INTRODUCTION

Breast cancer is one of the most common cancers and a leading cause of death all over the world ***(Weigel and Dowsett, 2010).*** In Egypt, it represents 18 % of total cancer cases ***(Salem at al., 2010).***

However, prognosis improves if early diagnosed, with selection of appropriate therapeutic strategies, and efficient follow up ***(Karimi et al., 2014).***

Serum biomarkers are currently used for the diagnosis and monitoring of breast cancer. However, their sensitivities and negative predictive values are not satisfactory for primary diagnosis of breast cancer ***(Misek and Kim, 2011).***

Several studies have shown that tumor cells adapt their metabolism to the microenvironment by suppressing mitochondrial function ***(Jang et al., 2013).*** Mitochondria, whose principle function is to generate energy through aerobic respiration, is the major source and target of intracellular reactive oxygen species that plays an important role in breast carcinogenesis ***(Thyagarajan et al., 2013).***

Various types of Mt.DNA alterations have been reported including point mutation, large deletion and copy number changes in several types of cancers ***(Ghaffarpour et al.,***

2014). Decreased Mt.DNA has been reported for renal, gastric, breast, ovarian and hepatic cancer ***(Xia et al., 2009).***

The discovery of Mt.DNA has sparked the interest of scientists as the Mt.DNA changes might serve as a sensitive early biomarker for non-invasive detection of several types of solid cancer including breast cancer ***(Duchen and Szabatkai, 2010).***

AIM OF WORK

The aim of the present work was to study circulating cell-free mitochondrial DNA (Mt.DNA) detected by real-time polymerase chain reaction (RT-PCR) in a group of breast cancer patients in order to evaluate its clinical utility as an early diagnostic marker. This may allow intervention at an early stage and may have its impact on the therapeutic regimens.

I-BREAST CANCER

A) Epidemiology:

Breast cancer is the most common cancer in women and accounts for 29% of all cancers diagnosed every year worldwide. It is the second leading cause of cancer death in women, exceeded only by lung cancer, but it is the first in women under the age of 55 ***(Fakhrjou et al., 2016)***.

In Egypt, breast cancer is estimated to be the most common cancer among females accounting for 12,621 new cases in 2008. It is also the leading cause of cancer-related mortality. These estimates are confirmed in many regional Egyptian cancer registries as well as in hospital-based frequencies ***(The National Cancer Registry Program of Egypt, 2012).***

Since the 1990s, profound changes have taken place in the clinical presentation and management of breast cancer due to mammography screening, implementation of effective hormone treatments and chemotherapy as well as progress in radiotherapy and surgery. These innovations have probably contributed to the observed improvements in breast cancer survival ***(Autier et al., 2010).***

B) Risk Factors:

Breast cancer is a heterogeneous disease with no single characterized cause. However, epidemiological studies have identified many risk factors that increase the chance for a woman to develop breast cancer. These factors include personal and family histories, dietary, hormonal factors and pathological entities ***(Siegel et al., 2016).***

1- Personal History:

a) Age:

Age is one of the major risk factors for breast cancer in women. The incidence of breast cancer increases steeply with age with the greatest rate increase in postmenopausal women, where the risk doubles with each decade of life up to age 80. The decline in incidence rates after age 80 may reflect lower rates of screening leading to incomplete detection ***(Bogusz et al., 2016).***

b) Race:

White women have a higher incidence of breast cancer than African American women after age 40. In contrast, African American women have a higher incidence rate

before age 40 and are more likely to die from breast cancer at every age ***(Chuang et al., 2015)***.

Breast cancer is 5 to 6 times higher in Western countries than in Asia and Africa. Several studies on Japanese immigrants to North America demonstrate an increase breast cancer risk in subsequent generations suggesting that environmental rather than genetic factors are responsible for the observed international differences ***(Leopold et al., 2016)***.

c) Prior history of breast disease:

The major risk factor for development of primary breast cancer is a personal history of cancer in the other breast. However, many second cancers can appear in the same breast. Most recurrent breast cancers arise within the first five years following treatment. Recurrence rates are very low in patients with primary tumors smaller than 1 cm and negative axillary nodes ***(Malone et al., 2010).***

Most studies indicate that women with a history of a biopsy proven benign breast disease are at an increased risk of subsequent breast cancer ***(Nelson et al., 2012).***

d) High breast density:

High mammographic breast density is considered one of the strongest risk factors for breast cancer. Among women with more than 75% breast density, the risk of breast cancer is more than 4 times that of women with much less dense breasts ***(Winkel et al., 2016).***

Mammographic density is defined and measured by the amount of radio-dense areas, which represent epithelial tissue and stroma, where breast density is associated with epithelial proliferation and with stromal fibrosis**.** The association between extensive mammographic density and an increased risk of breast cancer is not only because of a masking effect of the breast density, which could obscure a cancer, but also because of a biologic connection between breast density and breast cancer ***(Onega et al., 2014).***

2- Family History:

The presence of significant family history is the most important risk for the development of breast cancer. It was reported that the risk of breast cancer is 1.5 to 2.0 times greater with one first-degree relative with breast cancer, and 4-6 times greater with two affected relatives. The risk increases when the relative is affected at a younger age and

has bilateral disease. However, patients with family history of breast cancer have a survival advantage over those without family history ***(Mavaddat et al., 2015).***

3- Dietary Factors:

A number of studies have linked a low intake of vitamin A and carotenoids, such as beta-carotene, with an increased risk of breast cancer. Vitamin A is found in high amounts in green and yellow vegetables and some fruits and is important for cell growth. Carotenoids are powerful antioxidants that can help protect cells from the damaging effects of oxygen free radicals in the body ***(Aune et al., 2012).***

A high intake of fat, especially unsaturated fatty acids has been reported to be weakly associated with an increased breast cancer risk. A particular type of polyunsaturated fatty acids, omega-3 polyunsaturated fatty acids, seems to be protective ***(Jung et al., 2013).***

4- Obesity:

Obesity has been linked to an increased risk of developing breast cancer by many scientific studies. There is evidence to suggest that excess body fat at the time of breast cancer diagnosis is associated with higher rates of cancer

recurrence and death ***(Ligibel., 2011).*** Furthermore, studies have shown that obese women are more likely to have large tumors, greater lymph node involvement, and poorer breast cancer prognosis with 30% higher risk of mortality ***(Protani et al., 2010).***

5- Hormonal Factors:

a) Endogenous:

The early age of menarche; less than 12 years of age; has been associated with an increase in breast cancer risk. This may be due to a prolonged exposure of breast epithelium to estrogens and progesterone due to early regular ovulatory menstrual cycles. Moreover, significantly higher levels of estradiol in women with early menarche during their adolescence as well as higher follicular but not luteal phase estradiol levels and a lower sex hormone binding globulin (SHBG) are additional factors. Breast cancer is therefore very uncommon in Turner syndrome, as they rarely ovulate ***(Lancet, 2013).***

Similarly, delayed menopause maximized the number of ovulatory cycles and therefore may lead to an increased breast cancer risk. Indeed, it has been shown that the risk of breast cancer increases approximately 3% for every 1-year

increase in the age at menopause. In addition, surgically induced menopause (ovariectomy or hysterectomy) before the age of 35 results in a decrease of breast cancer risk. These women have only 40% risk of the women experiencing natural menopause. Even unilateral ovariectomy performed before the age of 45 has been demonstrated to be protective ***(Press et al., 2011).***

b) Exogenous:

Exogenous estrogens, either the combined oral contraception (COC) or hormonal replacement therapy (HRT), also confer increased risks of breast cancer, depending on the duration of exposure and whether the estrogen is used alone or in combination with progesterone. Furthermore, using COC at a younger age, especially before age of 20 years, results in a higher increase of breast cancer risk than using COC at an older age ***(Chlebowski et al., 2013).***

Hormonal replacement therapy is a further area of controversy. Long-term treatment for more than 10 years after the menopause is associated with a significant increase in risk. However, it was found that the risk from combined estrogen/progesterone HRT is greater than for estrogen only.

The risk appears to increase cumulatively by 1–2% per year, but to disappear within 5 years of cessation of HRT**.** In addition, HRT use may increase the risk of late-stage diagnoses by increasing breast tissue density, thereby reducing the effectiveness of mammograms ***(Shapiro et al., 2012).***

6- Physical Activity:

Physical activity performed in adolescence and young adulthood (12- 24 years old) has shown a consistent 20% reduction for developing breast cancer. Physical activity may reduce the risk by delaying the onset of menarche and modifying the bioavailable hormone levels ***(Coughlin and Smith, 2015).***

C) Genetics of Breast Cancer:

Like most malignancies, breast cancer is the result of a complex and heterogeneous combination of genetic alterations that promote clonal selection of cellular immortality. The occurrence of these changes leading to breast cancer includes an interaction between inherited genetic susceptibility (germ like mutations) and acquired genetic changes (somatic mutations) resulting from

endogenous and exogenous environmental factors ***(Eva and William, 2010).***

1- Inherited Genetic Susceptibility:

Development of a clinically recognizable tumor requires multiple genetic changes or mutations. If one or more of such mutations is transmitted in the germ cell, the affected person has an increased chance of developing a malignancy, tends to develop this disease earlier in life than usual and may develop multiple primary tumors ***(Eva and William, 2010).***

Clinical investigations of familial aggregation of breast cancer have identified at least five genetic syndromes that feature breast cancer. Each syndrome has associated genetic mutations that appear consistently with each syndrome. The genes involved include BRCA1 and BRCA2 (breast-ovarian cancer syndrome 1 and 2), p53 (Li-Fraumeni syndrome), PTEN (Cowden's disease), and ATM genes (ataxia telangectasia mutated genes) ***(Kim et al., 2010).***

Table (1): Principle Genes Mutated in Familial Breast Cancer

Gene	Risk Range	Syndrome	Other ancers
BRCA1	56%-87%by age 70 years	Hereditary breast/ovarian cancer	Ovary, prostate
BRCA2	37%-84% by age 70 years	Hereditary breast/ovarian cancer	Male breast cancer
p53	50%-89% by age 50 years	Li-Fraumeni syndrome	Brain, adrenal, sarcomas, leukemia
PTEN	30%-40% by age 50 years	Cowden's disease	Thyroid, hamartomas, prostate
ATM	Fivefold increase in heterogenous women lifetime	Ataxia telangiectasia	Leukemia, lymphoma

(Suter and Marcum, 2007)

a) BRCA 1 and 2:

BRCA1 is located on long arm of chromosome 17, and BRCA2 is on long arm of chromosome 13. The exact functions of BRCA1 and 2 are still not clearly identified. Data support the suggestion that ATM protein, in response to DNA damage, phosphorylates and thereby activates BRCA1

protein. Subsequently, the phosphorylated BRCA1 can complex with BRCA2 and Rad51, activating DNA repair by homologous recombination ***(Aleskandarany et al., 2015).*** As Rad51 is required for meiotic and mitotic recombination events and for the repair of double- strand DNA breaks, its linkage to BRCA1 and BRCA2 proteins allows these proteins to control the genomic and stability ***(Fridlich et al., 2015).***

The majority of families with breast and ovarian cancers are the result of mutations in BRCA1, with up to 20% resulting in mutations in BRCA2 ***(Yang et al., 2015).***

In women, mutations in these genes confer 40%-70% lifetime risk of breast cancer. Mutations in BRCA1 and BRCA2 also increase the risk of affected men developing breast cancer, although not to the same absolute risk as in women ***(Gargiulo et al., 2016).***

b) p53:

The p53 gene; a 53 KDa protein; located on chromosome 17 at region p13.3. It encodes the transcription factor p53, which is a key regulator of the gap 1 (G1) checkpoints of the cell cycle ***(Suter and Marcum, 2007).***

DNA damage, activates p53 that upregulates a variety of target genes resulting in: i) Cell cycle arrest, ii) DNA repair, or iii) apoptosis (if cellular DNA failed to be repaired). Therefore, the role of p53 is central to normal cellular homeostasis. Failure of the normal function of p53 (e.g. loss or mutation) places the cell at risk for genomic instability and accumulation of additional genetic mutations, giving rise eventually to a malignant phenotype ***(Walerych et al., 2012).***

c) ATM (Ataxia- Telangiectasia - mutated) gene:

Ataxia Telangiectasia (AT) is an autosomal recessive disease characterized by cerebellar ataxia, telangiectasias, immune defects, and a predisposition to malignancy. The ATM gene located on 11q encodes a protein involved in the cell cycle control and DNA repair ***(Choi et al., 2016).***

This disease is clinically manifest only in the homozygous state and heterozygous states are only carriers of the ATM gene. Unlike the disease, breast cancer is associated with the heterozygous carrier state and they are at a five-fold risk of developing breast cancer ***(Goldgar et al., 2011).***