Frequency of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) mutations in Egyptian women with breast cancer compared to healthy controls

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ABSTRACT

Background: The three founder mutations of BRCA1/2 (185delAG, 5382insC and 6174delT) have been reported to be associated with breast cancer. This work was designed to check for the frequency of these genetic mutations in Egyptian women affected with breast cancer compared to healthy first-degree relatives and unrelated controls.

Subjects and methods: This work is a case control study including 43 women diagnosed with breast cancer. Their genetic data were compared to controls including 63 first degree relatives in addition to 91 healthy unrelated controls from the same locality. DNA deletion or insertion mutations were characterized in blood samples of all participants using the PCR technique.

Results: The frequency of BRCA1 (185delAG) mutation in BC patients and their first-degree relatives was higher than healthy controls (2.3% and 3.2% vs. 1.1%). However, the other two mutations BRCA1 (5382insC) and BRCA2 (6174delT) showed higher frequencies among healthy controls than BC patients and their first-degree relatives (49.5% vs. 11.6% and 6.3%; 61.5% vs. 11.6% and 14.3%, respectively). Furthermore, BC patients with BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) mutations showed no significant difference compared to others regarding their clinical and laboratory markers.

Conclusions: This study indicates that BRCA1 (185delAG) mutation might contribute to the incidence of breast cancer among Egyptian women, but not with BRCA1 (5382insC) and BRCA2 (6174delT) mutations. Furthermore, there was no significant association between these three founder mutations and the clinical presentation of BC among Egyptian women.

1. Introduction

Breast carcinoma (BC) is one of the elementary cause of breast malignancies as well as cancer mortality among women worldwide (Tiberi et al., 2017; Parker et al., 1997). In Egypt, BC is also considered as the primary cancer disease affecting women with a prevalence of approximately 30% of the total cancer cases (Ibrahim et al., 2010; Omar et al., 2003; Schairer et al., 2013). The etiology of breast carcinoma is unclear, although hypothesized to be due to several risk factors including environmental elements, reproductive changes, cellular abnormalities, epigenetic transformations and genetic aberrations that promoting the incidence and proliferation of breast malignancies (Miki et al., 1994; Wooster et al., 1994). The accumulation of anomalies in the germline mutations of various founder genes has been also associated with the progression of breast carcinoma (Fodor et al., 1998).

For instance, the predisposition of breast malignancies might be associated with the inheritance of germline mutations in several tumor suppressor genes, particularly, BRCA1 (OMIM#: 113705) and BRCA2 (OMIM#: 600185) (Smith et al., 1992; Wooster et al., 1995; Miki et al., 1994). The germline mutations in BRCA1 and BRCA2 generate premature stop codons within the open reading frame (ORF) region, permitting their escape from surveillance mechanisms such as nonsense-mediated mRNA decay (NMD) resulting in truncation the translated protein (Lejeune and Maquat, 2005; Buisson et al., 2006). BRCA1 is
situated in chromosome # 17 (17q21), and encoded a transcript of 1863 amino acids, while BRCA2 is located on chromosome # 13 (13q12-13), and encoded another transcript of 3418 amino acids (Esteves et al., 2009; Osorio et al., 1998; Ramus and Gayther, 2009). These two transcripts function in a rhythm matter to block the activation and initiation of cancer malignancies in a (Rikhi et al., 1994; Collins et al., 1995; Welch et al., 2000). Besides this action, they encode tumor suppressor markers which function in specific way to control the regulation of different cellular processes including, blockage of uncontrolled cellular proliferation, apoptosis boosting, transcription regulation and repairing injuries in double strands of DNA (Billack and Monteiro, 2005; Welch et al., 2000). However, the mutations in these two genes have high susceptibility rate for the incidence of breast malignancies, especially among family studies (Easton et al., 1993; Buisson et al., 2006; Finkelman et al., 2012). The risk of developing the breast malignancies in women is increased dramatically, in which it was reported that women who inherit one copy of a mutant allele of these germline mutations, may develop a familial breast cancer by ratio 50–55% (Antoniou et al., 2003; Laitman et al., 2011). Recently, three founder mutations within BRCA1 and BRCA2 genes were identified, including 185delAG [c.68,69delAG] within exon 2 and 5382insC [c.5266dupC] within exon 20 in BRCA1 gene, while 6174delT [c.5946delT] within exon 11 in BRCA2 gene (Ewald et al., 2011; Abeliovich et al., 1997; Ford et al., 1998). Germline mutations in BRCA1 and BRCA2 have been reported in several studies with different ethnic populations (Neuhausen et al., 2009; Abeliovich et al., 1997; Hartwig et al., 2013), but with conflicting conclusions. The high prevalence of these founder mutations was identified among Ashkenazi Jewish (Hartge et al., 1999; Struwing et al., 1997; Warner et al., 1999). Furthermore, these germline mutations also observed among Byelorussian (Bogdanova et al., 2010), Egyptian (Abdel-Mohsen et al., 1999), British (Ford et al., 1995), Spanish (Osorio et al., 1998; Ramus and Gayther, 2009), Brazilian (Ewald et al., 2011) and Russian (Sokolensko et al., 2006) populations. The purpose of this study is to test for the frequency of the three founder mutations BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) in Egyptian women affected with cancer breast compared to healthy first degree relatives and unrelated controls.

2. Subjects and methods

This study including 197 women stratified into three groups; breast cancer (BC) patients, their first-degree relatives and unrelated healthy women controls. Breast cancer patients involved forty-three women derived from 43 families diagnosed with breast cancer, with an age mean ± SD was 45.3 ± 9.4 years. The basis of diagnosis of breast cancer (BC) was performed using histopathological screening together with other medical imaging concerning cancer breast including mammography/ultrasound/magnetic resonance imaging (MRI) (Burcsoş et al., 2013). All the patients were recruited from the Outpatient Clinic of the Oncology unit, Specialized Internal Medicine Hospital, Mansoura University, Mansoura, Egypt in the time extending from July 2016 to January 2017. The breast cancer patients with other types of cancers and malignancies as well pregnant and breast-feeding patients were excluded from this study. The permission to perform genetic screening was carried out after all the participants in this study were informed about genetic testing related to breast carcinoma. Thus, an informed written consent was signed from all participants before their enrollment into the study. This survey was designed after obtaining an authorization of ethical and scientific committees from Horus University and Mansoura University, Egypt. The clinical data of BC patients were extracted from pathological and medical records. The collected information involving age, diabetes mellitus status, hypertension status, marital status, parity and menstrual phase. Breast cancer tumor types can be classified into non-invasive and invasive breast cancer. Furthermore, the histological differentiation of breast cancer was stratified into four categories as follows: (Abdel-Mohsen et al., 2016) well differentiated; (Abeliovich et al., 1997) moderately differentiated; (Abugattas et al., 2015) poorly differentiated and (Antoniou et al., 2003) undifferentiated (Rakha et al., 2010). In all breast cancer women, the biopsy specimens were obtained and tested for the existence of breast cancer hormone receptors involving estrogen receptor (ER) and progesterone receptor (PR), in addition to the screening the degree of tumor proliferation by determining human epidermal growth factor receptor 2 (HER2/neu) (Gutierrez and Schiff, 2011). The second group is conducted on first-degree female relatives (either sisters and/or daughters) of breast cancer women (group I). This group included 63 women who were not affected by breast cancer themselves, with an age mean ± SD was 25.4 ± 8.9 years. The third group involved 91 control healthy women from the Delta district of Egypt, with an age mean ± SD of 39.7 ± 6.7 years.

3. Extraction and genotyping of DNA

For all participants, the extraction and manipulation of genomic DNA was carried out using DNA extraction kit provided by Gentra Systems, USA. The extraction protocol was performed according to the instructions provided by the manufacturer for peripheral blood samples. Genotyping of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) mutations were performed via a multiplex-PCR technique. For rapid detection of these three mutations, three primers were applied (one common primer and two specific primers; one specific for the mutant-type allele and the other specific for wild-type allele) (Chan et al., 1999; Fattahi et al., 2009). The specific primers for these three mutations are as follow: [for BRCA1 (185delAG); Fc (p1): (5′-gggtggacgcaaatgtgaa), Rf (p2): (5′-gtctgctaccagaggtgctc) and Rm (p3): (5′-cccacaaacatccctccctgacaggaatgagagacag)]. [for BRCA2 (5382insC)]; Rc (p4): (5′-cagggagctaacacatcag), Fw (p5): (5′-aaggagagacagatacag) and Fm (p6): (5′-aaggagagacagatacag)]. [for BRCA2 (6174delT)]; Rf (p7): (5′-agctgctgaattgctgta), Fw (p8): (5′-tgctgctgaattgctgta) and Fm (p9): (5′-agctgctgaattgctgta)]. The PCR amplification was performed using Veriti® Thermal Cycler (Applied Biosystems, USA) in a volume containing about 25 ng of genomic DNA, 10 mmol/l tris-HCl, 50 mmol/l KCl, 3.25 mmol/l MgCl₂, 0.2 mmol/l of each specific primer and 50 kU/l AmpliTaq® (Thermo Fischer Scientific, USA). This amplification process was carried out within an initial denaturation step at 95 °C for 12 min followed by 35 cycles with denaturation at 94 °C for 1 s, annealing at 57 °C for 15 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min (Chan et al., 1999). The BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) mutations were detected by electrophoresis in 2.5% agarose gel stained with ethidium bromide permitting the differentiation of the amplified products under UV light. The amplified products were detected by specific fragment size, in which the mutant and wild variants were 354 and 335 bps for BRCA1 (185delAG), 295 and 271 bps for BRCA1 (5382insC), and 171 and 151 bps for BRCA2 (6174delT).

3.1. Statistical analysis

The data in this study were analyzed and performed using the IBM Statistical Package of Social Science (IBM SPSS, version 23.0). The distribution of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) mutations among breast cancer women, their first-degree relatives and healthy controls were compared using Fisher’s exact test and odds ratio (OR) with the 95% confidence interval (95% CI). Clinical presentations including age, gender, diabetes status, hypertension status, marital status, menstrual phase, histological differentiation, tumor types, estrogen receptor, progesterone receptor and HER2/neu status compared in the patients with positive mutations vs. negative ones. A minimum level of statistical significance was considered at a p level of < 0.05.
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