

Loss of Heterozygosity at BRCA1, TP53, nm-23 and Other Loci on Chromosome 17q in Human Breast Carcinoma

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ABSTRACT

Background: In Egypt, breast cancer ranks number one among the female malignancies. Activation of oncogenes and inactivation of tumor suppressor genes are thought to play an important role in the development and progression of breast cancer.

Purpose: The present study is a trial to investigate the role of chromosome 17 in sporadic invasive ductal carcinoma of the breast through detection of LOH for 6 highly polymorphic microsatellite loci, two of which are located at BRCA1 gene (D17S855 and D17S856), one at TP53 gene, one at nm 23 gene and finally two at 17q12-12.3 (D17S183 and D17S250).

Material and Methods: Tissue samples and their corresponding safety margin normal tissues were collected from 25 patients with invasive ductal carcinoma of the breast of grades 2 and 3. LOH was detected for the 6 highly polymorphic microsatellite markers mentioned previously using PCR assay.

Results: The percentage of overall LOH recorded was 68% of the cases examined. The highest LOH was recorded in D17S855 and D17S856 (43% and 32% respectively), both markers are located at BRCA1 gene, followed by 32% LOH in nm-23 gene. D17S183 and D17S250, which are localized telomeric and centromeric to BRCA1 gene, showed 24% and 28% LOH, respectively. The lowest percentage of LOH was observed in the TP53 gene (14%). No significant correlation was found between each of the six markers used and lymph node status, grade, or menopause status. LOH at the nm-32 marker exhibited a significant association with lymph node involvement.

Conclusion: It can be concluded from the present study that BRCA1 gene may be involved in carcinogenesis of some sporadic breast cancer cases. Deletion in nm-23 gene is associated with the advanced stage of the disease. Finally, another gene located at 17q12-12.3 region may be involved in some sporadic breast cancer cases.

Key Words: *Loss of heterozygosity (LOH) - Breast cancer- BRCA1 - nm-23 - p53 genes.*

INTRODUCTION

In Egypt, breast cancer is number three in rank after urinary bladder tumors and malignant lymphomas. However, it is number one (27.3%) among the female malignancies. Common breast carcinomas, namely ductal and lobular, constitute the majority (92.11%) of the cases. Among the histopathological types, invasive ductal carcinoma constitutes 83.36%, whereas intraductal carcinoma represents 1.5% of all the histotypes [1].

The study of genetic alterations and gene expression in breast cancer underwent a revolution during the 1990's. The principle genetic lesions including gene amplification, gene deletions, point mutations, loss of heterozygosity, chromosomal rearrangements, overall aneuploidy and microsatellite instability have been reported in advanced-stage cancer of the breast [2]. The most common genetic abnormality in breast cancer is the loss of heterozygosity (LOH) [3]. LOH on several chromosomes, especially chromosome 17, has been implicated in different types of cancer such as ovarian carcinoma [4], and endometrial carcinoma [5].

The pattern of loss of heterozygosity on chromosome 17 in human breast cancer is complicated and shows many different regions of loss. In the 1990's, chromosome 17q21 was identified as the location of a susceptibility gene for early onset of breast cancer termed breast cancer 1 (BRCA 1) [6]. Analysis of 42 sporadic cases of infiltrating ductal cancer of the breast for abnormalities on chromosome 17

showed different regions of allelic loss on chromosome 17q [7]. It has been observed that allelic loss at the BRCA 1 region takes place in breast cancer patients [8]. The p53 tumor suppressor gene, which is located on the short arm of chromosome 17 (17 p13.1), has been demonstrated to encode for a 53 KDa nuclear phosphoprotein involved in the regulation of the cell cycle and growth [9]. LOH, at or near the p53 gene locus, occurred frequently in a variety of human tumor types. It is believed to favor malignancy by removal of normal copy of this tumor suppressor gene and the remaining p53 allele is often inactivated by point mutation [10]. The nm-23 gene is a known metastatic-suppressor gene located on 17q22 chromosome [11]. Three nm-23 genes were identified in human; nm-23H1, nm-23H2 and DR-nm-23 [12]. The three genes encode proteins of about 17KD and are about 90% identical. The nm-23 genes and their proteins were found to correlate with non metastatic behavior of cancer cells both in vivo and in vitro [11,13].

The aim of the present study is first, to try to investigate the role of chromosome 17 areas in the development and progression of sporadic breast cancer. This is achieved through studying loss of heterozygosity in BRCA 1, p53, and nm-23 genes that are located on chromosome 17; second, to evaluate the status of other microsatellite markers along the same chromosome.

MATERIAL AND METHODS

Tissue samples and histological diagnosis:

Fresh tumors and their corresponding safety margin of normal tissues were obtained at the same time of surgical resection from 25 primary breast cancer female patients who underwent radical mastectomy between 1998 and 1999 at the NCI, Cairo University. Their ages ranged from 20 to 70 years. Each tumor specimen was divided into two pieces, one was preserved in 10% formaline for histopathological examination, the other was used to isolate genomic DNA. All the 25 tumor samples were diagnosed as invasive ductal carcinoma. According to Bloom-Richardson system [14], sixteen tumors were grade 2 and nine were grade 3. These patients had received neither chemotherapy nor radiotherapy prior to surgery.

DNA extraction:

DNA extraction from the tumor and safety margin tissues obtained from the 25 patients was performed using QIAamp DNA Minikit method (Qiagen, Germany) as described in the manufacturer's protocol. This method depends on degradation of the proteins and cell membranes using proteinase K enzyme, precipitation of DNA by ethanol (96-100%) followed by purification of DNA using QIAamp spin columns. The final step depends on proper adjustment of the lysate buffering conditions to allow optimal binding of the DNA to the QIAamp silica gel membrane where proteins and other contaminants are not retained. The retained DNA is finally eluted from the membrane using distilled water or TRIS-EDTA.

Detection of loss of heterozygosity:

Six highly polymorphic microsatellite markers were used in the present study. All the six markers were dinucleotide repeats (CA)_n, Tp53 at 17p13.1 [15]; D17S250 at 17q12 [16]; D17S856 at 17q21 [17]; D17S855 at 17q21 [18]; D17S183 at 17q21.3 [19]; and nm-23 at 17q22 [20]. Analysis for LOH in the previous stated markers was performed on DNA isolated from tumor and their corresponding matched safety margin tissues. PCR was performed in 10 ul volumes which contained 1 ul 10X PCR buffer (100mM Tris-HCl, pH 8.3; 500mM KCl; 30mM MgCl₂; 0.1% w/v gelatine), 50 pm of each primer, 200mM each of dCTP, dGTP, dCTP, and dATP, 1 uCi of α -³²P (dCTP) (3000 Ci/mmol) and 0.25 unit of Taq DNA polymerase and finally 100ng of DNA was added. The PCR reaction was programmed as follows: reaction were heated at 95°C for 10 min. and then cycled for 35 times: each cycle consisted of 1 min. at 94°C, 1 min. at the annealing temperatures (55°C for D17S250 and nm-23, 60°C for TP53, D17S856, D17S855 and D17S183). Final elongation for 10 min at 72°C was allowed. Ten ul of loading buffer (containing 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 20mM EDTA) were added to the PCR products then heated at 94°C for 4 min and chilled in ice, 5 ul of the mixture were electrophoresed in 6% acrylamide containing 7M urea in 1XTBE buffer for 2-3 hours at constant watt (60W). The gel was dried and exposed to a Kodak-X-ray film at -80°C for 1 or 2 days. The bands for each sample were analyzed densitographically using BioDoc Analyzer (Biometra, Germany).

The samples were considered informative for each particular chromosomal marker if two alleles could be identified with normal DNA. Allelic loss (LOH) was scored if the autoradiograph signal of one allele was reduced or disappeared in the tumor DNA compared with corresponding normal allele.

Statistical analysis:

The results were analyzed using GraphPad prism computer system (GraphPad software, San Diego, USA). Fisher test was used to test the association between LOH with each of clinicopathological parameters and the association was considered significant when $p \leq 0.05$.

RESULTS

Six different markers that are distributed in BRCA1, p53, and nm-23 tumor suppressor genes were used to clarify their involvement in the genesis of breast cancer. LOH study for the 25 breast cancer cases were recorded. Seventeen out of 25 cases (68%) exhibited LOH in at least one or more of the six markers used. Table (1) shows the association between LOH at the different studied loci with tumor grade, menopausal status and lymph node involvement. Sixteen out of 25 cases examined were diagnosed as grade 2 whereas 9 cases were grade 3 and none of the cases were grade 1. These results indicated that LOH was observed in both grades by almost the same percentage, 68.75% and 66.7% for grades 2 and 3, respectively, and this association was statistically insignificant ($p=1$). The patients under investigation were classified as premenopause (10 cases) and postmenopause patients (15 cases). LOH was observed in the 10 premenopause cases (100%) whereas only 7 of 15 postmenopause patients (46.7%) had LOH and this association was statistically significant ($p=0.0077$). Thirteen out of 18 cases (72%) who revealed positive lymph node metastasis exhibited LOH. Meanwhile, 4 out of 7 cases (57%) who were lymph node free had LOH ($p=0.06396$).

Table (2) shows the name of markers used, their location on chromosome 17, the number and percentage of informativity, as well as the number and percentage of LOH for each marker. The most common region that showed the highest percentage was located at 17q21.2 and represented by the marker D17S855 which is located at BRCA 1 gene, the percentage recorded

was 40% followed by 32% deletions in both the D17S56 and nm-23 markers. D17S183 and D17S250 markers exhibited 24% and 28% deletions, respectively. The lowest percentage of LOH observed was found in the region of TP53 gene which was only about 14%. Fig. (1) indicates some representative LOH samples for different markers used in the present study. Deletion or significant reduction of intensity in one of the two alleles was observed as compared to its corresponding normal tissue.

There was no significant relation between LOH at each of the six markers on chromosome 17 with different clinicopathological criteria including tumor grade, menopause status, lymph node status except for the nm-23 marker which showed a significant association between LOH with lymph node involvement (Table 3) where the six cases exhibited LOH were diagnosed as positive for lymph node metastasis (100%) whereas none of the cases that were free of lymph node metastasis showed LOH at that gene ($p=0.0237$).

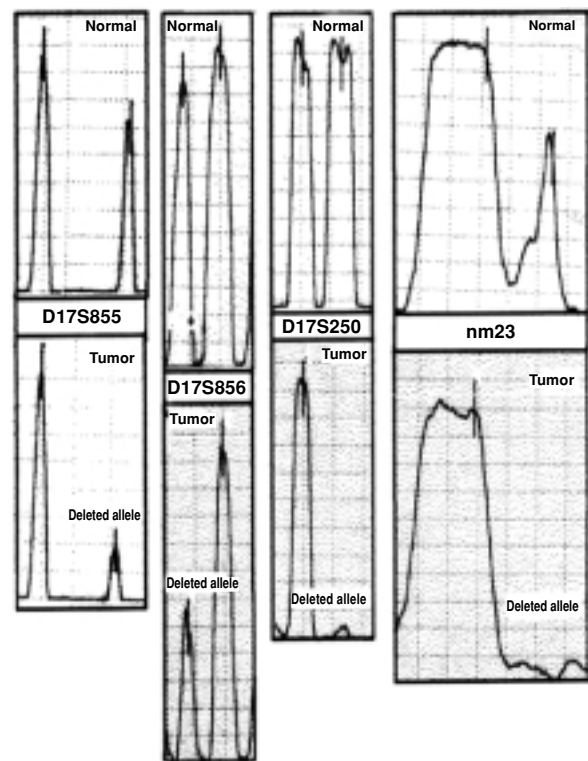


Fig. (1): Loss of heterozygosity for different microsatellite markers at chromosome 17q in carcinoma of breast. A deletion in one allele is observed in tumor as compared to its corresponding normal one.

Table (1): The Association between loss of heterozygosity (LOH) on chromosome 17 with different clinico-pathological criteria.

Criteria	LOH	Percentage (%)	p-value
<i>Menopause:</i>			
Pre-menopause	10/10	100	0.0077*
Post-menopause	7/15	46.7	
<i>Tumor grade:</i>			
2	11/16	68.75	1.000
3	6/9	66.75	
<i>Lymph node:</i>			
Positive	13/18	72	0.6396
Negative	4/7	57	

* significant

Table (2): Loss of heterozygosity (LOH) of different microsatellite markers on chromosome 17 in breast cancer patients.

Marker name	Location	Informativity (%)	LOH (%)
TP53	17p13.1	21/25 (84)	3/21 (14.3)
D17S855	17q21.2	14/25 (56)	6/14 (42.9)
D17S856	17q21.1	19/25 (76)	6/19 (31.6)
D17S183	17q12.3	25/25 (100)	6/25 (24)
D17S250	17q12	18/25 (72)	5/18 (27.8)
nm-23	17q22	19/25 (76)	6/19 (31.6)

Table (3): Association between loss of heterozygosity (LOH) at different markers on chromosome 17 with some clinico-pathological criteria in breast cancer patients.

Pathological Parameters	Microsatellite markers					
	TP53	D17S250	D17S856	D17S855	D17D183	nm-23
<i>Menopause</i>						
Pre-	2/8	4/7	4/8	4/6	4/10	3/8
Post-	1/13	1/11	2/11	2/8	2/15	3/11
p-value	0.538	0.155	0.378	0.628	0.369	1.00
<i>Grade</i>						
2	3/13	1/11	4/12	5/9	5/16	4/13
3	0/8	4/7	2/7	1/5	1/9	2/6
p-value	0.525	0.155	1.00	1.00	0.634	1.00
<i>Lymph node</i>						
Positive	3/7	2/13	5/13	4/10	6/18	6/9
Negative	0/4	3/5	1/6	2/4	0/7	0.11
p-value	1.00	0.296	0.637	1.00	0.292	0.023*

* significant

DISCUSSION

Two strategies are now currently used by most research centers that are interested to improve breast cancer treatment. The first strategy is directly toward improving the outcome of conventional methods of treatment such as chemotherapy, radiotherapy and surgery [21]. The second strategy is directed towards detection of the molecular changes that lead to the formation of cancer aiming to use these changes as either molecular markers for early detection, diagnosis or as tools for prognosis of the disease or as targets, for therapy [22]. The full picture for understanding the mechanism of development of breast cancer at the molecular level is still unknown. The main aim of this study was to gain insight in the locations and frequencies of regional chromosomal alterations of tumor

suppressor genes, mainly TP53, BRCA 1, and nm-23 genes, in addition to other loci on the same chromosome in invasive ductal carcinoma of the breast.

It is known that p53, a tumor suppressor gene that is located on chromosome 17p13 and has 10 coding exons, plays more than one role in the cell including its role in controlling transcription of some genes and cell cycle regulation and apoptosis [23,24]. One of the molecular changes that occur in p53 gene and are detected in many tumors is LOH which has been reported for chromosome 17 in several types of cancer including those of breast [8]. High percentage of LOH (85%) at different regions of chromosome 17p in breast tumors whereas LOH restricted on TP53 gene was reported in few tumors [20]. Vos et al. [24] reported 70% LOH

at chromosome 17 in poorly differentiated ductal carcinoma in situ (DCIS) cases versus 17% in well differentiated DCIS which indicated that p53 is the target gene in most cases. Another study [16] suggested the possible existence of two suppressor genes on chromosome 17, namely p53 gene at 17p13.1 and another one telomeric to it, and these two genes may be involved in the carcinogenesis of breast cancer. It was recently reported that LOH at 17p13.3 region is detected in breast cancer and it was shown to be a significant predictor of lymph node metastasis and may serve as a negative prognostic indicator [8]. As shown from the present results, LOH at TP53 region was reported in 14.3% of the informative cases examined. This low percentage of LOH as compared to other studies may be attributed to: first, the involvement of alternative mechanisms for inactivation of p53 gene rather than LOH such as different types of mutations; second, the deletions are targeted to another regions in the short arm of the chromosome; third, sample number limitation (25 cases only) which may decrease the possibility of high LOH appearance.

BRCA 1 gene is another tumor suppressor gene that is located at 17q21 and it encodes a large protein which is involved in DNA damage response checkpoint [25]. Increasing evidence suggests that BRCA 1 is involved in DNA damage repair [26]. BRCA 1 has been characterized and shown to be mutated in patients with familial breast and ovarian cancers [27]. LOH at BRCA 1 locus in breast cancer of Arabic women appeared to be higher than in other populations studied [28]. In the present study, search for chromosomal deletions by studying LOH at D17S855 and D17S856 microsatellite markers located in BRCA 1 region was done. The idea behind this was to investigate the involvement of BRCA 1 in sporadic breast carcinoma. The percent of LOH at the previously mentioned markers was 32% and 43%, respectively. These results were in agreement with those reported by Craig et al. [19] who found that the smallest common region that was deleted occurred in the approximately 120-kilobase interval between the D17S846 and D17S746 loci within BRCA 1 region. Silva et al. [29] reported an allelic loss at marker D17S856 in breast cancer patients and there was a correlation between LOH observed and poor prognosis. Similar results were obtained in the study of

Fukino et al. [30] who identified commonly deleted region including the BRCA 1 gene in sporadic breast cancers. LOH detected in D17S855 marker was shown to be associated with tumor grades, where 31% were grade 2 and only 11% were grade 3, while none of the cases examined were grade 1. These results indicated that LOH at BRCA1 gene may occur with low grade cases rather than in high grades. In previous studies, LOH detected at BRCA 1 and p53 genes correlated with early-onset sporadic breast cancer [8,31].

It was reported that BRCA 1 interacts with p53 and function as a transactivator in both p53 dependent and independent fashions [22]. The data reported showed no significant correlation between LOH in both onco-suppressor genes, p53 and BRCA 1. These results suggested the involvement of other molecular changes such as mutations which affect p53 gene and may have a role in tumorigenesis in BRCA 1 tumors. This suggestion was confirmed by the results of Tseng et al. [3] who found a higher frequency of LOH at chromosome 17 in BRCA 1 gene in tumors with an abnormal TP53. Their results revealed a possible link between an abnormal TP53 and specific genomic deletions of breast cancer susceptibility loci which may provide clues to the role of TP53 during breast tumorigenesis. On the contrary, results of this study disagreed with those of Niederacher et al. [33] who studied loss of heterozygosity in p53 and BRCA 1 genes in 121 cases of invasive ductal carcinoma using fluorescent PCR. They found a significant correlation between LOH in p53 and BRCA 1 genes. This disagreement may be due to differences in the sensitivity of the techniques used or to the limited number of samples in this study.

nm-23, non metastatic gene, is located on 17q22 and it has been proposed as a gene implicated in the control of metastasis on the basis of several reports indicating that the nm-23 gene or its protein expression decreased when the metastatic ability of the cell or tumor increased [34]. In the present study, LOH in nm-23 microsatellite marker was analyzed in invasive ductal carcinoma of breast samples to find out the correlation between LOH in nm-23 gene with lymph node metastasis. Thirty two percent of the samples analyzed showed LOH in nm-23 and the results indicated that

samples which exhibited deletion in one allele of nm-23 gene had a high number of metastatic lymph nodes. These results were in agreement with those reported by Generossonet et al. [35] and Vivi et al. [36] who found that nm-23 RNA levels were differentially expressed in human breast tumors and that low nm-23 RNA levels were associated with histopathological indication of high metastatic potential.

LOH of D17S250, which is centromeric to BRCA 1 gene, and D17S183, which is telomeric to the same gene occurred in 28% and 24% of the cases, respectively. These results suggested the presence of two independent regions on chromosome 17q that may contain tumor suppressor gene(s) and could be involved in sporadic breast carcinogenesis. Craig et al. [19] studied the involvement of several markers on chromosome 17 in sporadic breast cancers including D17S250 and D17S183 and the percentages of LOH recorded were 21% and 22%, respectively. Similarly, Futreal et al. [20] found that LOH at D17S250 marker was 50% in sporadic breast carcinoma and they concluded that a common region of deletion was flanked by D17S250 to D17S579. All markers were located in this region map within the region of an early-onset familial breast cancer locus (BRCA 1). They suggested also that the same gene or other genes may be involved in both familial and sporadic breast tumors. Deletion in D17S183 loci was shown to be associated with positive rather than negative lymph node cases which indicated that this gene may be associated with the metastatic potentiality of the tumor.

From the present study, it can be concluded that [1] Molecular changes represented by LOH in BRCA 1 gene could be associated with the development of sporadic breast cancer, [2]. Deletion in the non metastatic gene nm-23 is associated with the metastatic potentiality of the breast cancer, [3]. Allelic imbalance in p53 gene does not correlate with allelic imbalance in BRCA 1 gene, (4) The results throw a light on two independent regions (D17S250 and D17S183) on chromosome 17q12-12.3 that may contain a potential tumor suppressor gene involved in the development of sporadic breast cancer, [5]. Chromosome 17 is considered to be the most closely associated chromosome with the carcinogenesis of breast cancer, this is essentially due to the fact that it carries many

important tumor suppressor genes such as p53, BRCA 1 and nm-23 genes which are involved in such type of tumor.

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