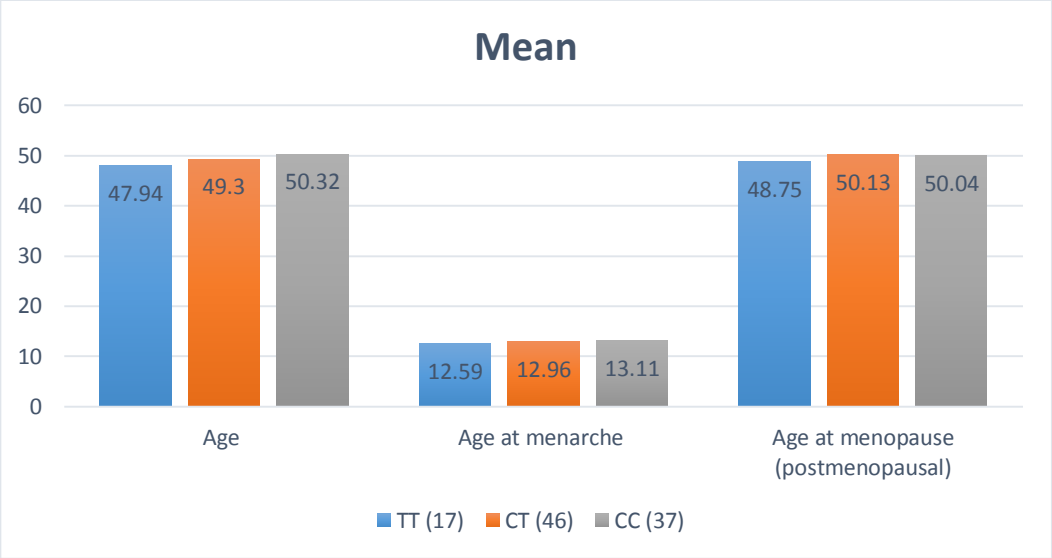

Fig. (35): Quantitative demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of BC group.

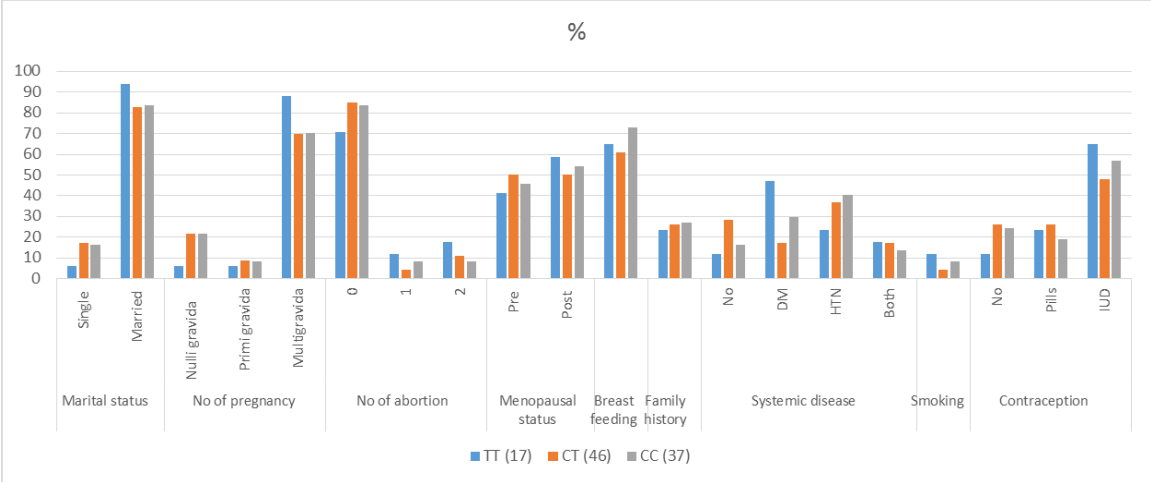

Fig. (36): Qualitative Demographic and clinical characteristics among different lncRNA-H19 rs217727 genotypes of BC group.

Table (18) and fig. (35, 36) show non-significant statistical differences regarding include marital status, age, age at menarche, age at menopause, no of pregnancy, no of abortion, menopausal status, breast feeding , family history, systemic diseases, smoking and contraception between different lncRNA-H19 rs217727 genotypes of BC group (*p* values > 0.05).

Table (19): Association of H19 long noncoding RNA polymorphisms and specific molecular subtypes for breast cancer patients (ER, PR, HER2).

BC group (100)	TT (17)	CT (46)	CC (37)	Statistical test	P value
	No (%)	No (%)	No (%)		
ER status **Yes** **No**	 15 (88.2%) 2 (11.8%)	 33 (71.7%) 13 (28.3%)	 26 (70.3%) 11 (29.7%)	X2= 2.18	0.34
P value	0.14	0.63	0.52		
OR (95% CI)	3.05 (0.65-14.37)	0.81 (0.33-1.97)	0.74(0.30-1.84)		
PR status **Yes** **No**	 10 (58.8%) 7 (41.2%)	 35 (76.1%) 11 (23.9%)	 27 (73%) 10 (27%)	X2= 1.86	0.39
P value	0.18	0.40	0.87		
OR (95% CI)	0.48 (0.16-1.43)	1.46 (0.60-3.55)	1.08(0.44-2.68)		
HER2 status **Yes** **No**	 11 (64.7%) 6 (35.3%)	 36 (78.3%) 10 (21.7%)	 28 (75.7%) 9 (24.3%)	X2= 1.23	0.54
P value	0.28	0.49	0.91		
OR (95% CI)	0.54 (0.18-1.67)	1.39 (0.55-3.47)	1.06 (0.41-2.72)		

ER: estrogen receptors, PR: progesterone receptors, Her2/neo: human epidermal growth factor receptor 2, +ve: positive, -ve: negative; χ^2*: Chi-square;* ***OR****:odd ratio;* ***CI****: confidence interval;* ***OR(CI)****>1:risk &<1: protective; p*>0.05: Non-significant difference.

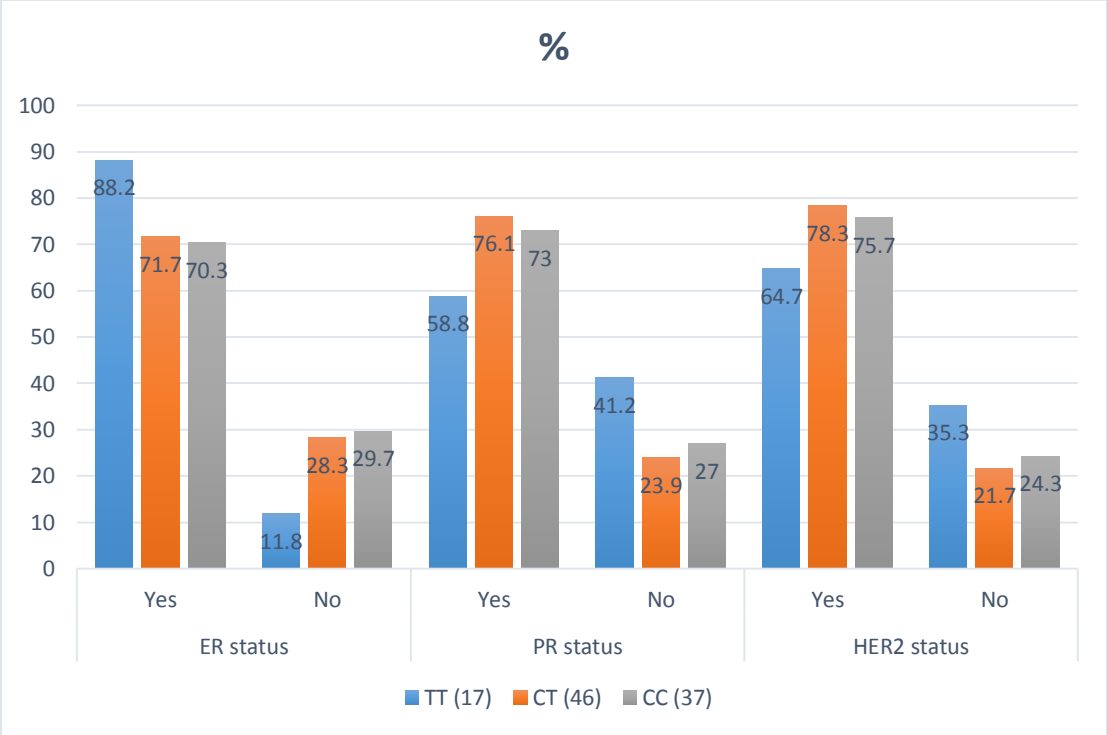

Fig. (37): Association of H19 long noncoding RNA polymorphisms and specific molecular subtypes for breast cancer patients.

Table (19) and fig. (37) show that 70.3% (26/37) of the CC genotype was ER +ve, 73% (27/37) was PR +ve and 75.7% (28/37) was HER2 +ve. While CT genotype had 71.7% (33/46) ER +ve , 76.1% (35/46) PR +ve and 78.3% (36/46) HER2 +ve. On the hand, TT genotype showed 88.2% (15/17) ER +ve , 58.8% (10/17) PR +ve and 64.7% (11/17) HER2 +ve. This table shows non-significant statistical differences regarding hormonal status among different rs217727 lncRNA-H19 genotypes of BC group (*p* values > 0.05).

Table (20): H19 lncRNA expression in BC, benign and control group.

		Patient groups		Statistical test	P value	
	Healthy control (50)	Benign group (50)	BC (100)			
	Mean ± SD	Mean ± SD	Mean ± SD			
RQ value	1.0 ± 0.08	1.27 ± 0.07	2.04 ± 0.22	F= 821.9	<0.001**	**P1**=<0.001**
						P2=<0.001**
						P3=<0.001**

P1: BC compared to healthy control group, **P2:** BC compared to benign group, **P3:** BC compared to non-malignant females; χ^2**:** *Chi-square; p*>0.05: Non-significant difference.

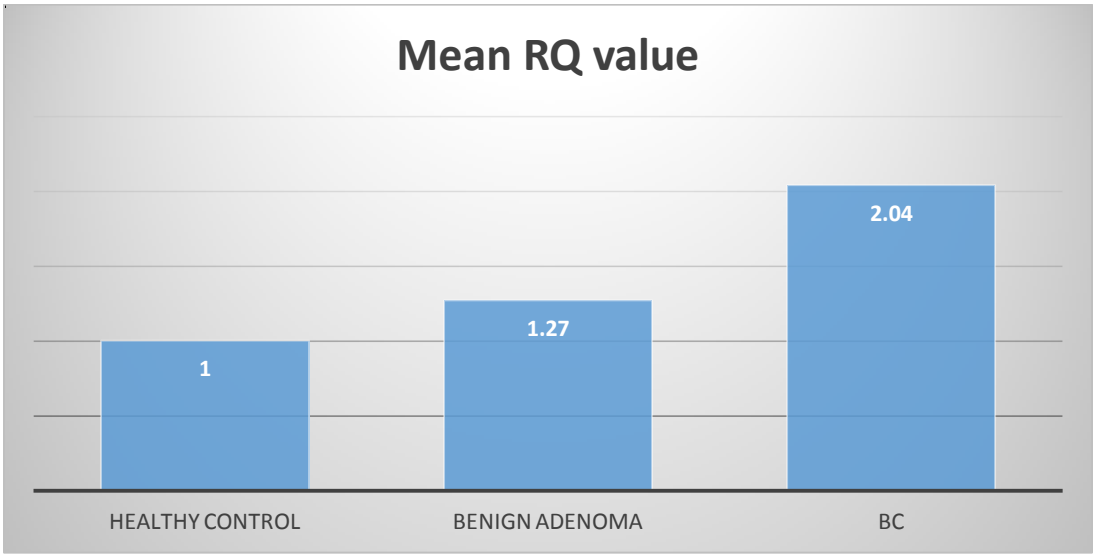

Fig. (38): H19 lncRNA expression in BC, benign and control group.

Table (20) and fig. (38) show that H19 lncRNA expression levels were significantly increased in BC group compared to benign and control group (*p* value < 0.001).

Table (21): lncRNA-H19 gene expressions of BC, benign and control group according to the qualitative demographic & clinical characteristics.

Variable	BC group (100)			Benign group (50)			Control group (50)		
	RQ value	Statistical test (st t)	P value	RQ value	Statistical test (st t)	P value	RQ value	Statistical test (st t)	P value
	Mean ± SD			Mean ± SD			Mean ± SD		
Marital status **Single** **Married**	 2.0 ± 0.14 2.05 ± 0.23	0.78	0.44	 1.30 ± 0.06 1.26 ± 0.065	1.49	0.144	 0.97 ± 0.07 1.01 ± 0.08	1.45	0.15
No of pregnancy **Nulli gravida** **Primi gravida** **Multigravida**	 1.99 ± 0.14 2.03 ± 0.21 2.05 ± 0.23	F= 0.753	0.473	 1.31 ± 0.061 1.24 ± 0.064 1.26 ± 0.058	F= 2.78	0.072	 0.98 ± 0.06 1.03 ± 0.04 1.0 ± 0.09	F= 0.68	0.51
No of abortion **0** **1** **2**	 2.03 ± 0.21 2.11 ± 0.30 2.05 ± 0.23	F= 0.453	0.637	 1.31 ± 0.058 1.24 ± 0.061 1.26 ± 0.064	F= 0.499	0.61	 0.999 ± 0.08 0.993 ± 0.078 1.01 ± 0.11	F= 0.07	0.94
Menopausal status **Pre** **Post**	 2.039 ± 0.22 2.04 ± 0.21	0.019	0.99	 1.28 ± 0.069 1.25 ± 0.057	1.79	0.081			
Breast feeding **Yes** **No**	 2.03 ± 0.23 2.06 ± 0.20	0.62	0.54	 1.26 ± 0.066 1.28 ± 0.063	0.98	0.33	 0.996 ± 0.081 1.01 ± 0.084	0.42	0.68
Family history **Yes** **No**	 2.0 ± 0.19 2.05 ± 0.22	1.02	0.31	 1.29 ± 0.07 1.26 ± 0.063	1.27	0.211	 0.98 ± 0.06 1.01 ± 0.09	0.81	0.42
Systemic disease **No** **DM** **HTN** **Both**	 2.01 ± 0.18 2.05 ± 0.24 2.04 ± 0.22 2.05 ± 0.22	F= 0.19	0.91	 1.28 ± 0.07 1.27 ± 0.067 1.27 ± 0.07 1.23 ± 0.024	F= 1.07	0.371	 0.994 ± 0.081 1.02 ± 0.095 0.994± 0.071 0.996 ± 0.092	0.293	0.831
Smoking **Yes** **No**	 2.08 ± 0.28 2.04 ± 0.21	0.53	0.60	 1.25 ± 0.062 1.27 ± 0.066	0.55	0.59	 0.95± 0.11 1.0 ± 0.08	1.03	0.31
Contraception **No** **Pills** **IUD**	 2.01 ± 0.16 2.05 ± 0.20 2.05 ± 0.24	F= 0.36	0.70	 1.28 ±0.066 1.30 ±0.062 1.25±0.063	2.04	0.14	 0.977 ± 0.06 1.01 ± 0.098 1.012 ± 0.085	0.952	0.393

Data are qualitative data presented as **mean, ± SD**; and quantitative presented as **numbers, percentages**; ; χ^2**:** *Chi-square; p*>0.05: Non-significant difference.

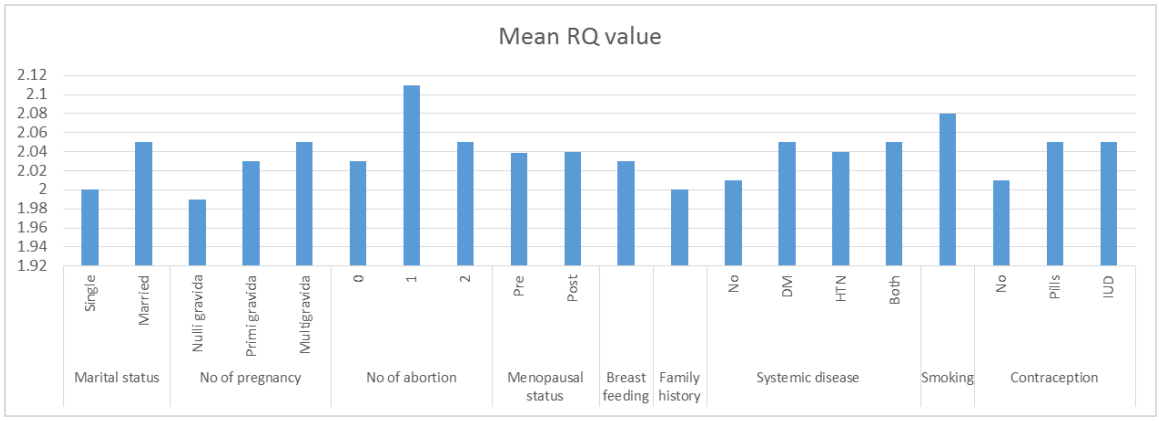

Fig. (39): lncRNA-H19 gene expressions of BC group according to the qualitative demographic & clinical characteristics

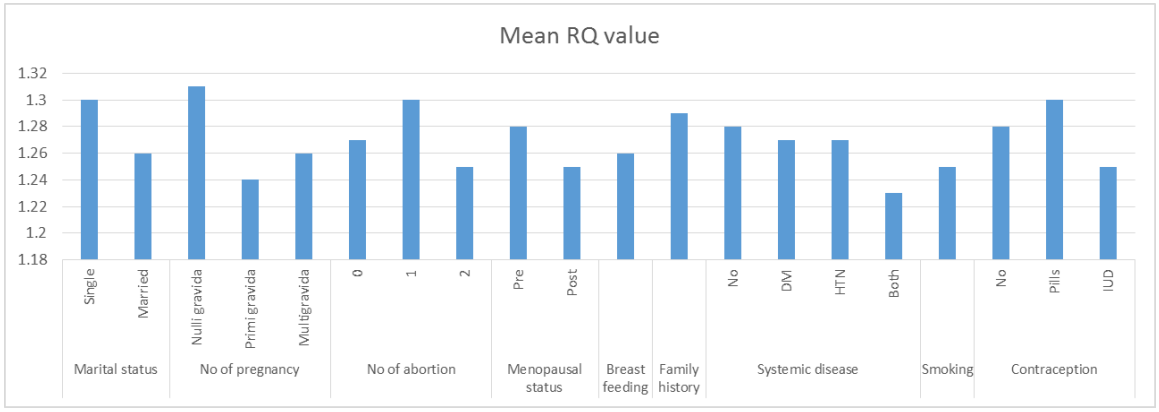

Fig. (40): lncRNA-H19 gene expressions of benign group according to the qualitative demographic & clinical characteristics**.**

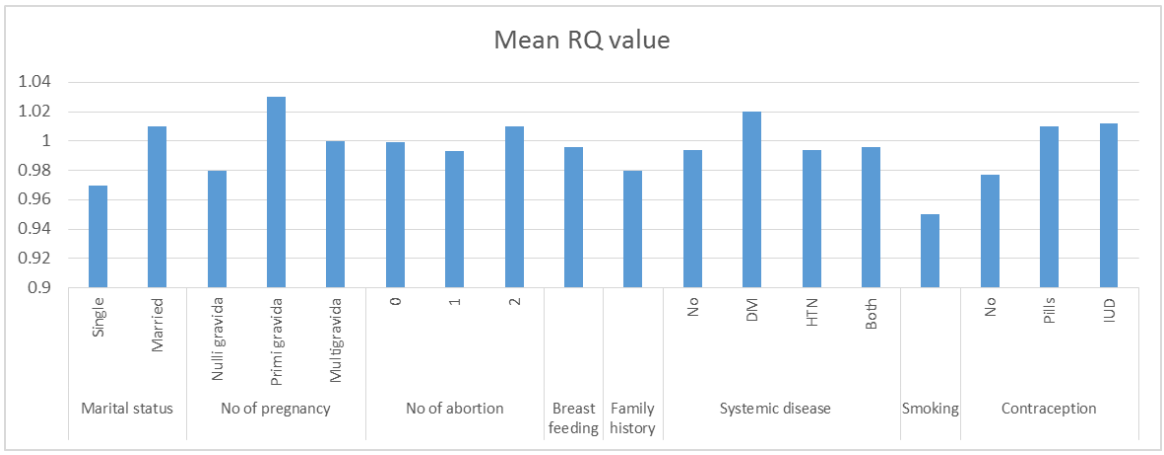

Fig. (41): lncRNA-H19 gene expressions of benign group according to the qualitative demographic & clinical characteristics.

Table (21) and fig. (39, 40, 41) show that there were non-significant statistical differences in lncRNA-H19 gene expressions levels regarding include marital status, no of pregnancy, no of abortion, menopausal status, breast feeding, family history, systemic diseases, smoking and contraception in BC benign and control group (*p* values > 0.05).

Table (22): H19 lncRNA expression of the BC group according to the quantitative demographic & clinical characteristics.

	Patient groups				Control group (50)	
	BC (100)		Benign group (50)			
	RQ value	P value	RQ value	P value	RQ value	P value
Age	-0.01	0.925	-0.151	0.297	0.096	0.507
Age at menarche	-0.241	0.016*	0.202	0.16	-0.092	0.525
Age at menopause (postmenopausal)	-0.091	0.459	-0.111	0.574	0.04	0.823

Data presented as **mean, ± SD**; *p*>0.05: Non-significant difference.

Table (22) shows that there were significant statistical differences in lncRNA-H19 gene expressions levels regarding age of menarche (P= 0.016), but there were no significant differences in age and age at menopause in BC group.

- There were non-significant statistical differences in lncRNA-H19 gene expressions levels regarding age, age of menarche and age at menopause in the benign group (*p* values > 0.05).

- There were non-significant statistical differences in lncRNA-H19 gene expressions levels regarding age, age of menarche and age at menopause in the control group (*p* values > 0.05).

Table (23): Distribution of H19 lncRNA gene expression between metastasis and non- metastasis groups.

BC group (100)	RQ value	Statistical test (F test)	P value
	Mean ±SD		
Distant metastasis **Yes** **No**	2.07 ± 0.22 1.96 ± 0.19	st t= 2.05	0.043*

p>0.05: Non-significant difference.

Table (23) show significant statistical decrease in H19 lncRNA gene expression in metastatic group than non-metastatic group being higher in metastatic group (*p* values < 0.05).

Table (24): Association of H19 lncRNA gene expression and specific molecular subtypes for breast cancer patients (ER, PR HER2).

BC group (100)	RQ value	Statistical test (st t)	P value
	Mean ± SD		
ER status **Yes** **No**	 2.06 ± 0.22 2.0 ± 0.18	1.23	0.22
PR status **Yes** **No**	 2.03 ± 0.21 2.08 ± 0.22	1.02	0.31
HER2 status **Yes** **No**	 2.02 ± 0.21 2.09 ± 0.22	1.27	0.21

ER: estrogen receptors, PR: progesterone receptors, Her2/neo: human epidermal growth factor receptor 2, +ve: positive, -ve: negative; *p*>0.05: Non-significant difference.

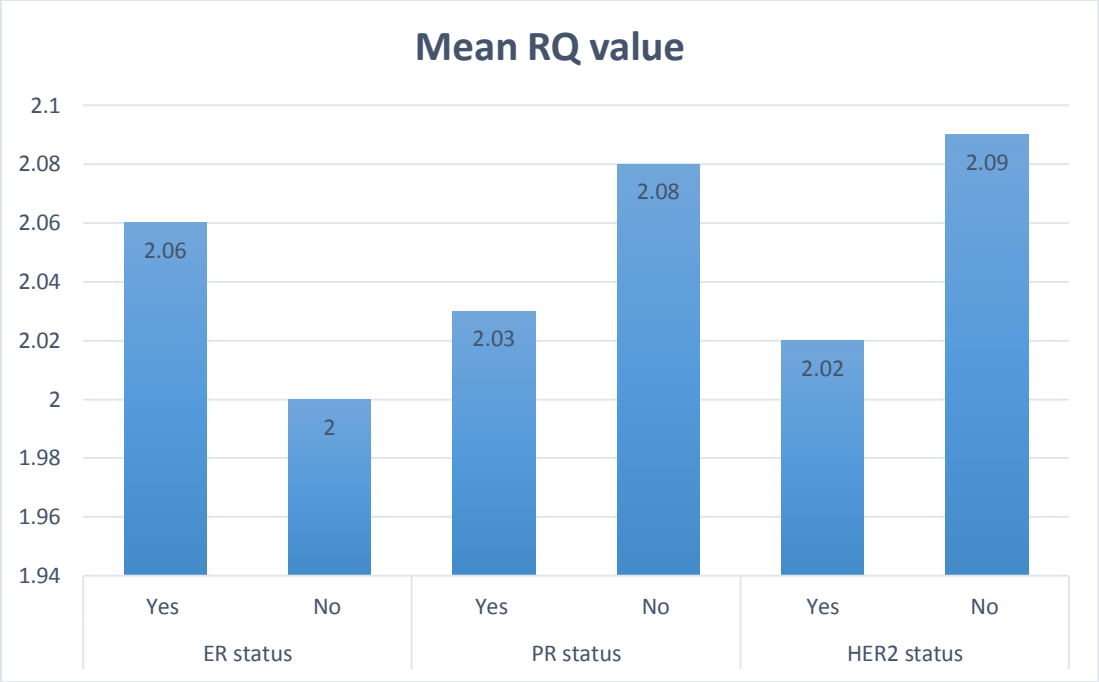

Fig. (42): Association of H19 lncRNA gene expression and specific molecular subtypes for breast cancer patients.

Table (24) and fig. (42) show that there were non-significant statistical differences regarding hormonal status among different lncRNA-H19 gene expression levels of BC group (*p* values > 0.05).

Table (25): Association of H19 lncRNA gene expression and grading.

BC group (100)	RQ value Mean ± SD	Statistical test (F test)	P value
Grade			
I	2.07 ± 0.233	F= 0.403	0.669
II	2.02 ± 0.230		
III	2.06 ± 0.173		

p>0.05: Non-significant difference.

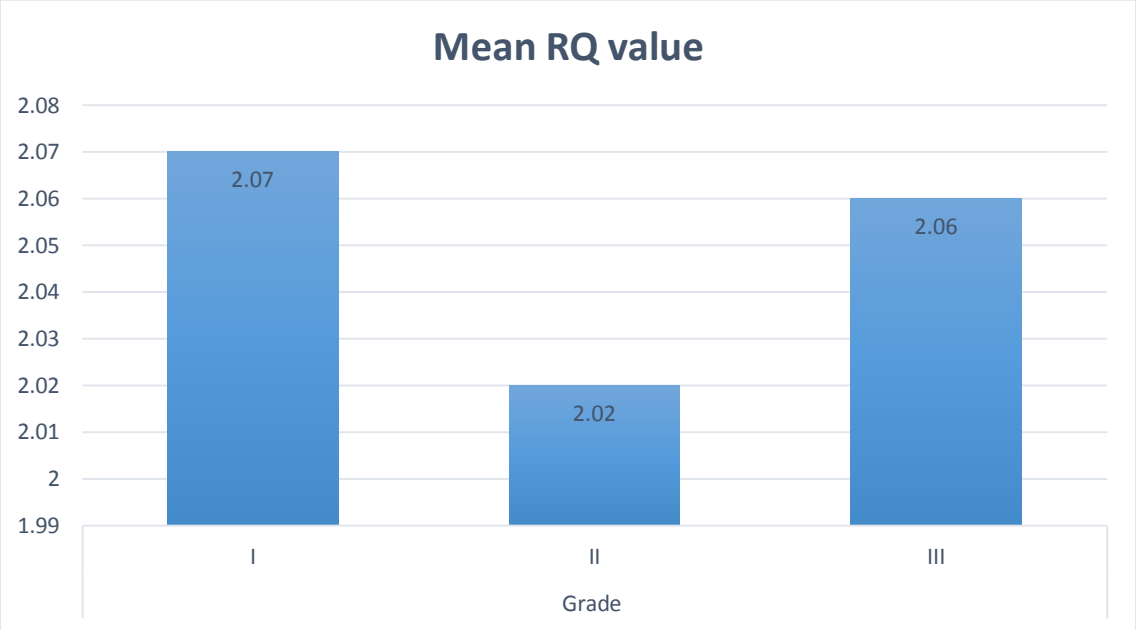

Fig. (43): Association of H19 lncRNA gene expression and grading

Table (25) and fig. (43) show that there were non-significant statistical differences regarding tumor grading between different lncRNA-H19 gene expression levels of BC group (*p* values > 0.05).

Table (26): Association of lncRNA-H19 gene expression and staging**.**

BC group (100)	RQ value	Statistical test (F test)	P value
	Mean ± SD		
Stage			
I	2.01 ± 0.24	F= 1.54	0.21
II	2.05 ± 0.22		
III	2.09 ± 0.191		
IV	1.96 ± 0.193		

p>0.05: Non-significant difference.

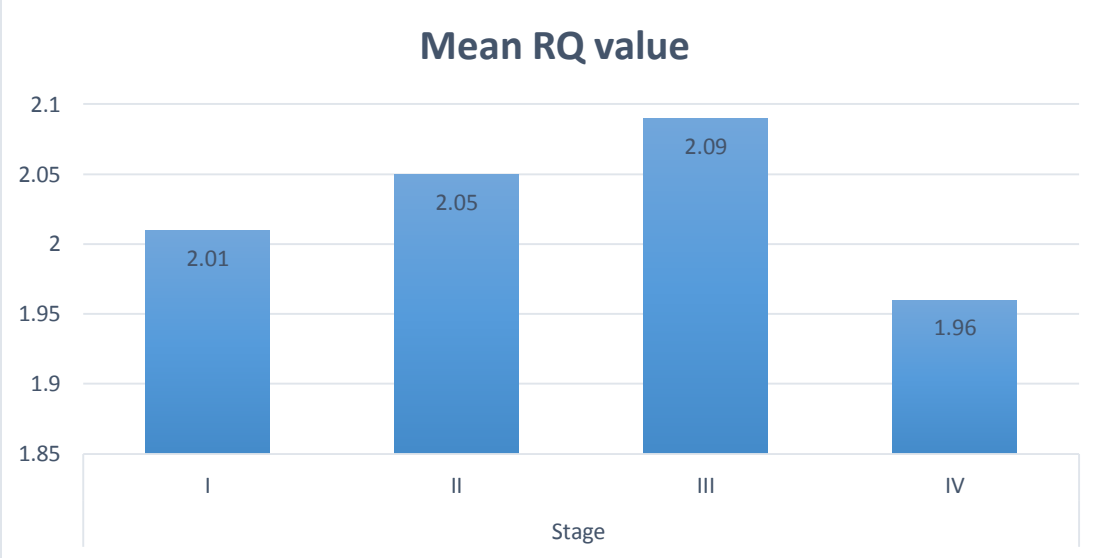

Fig. (44): Association of lncRNA-H19 gene expression and staging.

Table (26) and fig. (44) show that there were non-significant statistical differences regarding tumor staging between different lncRNA-H19 gene expression levels of BC group (*p* values > 0.05).

Table (27): Effect of different rs217727 genotypes on progression-free survival in BC Patients.

	TT (17)	CT(46)	CC (37)	Statistical test	P value
	No (%)	No (%)	No (%)		
Survival **Died** **Alive**	 2 (11.8%) 15 (88.2%)	 3 (6.5%) 43 (93.5%)	 8 (21.6%) 29 (78.4%)	FET= 4.0	0.12

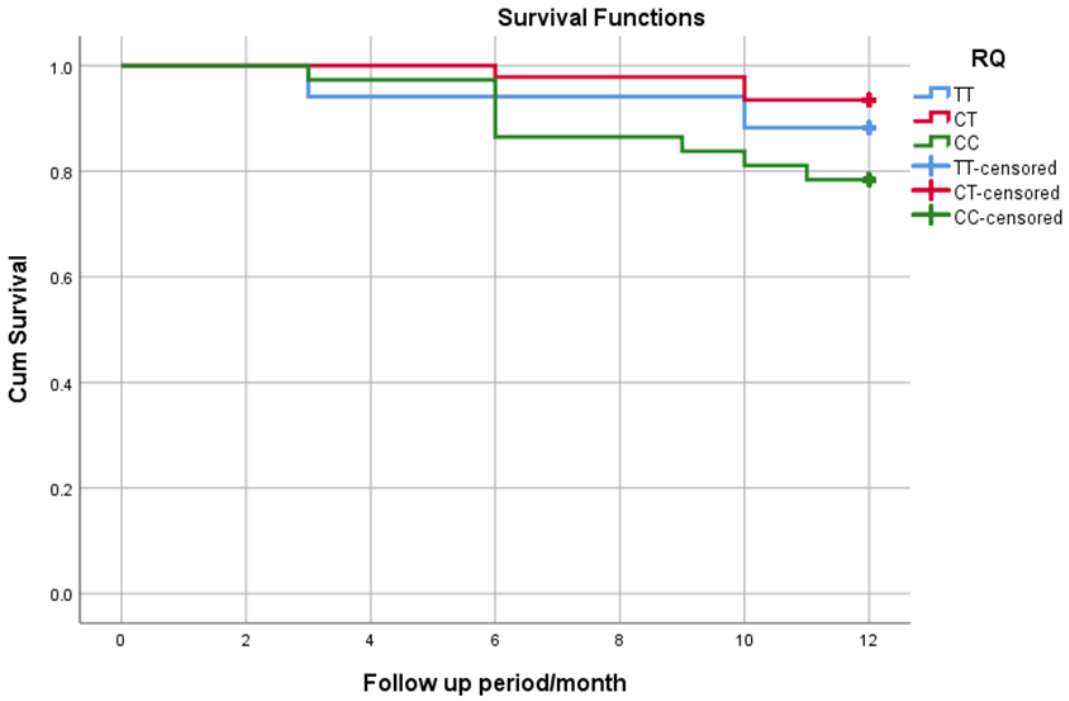

Fig. (45): Effect of different rs217727 genotypes on progression-free survival in BC Patients.

Table (27) and fig. (45) show that the survival rate was higher in CT genotype (93.5%), but there were no significant differences (*p* value < 0.05).

Table (28): H19 lncRNA expression levels among different rs217727 genotypes of BC, benign and control group.

Genotype		Patient groups		P value	
	Healthy control (50)	Benign group (50)	BC (100)		
	Mean ± SD	Mean ± SD	Mean ± SD		
TT	1.03 ± 0.086	1.36 ± 0.01	2.36 ± 0.09	TT= <0.001**	P1=<0.001**
					P2=<0.001**
					P3=<0.001**
CT	1.0 ± 0.085	1.31± 0.06	2.09 ± 0.12	CT= <0.001**	P1=<0.001**
					P2=<0.001**
					P3=<0.001**
CC	1.80 ± 0.081	2.01 ± 0.04	1.00 ± 0.11	CC= <0.001**	P1=<0.001**
					P2=<0.001**
					P3=<0.001**
F test	0.221	24.63	129.49		
P value	0.803	<0.001**	<0.001**		

P1: BC compared to healthy control group, **P2:** BC compared to benign group, **P3:** BC compared to non-malignant females; $p>0.05$: Non-significant difference.

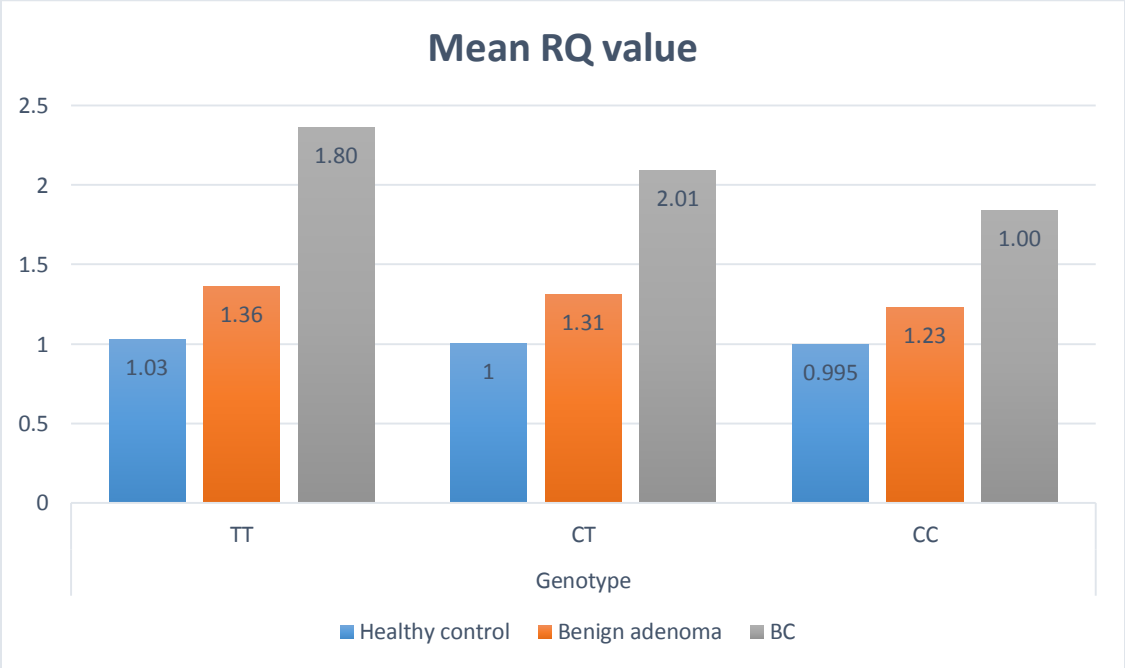

Fig. (46): H19 lncRNA expression levels among different rs217727 genotypes of BC, benign and control group.

Table (28) and fig. (46) show that there were significant differences in H19 lncRNA gene expression levels between different genotypes of BC and benign group (*p* value < 0.001).

- There were significant statistical increase in H19 lncRNA expression levels in TT&CT genotypes in BC compared to control, compared to benign and to non-malignant females (*p* value < 0.001).

- H19 lncRNA expression levels also show significant statistical decrease with CC genotypes in BC compared to control, compared to benign and to non-malignant females (*p* value < 0.001).

Discussion

The most common causes of cancer death in women include lung, breast, and colorectal cancer. The latest data estimated approximately 279,100 new cases and 42,690 deaths due to BC in the United States in 2020 ***(Siegel et al., 2020).*** According to the Global Burden of Disease Study 2017, in the same year, there were 16,697,282 BC patients globally, highlighting the enormous influence of this disease on public health ***(Li et al., 2019).***

The complexity and heterogeneity of BC includes multiple subtypes, as well as a variety of clinical, pathological and molecular profiles that result in a challenge for diagnosis and treatment ***(Pang et al., 2019).***

SNP may affect gene expression and function through indirect influence of related transcription factors or microRNAs, and further participate in the occurrence and development of tumors ***(Chen et al., 2017)***.

SNPs have been identified to be associated with an elevated risk of BC, so the identification of additional potential SNPs could have a great impact on risk estimation for BC and provide earlier application of proper therapeutic strategies to decrease its mortality rate ***(Fejerman et al., 2014).***

Nearly 10% of SNPs in cancers were associated with a change in the amino acid sequence, while a large proportion occurred in the coding or noncoding regions ***(Haemmerle and Gutschner, 2015).*** This led to the discovery of the role of the noncoding sites in cancer development. LncRNA is transcribed from noncoding sites and may be increase the susceptibility to cancer ***(Rahib et al., 2014).***

LncRNA-H19 is a carcinogenic gene located at 11p15.5 of human chromosome, which is abnormally expressed in some types of tumors and acts as a tumor suppressor gene. According to the evidence, it suggests that genetic changes in lncRNA- H19 play an important role in cancer development and it is suggested to be a novel biomarker for the diagnosis of cancer ***(Yang et al., 2015).***

Larger and well-designed studies are required to further confirm the exact role of these specific H19 polymorphisms in cancer development, progression, and severity. H19 rs217727 polymorphism could serve as a marker for and potentially therapeutic target in a variety of cancer subtypes ***(Hashemi et al., 2019).***

This current study aims to evaluate the efficacy of H19 lncRNA expression as potential molecular noninvasive tumor marker in diagnosis and prognosis of BC in Egyptian females, evaluate the rs217727 polymorphism as possible prognostic biomarker for BC and study the associations between H19 SNP (rs217727) and BC & its effect on the expression of H19 lncRNA.

This study was carried on 200 subjects of females selected from Department of General Surgery, Faculty of Medicine, Benha University Hospital.

The subjects were categorized into 3 groups:

Malignant BC group: included 100 females, diagnosed as breast cancer patients by clinical, radiological, and histopathological examinations.

Benign breast lesion group: included 50 females, diagnosed by clinical & radiological examination (US and mammography).

Control group: included 50 age matched females who were clinically, breast US and mammography free.

Regarding lncRNA-H19 rs217727 polymorphism, the CC genotype was observed in 37% of BC patients compared with 52% of benign group and 56% of the controls while the CT genotype was observed in 46% of BC patients, 40% of benign and 38% of the controls. Only 17% of BC patients had the TT genotype, 8% in benign group and 6% women in controls.

The frequency of lncRNA-H19 rs217727 T allele was higher in BC cases (40%) in BC patients and low in benign group (28%) and controls (25%), while the C allele was found in 60% of BC patients, 72% of benign group and 75% of the controls.

Our study that the TT&CT genotypes were significantly higher in BC patients compared to control group (P1=0.062, P1<0.001 respectively), also TT genotype was significantly higher in the BC patients compared to non-malignant group (P3=0.03). While the CC genotype was significantly lower in BC patients compared to control group (P1=0.027), benign breast lesion group (P2=0.08) & non-malignant group (P3=0.016).

This coincides with our results that the T allele was significantly higher in BC patients compared to the healthy control group (P1=0.01), benign breast lesion group (P2=0.04) & non-malignant group (P3=0.004) with odd ratio above 1 meaning that T allele confers risk to BC.

This also coincides with our results that the C allele was significantly lower in BC group compared to the healthy control group (P1=0.01), benign breast lesion group (P2=0.04) & non-malignant group (P3=0.004) meaning that C allele protective against the occurrence of BC.

In accordance with our results, ***Lin et al., (2017***) reported that the CT genotype was significantly higher in BC (46.9%) than control group (44.1%) *(P=0.023)*.

Also, the frequency of CC genotype (37%) and TT genotype (17%) in the BC group in our study is close to what was reported by ***Mathias et al., (2020***) (CC frequency=40.1% and TT frequency=13%).

Mathias et al., (2020) demonstrated that the C allele was found in 51.29 % of breast cancer patients and 52.46 % of healthy donors, while the T allele was found in 48.71 % of patients and 47.54% of controls. They also found that the frequency of T allele was significantly higher in BC patients compared to the controls ($P < 0.05$).

In addition, ***Lin et al., (2017), Hassanzarei et al., (2017) and Wang et al., (2019)*** demonstrated that T allele carriers have a significantly higher risk for the development of BC.

On the other hand, ***Abdollahzadeh S. and Ghorbian S. (2019), Xia et al., (2016), Lu et al., (2016), Lv et al., (2017) & Verhaegh et al (2008)*** found that the H19 rs217727 polymorphism was not associated with the susceptibility to breast cancer in the studied population. They observed no significant difference in the rs217727 polymorphism frequency between BC cases and control groups.

Our results may be explained as the polymorphism can generate effect at several levels of lncRNA regulation. Some of these repercussions include alterations in transcription regulation expression, change of miRNA target sites and modification of the RNA secondary structure. For example, it is well known that lncRNA H19 interacts with several miRNAs, such as miR-152, miR-675-5p and let-7 in several tumorigenesis processes ***(Zhang et al., 2017).*** A single nucleotide alteration in lncRNA target inside a miRNA binding site sequence can block the interaction with lncRNA-miRNA and modulate the process in a cell tumor ***(Fu et al., 2020).***

As regarding the demographic data between different lncRNA-H19 rs217727 genotypes of BC, benign and control groups, there were non-significant statistical differences, being elevated in old age (age: 50 years*)*, married, multigravida and postmenopausal women compared to the CC, CT, TT genotypes *(p values > 0.05).*

These findings closely similar to those reported by ***Hassanzarei et al., (2017)*** who reported that the CT and TT genotypes higher in age>50 with non-significant statistical differences *(P= 0.884).*

On the other hand, ***lin et al. (2016***) reported that the T carriers of rs217727 (CT + TT genotypes) showed elevated risks of BC were more likely to be evident in subgroups of younger patients (age: 40 years), premenopausal women, and subjects with later menarche, later menopause, earlier age at first live birth, and fewer pregnancies. However, none of these subgroups passed the threshold for Bonferroni correction (*P*,0.0035). No significant heterogeneity was detected within any of the subgroups either.

Regarding H19 lncRNA expression, this work demonstrated that H19 lncRNA expression levels were significantly increased in BC group (2.04±.22) compared to benign (1.27±.07) and control group (1.0±.08) *(p value < 0.001)* (table 20, page: 78).

This finding was in agreement with a recent study by ***Zohng et al., (2020).***Also, ***Vennin et al. (2017)*** showed that H19 lncRNA expression levels were significantly increased in BC group compared to control subjects *(P <0.0001)*. They identified that H19 lncRNA increases cell tumorigenic capacities in vitro and in vivo and acts as an oncogene by masking methylation site and H19 promoter regulates expression of the H19/IGF2 imprinted locus.

Li et al., (2017) releaved that H19 was aberrantly upregulated in breast tumor tissues who explained that H19 upregulates DNA methyltransferase DNMT1 by sponging miR-152, thereby promoting BC cell proliferation and invasion, so H19 may serve as a potential biomarker and a therapeutic target for breast cancer progression and diagnosis.

On the other hand***, Han et al., (2016)*** reported that there were non-significant differences between the BC patients and the healthy controls in the expression levels of H19 *(P = 0.554)*.

Our study may be explained by that the H19 lncRNA promoter was activated by E2F transcription factor 1 (E2F1), which promoted cell cycle progression (particularly in the S-phase). Furthermore, H19 contributed to the epigenetic regulation of gene expression in BC. H19 bound to and inhibited S-adenosylhomocysteine hydrolase, the sole enzyme that can hydrolyze S-adenosylhomocysteine (SAH) in humans. SAH can markedly

suppress S-adenosylmethionine-dependent methyltransferases, which can methylate multiple cellular components, including DNA, RNAs and proteins, through a feedback mechanism. H19 knockdown increased the DNMT3B-mediated methylation of Nctc1, a gene encoding lncRNAs, within the Igf2-H19-Nctc1 locus. Thus, H19 altered DNA methylation and led to breast tumorigenesis ***(Zhou et al., 2015).***

Our study showed that there was significant statistical increase in H19 lncRNA gene expression in metastatic group (2.07±.22) than non-metastatic group (1.96±.19) (*p* values < *0.05).*

***Zhong et al., (2020*)** found that H19 lncRNA expression levels to be significantly higher with distant metastasis (7.16±1.18) *(P = 0.008),* which was close to our result.

Another study conducted by ***Sun et al., (2019)*** revealed that the expression level of H19 was significantly associated with metastasis *(P = 0.049).*

The current work revealed that H19 lncRNA expression was significantly higher in TT&CT genotypes & significantly lower in CC genotype in BC compared to benign and control groups *(P<0.001).*

Lin et al., (2017) studied the association between rs217727 genotypes and the expression level of H19 in BC patients and corresponding normal tissue. He revealed that the expression level of H19 in BC tissue was significantly higher than in normal tissue *(P=0.022).* The rs217727 CT or TT genotype was also found to be significantly correlated with the elevated

expression of H19 in BC patients compared with the CC genotype *(P=0.013 and P=0.001*, respectively).

These results may be explained as rs217727 is located in exon 5 of the H19 gene, SNPs are the simplest form of DNA variation, so it affect promoter activity (gene expression), mRNA conformation (stability), and translational efficiency. The rs217727 polymorphism affect H19 mRNA expression levels, mutation may alter the translational efficiency, potentially leading to alterations in H19 structure, which may ultimately influence the function of H19. So there were association between rs217727 genotypes and the expression level of H19 in occurrence of BC.

Our results demonstrated that H19 rs217727 SNP & gene expression of H19 lncRNA were not significantly related to BC staging & grading, but significantly related to metastasis indicating that it can be a possible prognostic biomarker of BC.

Conclusion & Recommendation

In conclusion:

- High lncRNA rs217727 SNP T allele confers increase risk to BC.
- H19 lncRNA expression can be possible diagnostic & prognostic biomarker of BC.
- It is recommended to perform further large-scale studies to confirm our findings and further functional analyses are also necessary to uncover the underlying mechanism.

Summary

Breast cancer (BC) is one of the most frequently occurring cancer and cancer-related deaths in women. BC become a major public health challenge. In Egypt, BC is the most frequent cancer among Egyptian females. It represents about 38% of all reported cancer cases in Egyptian females.

LncRNAs can be genomically located between two protein coding genes (intergenic lncRNA), transcribed from a promoter of a protein-coding gene.

One of the lncRNA found in humans is H19, it is expressed exclusively from the maternal allele on chromosome 11p15.5 after 10 weeks gestation, H19 is highly expressed in the developing embryo. It promotes biological processes such as apoptosis, angiogenesis, inflammation and cell death.

Accumulating evidence has demonstrated that H19 lncRNA is abnormally expressed and promotes cancer-cell proliferation in many tumors, such as BC and hepatocellular, esophageal, and bladder cancers sug-gesting an oncogenic function.

SNPs are one of the most common types of genetic variations in the human genome. SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are associated with genetic susceptibility to cancer. SNPs have been confirmed to have profound effects on gene expression and function, and participate in carcinogenesis.

Some original studies and previous meta-analyses reported the relationship between H19 rs217727 and cancer risk, but the results were inconsistent. In addition, several recently published studies provide the basis for updating data sets and more accurately evaluating the relationship between H19 rs217727 and cancer risk.

The aim of this study was to evaluate the efficacy of H19 lncRNA expression as potential molecular noninvasive tumor marker in diagnosis and prognosis of BC in Egyptian females, evaluate the rs217727 polymorphism as possible prognostic biomarker for BC and study the associations between H19 SNP (rs217727) and BC & its effect on the expression of H19 lncRNA.

Our study was performed on 100 breast cancer (BC) patients, 50 women with benign breast lesion and 50 cancer- free controls.

All patients were subjected full history taking, complete clinical examination, laboratory investigations, radiological assessment, diagnostic biopsy for histopathology and molecular study of the gene variations.

The data analysis of rs217727 lncRNA-H19 revealed a significant increase in the frequency of the heterozygous variant CT genotype in BC patients compared with benign group and the controls *($p < 0.001$).*

The polymorphic genotype (TT) was likely to be significantly increased in BC patients as compared to non-malignant group, the CC genotype was observed to be significant lower in BC compared to control group and significant higher in BC to non-malignant group *($P<0.05$).*

Moreover, T allele was significant high in BC compared to benign and control group & C allele showed significant decrease in BC compared to control group and benign group *(P<0.05).*

In addition, the H19 lncRNA expression levels were significantly increased in BC group compared to benign and control group (*p value* < *0.001).*

There were significant statistical increase in H19 lncRNA expression levels in TT&CT genotypes in BC compared to benign & control groups. Also show significant statistical decrease with CC genotypes in BC compared to benign & control groups (*p* value < 0.001).

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الملـخص العربى

المقدمة:

يُعد سرطان الثدي واحد من أكثر أنواع السرطان شيوعًا والسبب الرئيسي للوفيات الناجمة عن السرطان في السيدات في جميع أنحاء العالم. وتُمثل نسبة حدوث هذا المرض الخبيث نسبة عالية ويتم اكتشاف ما يقارب سبعة عشر مليون حالة إصابة بسرطان الثدي عالميًا كل عام.

عالميًا، يُمثل سرطان الثدي أكثرالاورام الخبيثة انتشارًا في السيدات وتظل نسبه حدوثه ونسبة الوفيات الناجمة عنه في تزايد مستمر في العشر أعوام القادمة، فمن المتوقع أن تكون نسبه حدوثه عالية جدًا في الدول النامية لتصل لنسبة 55% ونسبة الوفيات تصل إلي 58%.

أما بالنسبة لمصر، يُعتبر سرطان الثدي في تزايد مستمر حيث أنه يُمثل حوالي 37.7% من حالات السرطان المبلغ عنها في السيدات المصريات مع ظهور 12,621 حالة جديدة خلال عام 2008.

يُعتبر سرطان الثدي متعدد الأسباب والتي تتمثل في عوامل بيئية وعوامل جينية، فقد أُثبت أن تقدم العمر والسمنة والإصابة السابقة بالأورام الحميدة في الثدي وإصابة أحد أفراد العائلة بنفس المرض والتاريخ الإنجابي وتاريخ الحيض للمرأة مرتبطة بتطور سرطان الثدي.

أما بالنسبة للعوامل الجينية، فإن تعدد الأشكال أحادي النوكليوتيدات يكون مرتبط بارتفاع نسبة حدوث سرطان الثدي وهذا يؤكد وجود عوامل جينية ضمن أسباب سرطان الثدي ولذلك يُعد التعرف علي وجود تعدد الأشكال أحادي النوكليوتيدات له دلالة خاصة لقياس إحتمالية حدوث سرطان الثدي ويمهد لإستخدام مبكر لتطبيقات علاجية لتقليل نسبه الوفاة.

يقع 7% فقط من تعدد الأشكال أحادي النوكليوتيدات علي المناطق المسئولة عن الشفرة الوراثية التي تُترجم الي بروتين ولكن 93% منه يقع علي المناطق الغير مسئولة عن الشفرة.

في الأعوام الحديثة، الأحماض النووية الريبوزية الطويلة الغير مشفرة حظيت بإنتباه شديد وذلك لوظيفتها المنظمة الواسعة المدي في الأمراض البشرية. يعرف الحمض النووي الريبوزي

الطويل الغيرمشفر علي أنه حمض ريبوزي منسوخ يتكون من أكثر من 200 نوكيوتيدة ولكنه لا يترجم الي بروتين.

يُعد الحمض النووي الريبوزي الطويل الغير مشفر، بالرغم من أن وظيفته ليست واضحة، له دور حيوي في حدوث السرطانات متضمنة التنظيم أثناء نسخ الحمض النووي وبعد نسخه وتنظيم الجينات المسببة للسرطان حيث ينتج عنه إنتشار وتقدم دورة الخلية وموت الخلايا المبرمج وكذلك غزو وهجرة الخلايا.

بالنسبة للحمض النووي الريبوزي فإنه يقع في جزء من الجين H19، الذي يتواجد على الكروموسوم البشري رقم 11. والذي ينتج عن نسخه حمض نووي ريبوزي طوله (2.3kb) ويملك العديد من الأدينين. والذي يلعب دورًا مهمًا في التطور الجيني والتحكم في النمو. يُمثل هذا الجين جينًا مطبوعًا من الكروموسوم الموروث من الأم.

هناك دراسات أثبتت أن الحمض النووي الريبوزي الطويل الغير مشفر الموجود علي H19 يُعبر عنه بطريقة غير طبيعية يزيد من حدوث الأورام الخبيثة مثل سرطان الكبد، إلخ. كما أن تعدد الأشكال النوكليوتيدية علي هذا الجين لها دور في حدوث سرطان الثدي.

هدف البحث:

تهدف هذه الدراسة الحالية إلى:

- إستكشاف فعالية الأحماض النووية الريبوزية الطويلة الغير مشفرة كدلالات أورام جزيئية بسيطة تستخدم فى تشخيص سرطان الثدي في السيدات المصريات.

- دراسة التعبير الجيني للحمض النووي الريبوزي الذي يقع علي الجين H19 للتفرقة بين الأورام الحميدة والخبيثة.

- دراسة تأثير تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H19 علي التعبيرالجيني لذات الجين علي سرطان الثدي.

مادة البحث:

اشتملت هذه الدراسة على مائة مريضا تم تشخيصهم على أنهم مرضى سرطان الثدي من خلال الفحوصات المتعددة وتمت مقارنة نتائج المرضى بـ خمسين حالة مصابة بالأورام الحميدة وخمسون شخصا من المتطوعين الأصحاء كمجموعة ضابطة.

طرق البحث:

تم عمل صحيفه شامله لجميع المرضى تضمنت الاتي:

- أخذ تاريخ مرضى كامل.
- الفحص السريري الكامل.
- فحوصات التحاليل المختلفة.
- الفحوصات الاشعاعية.
- أخذ عينة من الثدي للتشريح المرضي.
- الدراسة الجزيئية لاستكشاف الأحماض النووية الريبوزية الغير مشفرة وتعدد الأشكال أحادي النوكليوتيدات.

➢ **وقد تم جمع البيانات وجدولتها واخضاعها للتحليل الإحصائي.**

<u>وقد أظهرت نتائج البحث ما يلي:</u>

1- كشف تحليل بيانات تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H19 عن زيادة معنوية في تواتر النمط الجيني المتغاير CT في مرضى سرطان الثدي مقارنة بالمجموعة المصابة بالأورام الحميدة والمجموعة الضابطة.

2- كما أنه تم زيادة النمط الوراثي متعدد الأشكال (TT) بشكل كبير في مرضى سرطان الثدي مقارنة بالمجموعة غير الخبيثة، ولوحظ أن النمط الوراثي CC أعلى بشكل ملحوظ في

مرضي سرطان الثدي مقارنة بالمجموعة الضابطة وأعلى بشكل ملحوظ في مجموعة سرطان الثدي مقارنة بالمجموعة غير الخبيثة.

3- علاوة على ذلك ، كان T أليل مرتفعًا معنويًا في مرضي سرطان الثدي مقارنة بالمجموعة الحميدة والمجموعة الضابطة، وأظهر C أليل انخفاض معنوي في مرضي سرطان الثدي مقارنة بمجموعة التحكم والمجموعة الحميدة.

4- إضافة إلى ذلك، زادت مستويات التعبير الجيني للحمض النووي الريبوزي الطويل الذي يقع علي الجين H19 بشكل كبير في مجموعة مرضي سرطان الثدي مقارنة بالمجموعة الحميدة والمجموعة الضابطة.

الاستنتاج:

نستنتج من هذه الدراسة والبيانات المتوفرة لدينا أن وظيفة الورم للحمض النووي الريبوزي الذي يقع علي الجين H19 ترتبط بمرض سرطان الثدي حيث أنه يشارك في الهجرة والغزو وانتشارالأنواع المختلفة من السرطانات من خلال العديد من الآليات بما في ذلك التعديلات اللاجينية. علاوة على ذلك ، فإن مستوى التعبير الجيني للحمض النووي الريبوزي الذي يقع علي الجين H19 قابل للقياس حيث يتم تنظيمه في مرضى سرطان الثدي.

كما أنه تم استنتاج أن وجود تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H19 تلعب أدوارًا مهمة في التسبب في سرطان الثدي، لذلك قد تكون متغيرات الحمض النووي الريبوزي الطويل الغير مشفر الذي يقع علي الجين H19 علامة بيولوجية محتملة للاستعداد لحدوث سرطان الثدي. ومع ذلك ، هناك حاجة إلى مزيد من الدراسات واسعة النطاق لتأكيد نتائجنا والتحليلات الوظيفية الإضافية ضرورية أيضًا للكشف عن الآلية الأساسية.

الملخص العربى

المقدمة:

يُعد سرطان الثدي واحد من أكثر أنواع السرطان شيوعًا والسبب الرئيسي للوفيات الناجمة عن السرطان في السيدات في جميع أنحاء العالم. وتُمثل نسبة حدوث هذا المرض الخبيث نسبة عالية ويتم اكتشاف ما يقارب سبعة عشر مليون حالة إصابة بسرطان الثدي عالميًا كل عام.

عالميًا، يُمثل سرطان الثدي أكثرالاورام الخبيثة انتشارًا في السيدات وتظل نسبه حدوثه ونسبة الوفيات الناجمة عنه في تزايد مستمر في العشر أعوام القادمة، فمن المتوقع أن تكون نسبه حدوثه عالية جدًا في الدول النامية لتصل لنسبة ٥٥% ونسبة الوفيات تصل إلي ٥٨%.

أما بالنسبة لمصر، يُعتبر سرطان الثدي في تزايد مستمر حيث أنه يُمثل حوالي ٣٧,٧% من حالات السرطان المبلغ عنها في السيدات المصريات مع ظهور ١٢،٦٢١ حالة جديدة خلال عام ٢٠٠٨.

يُعتبر سرطان الثدي متعدد الأسباب والتي تتمثل في عوامل بيئية وعوامل جينية، فقد أُثبت أن تقدم العمر والسمنة والإصابة السابقة بالأورام الحميدة في الثدي وإصابة أحد أفراد العائلة بنفس المرض والتاريخ الإنجابي وتاريخ الحيض للمرأة مرتبطة بتطور سرطان الثدي.

أما بالنسبة للعوامل الجينية، فإن تعدد الأشكال أحادي النوكليوتيدات يكون مرتبط بارتفاع نسبة حدوث سرطان الثدي وهذا يؤكد وجود عوامل جينية ضمن أسباب سرطان الثدي ولذلك يُعد التعرف علي وجود تعدد الأشكال أحادي النوكليوتيدات له دلالة خاصة لقياس إحتمالية حدوث سرطان الثدي ويمهد لإستخدام مبكر لتطبيقات علاجية لتقليل نسبه الوفاة.

يقع ٧% فقط من تعدد الأشكال أحادي النوكليوتيدات علي المناطق المسئولة عن الشفرة الوراثية التي تُترجم الي بروتين ولكن ٩٣% منه يقع علي المناطق الغير مسئولة عن الشفرة.

في الأعوام الحديثة، الأحماض النووية الريبوزية الطويلة الغير مشفرة حظيت بإنتباه شديد وذلك لوظيفتها المنظمة الواسعة المدي في الأمراض البشرية. يعرف الحمض النووي الريبوزي

الطويل الغيرمشفر علي أنه حمض ريبوزي منسوخ يتكون من أكثر من ٢٠٠ نوكيوتيدة ولكنه لا يترجم الي بروتين.

يُعد الحمض النووي الريبوزي الطويل الغير مشفر، بالرغم من أن وظيفته ليست واضحة، له دور حيوي في حدوث السرطانات متضمنة التنظيم أثناء نسخ الحمض النووي وبعد نسخه وتنظيم الجينات المسببة للسرطان حيث ينتج عنه إنتشار وتقدم دورة الخلية وموت الخلايا المبرمج وكذلك غزو وهجرة الخلايا.

بالنسبة للحمض النووي الريبوزي فإنه يقع في جزء من الجين H١٩، الذي يتواجد على الكروموسوم البشري رقم ١١. والذي ينتج عن نسخه حمض نووي ريبوزي طوله (٢,٣kb) ويملك العديد من الأدينين. والذي يلعب دورًا مهمًا في التطور الجيني والتحكم في النمو. يُمثل هذا الجين جينًا مطبوعًا من الكروموسوم الموروث من الأم.

هناك دراسات أثبتت أن الحمض النووي الريبوزي الطويل الغير مشفر الموجود علي H١٩ يُعبر عنه بطريقة غير طبيعية يزيد من حدوث الأورام الخبيثة مثل سرطان الكبد، إلخ. كما أن تعدد الأشكال النوكليوتيدية علي هذا الجين لها دور في حدوث سرطان الثدي.

هدف البحث:

تهدف هذه الدراسة الحالية إلى:

- إستكشاف فعالية الأحماض النووية الريبوزية الطويلة الغير مشفرة كدلالات أورام جزيئية بسيطة تستخدم فى تشخيص سرطان الثدي في السيدات المصريات.

- دراسة التعبير الجيني للحمض النووي الريبوزي الذي يقع علي الجين H١٩ للتفرقة بين الأورام الحميدة والخبيثة.

- دراسة تأثير تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H١٩ علي التعبيرالجيني لذات الجين علي سرطان الثدي.

مادة البحث:

اشتملت هذه الدراسة على مائة مريضا تم تشخيصهم على أنهم مرضى سرطان الثدي من خلال الفحوصات المتعددة وتمت مقارنة نتائج المرضى بـ خمسين حالة مصابة بالأورام الحميدة وخمسون شخصا من المتطوعين الأصحاء كمجموعة ضابطة.

طرق البحث:

تم عمل صحيفه شامله لجميع المرضى تضمنت الاتي:

- أخذ تاريخ مرضى كامل.
- الفحص السريري الكامل.
- فحوصات التحاليل المختلفة.
- الفحوصات الاشعاعية.
- أخذ عينة من الثدي للتشريح المرضي.
- الدراسة الجزيئية لاستكشاف الأحماض النووية الريبوزية الغير مشفرة وتعدد الأشكال أحادي النوكليوتيدات.

➢ **وقد تم جمع البيانات وجدولتها واخضاعها للتحليل الإحصائي.**

<u>وقد أظهرت نتائج البحث ما يلي:</u>

١- كشف تحليل بيانات تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H١٩ عن زيادة معنوية في تواتر النمط الجيني المتغاير CT في مرضى سرطان الثدي مقارنة بالمجموعة المصابة بالأورام الحميدة والمجموعة الضابطة.

٢- كما أنه تم زيادة النمط الوراثي متعدد الأشكال (TT) بشكل كبير في مرضى سرطان الثدي مقارنة بالمجموعة غير الخبيثة، ولوحظ أن النمط الوراثي CC أعلى بشكل ملحوظ في

مرضي سرطان الثدي مقارنة بالمجموعة الضابطة وأعلى بشكل ملحوظ في مجموعة سرطان الثدي مقارنة بالمجموعة غير الخبيثة.

٣- علاوة على ذلك ، كان T أليل مرتفعًا معنويًا في مرضي سرطان الثدي مقارنة بالمجموعة الحميدة والمجموعة الضابطة، وأظهر C أليل انخفاض معنوي في مرضي سرطان الثدي مقارنة بمجموعة التحكم والمجموعة الحميدة.

٤- إضافة إلى ذلك، زادت مستويات التعبير الجيني للحمض النووي الريبوزي الطويل الذي يقع علي الجين H١٩ بشكل كبير في مجموعة مرضي سرطان الثدي مقارنة بالمجموعة الحميدة والمجموعة الضابطة.

الاستنتاج:

نستنتج من هذه الدراسة والبيانات المتوفرة لدينا أن وظيفة الورم للحمض النووي الريبوزي الذي يقع علي الجين H١٩ ترتبط بمرض سرطان الثدي حيث أنه يشارك في الهجرة والغزو وانتشارالأنواع المختلفة من السرطانات من خلال العديد من الآليات بما في ذلك التعديلات اللاجينية. علاوة على ذلك ، فإن مستوى التعبير الجيني للحمض النووي الريبوزي الذي يقع علي الجين H١٩ قابل للقياس حيث يتم تنظيمه في مرضى سرطان الثدي.

كما أنه تم استنتاج أن وجود تعدد الأشكال أحادي النوكليوتيدات التي تقع علي الجين H١٩ تلعب أدوارًا مهمة في التسبب في سرطان الثدي، لذلك قد تكون متغيرات الحمض النووي الريبوزي الطويل الغير مشفر الذي يقع علي الجين H١٩ علامة بيولوجية محتملة للاستعداد لحدوث سرطان الثدي. ومع ذلك ، هناك حاجة إلى مزيد من الدراسات واسعة النطاق لتأكيد نتائجنا والتحليلات الوظيفية الإضافية ضرورية أيضًا للكشف عن الآلية الأساسية.