Synchronous Breast Cancer and Gallbladder Diseases—A Chromosomal Analysis: A Pilot Study at a Tertiary Care Centre

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Abstract Out of a total of 50 study-eligible patients, 15 patients were found to have synchronous breast cancer and gallbladder diseases. Fourteen such patients had breast cancer and cholelithiasis while 1 patient had breast cancer with gallbladder cancer. Amongst these 15 patients, 7 patients tested positive for chromosomal aberrations on karyotyping. Aberrations in these patients were seen on chromosomes 13 and 17 with 2 patients having deletion 13q, 2 having deletion 17q, and 2 having deletions on 17p while one patient had multiple deletions on chromosomes 13q and 17p. The patient with multiple aberrations on 13q and 17p had a grade III infiltrating ductal carcinoma (NOS) of the breast with adenocarcinoma of the gallbladder. On the basis of our study and its analysis, we conclude that synchronous breast and gallbladder lesions are common amongst females and the occurrence of one should warn the clinician to search for the other. Also, chromosomal aberrations are common amongst patients with synchronous diseases and since these abnormalities are in the germ line cells, genetic counselling should be offered to the first- and second-degree female relatives.

Keywords Breast cancer · Gallbladder · Cholelithiasis · Cholangiocarcinoma · Gallbladder cancer · BRCA1 · BRCA2 · p53 · Lynch syndrome

Introduction

Breast cancer is a common disease in modern onco-surgical practice. With the advent of modern medicine, there has been a detailed study of breast malignancy in terms of its epidemiogenetics. With a resourceful infrastructure, we have been able to isolate the bizarre occurrences in the human genome that are responsible for tumorigenesis. Many genes are well known to us in modern medicine. Various aberrations at the gene level have been uncovered through years of research. Identification of BRCA1 and BRCA2 genes, the role of Her-2neu, etc. have facilitated superior management of the disease process overall. Yet, the disease remains elusive of a definite cure. Women are primarily affected. Amongst this population group, not only is breast cancer common, a lot of females also have gallbladder diseases. A dive into the historical aspects of gallstones and associated diseases has enlightened us regarding the similar nature of risk factors between gallbladder diseases and breast cancer. Gallbladder diseases especially gallbladder cancer have been analyzed for many years together with revelations of involved genes. The role of p53 in human cancers is irrefutable. With such familiar epidemiological and genetic factors, the synchronous presence of the two conditions warrants further investigations at the genome level. Nevertheless, assessment of synchronicity of these two common pathologies could unearth deeper understanding of the gene biology that underlies these two diseases.

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Aims and Objectives

- (1) To determine the incidence of gallbladder disease in patients with breast cancer

- (2) To study the clinical, radiological, biochemical and genetic profiles of patients with breast cancer and gallbladder diseases
- (3) To evaluate and stratify patients at risk of one disease in the presence of the other

Review of Literature

With the first description of hereditary breast cancer by Henry Lynch and subsequent detection of estrogen receptors within breast cancer cells, there was a lot of speculation regarding genetic alterations responsible for HBC and HBOC [1, 2].

In 1995, scientists from the National Institutes of Health (NIH) discovered that a particular alteration in the breast cancer gene called BRCA1 was present in 1 % of the general Jewish population. The researchers did a follow-up study in 1996 to estimate the cancer risk associated with this alteration as well as two other alterations subsequently reported to be present in the Ashkenazi Jewish population.

The primary purpose of the study was to estimate the risk of cancer associated with having three specific alterations in the breast cancer genes, BRCA1 and BRCA2. Two of the alterations tested were in the BRCA1 gene (185delAG and 5382insC) and one in the BRCA2 gene (6174delT).

With advances in gene amplification and mapping using DNA microarray, today, over 100 different alterations of BRCA1 and BRCA2 have been identified along with many other genes that are involved in the genesis of breast cancer.

In addition to inherited mutations, sporadic breast cancers exhibit “epigenetic” mechanisms for inactivating several important DNA repair genes including BRCA1, ATM, CHK2 and p53. In cancer, the main epigenetic mechanisms underlying abnormal gene expression include aberrant CpG-island-promoter methylation of specific TSGs, global changes in genomic DNA methylation and alterations in histone modification (deacetylation and methylation). These abnormalities can be reversed by inhibitors of both DNA methyltransferases and HDACs [3].

It has been postulated that promoter methylation may serve as the second hit in the Knudson two-hit model through inactivation of the normal allele of a TSG [4]. Hypermethylation of BRCA1 at promoter CpG islands occurs in a subset of sporadic breast tumors and may serve as a first hit by inactivating one BRCA1 allele followed by loss of the second BRCA1. BRCA1 methylation in sporadic breast cancer appears to result in a similar tumor phenotype as that seen in tumors of BRCA1 mutation carriers [4, 5]. In contrast, BRCA2 loss of expression via aberrant promoter methylation does not appear to occur in sporadic cancers.

One of the major epigenetic alterations that have been seen in patients with sporadic breast cancer is the expression of

estrogen receptor. This exists in 70 % of patients with sporadic breast cancers. ER is an epigenetically regulated gene that can undergo promoter methylation in a significant proportion of breast cancers [6].

Itoi et al. first discovered the role of p53 and K-ras genes in gallbladder cancer [7]. This was a pioneering discovery in the molecular biology of gallbladder cancer. Earlier, with Lynch having established the role of estrogen in p53 modulation in malignant cells, Wee et al. paved the path for molecular biology of gallbladder cancer having established the involvements of p53, p16, p21, K-ras and c-erbB2 [8].

Mutations in the ABCG5/G8 gene located on chromosome 2 have been held responsible for formation of cholesterol stones and also the same have been found in patients with cancer. A direct causal relationship is not yet proven, but with individuals having mutations in this gene, gallbladder stasis was longer and estrogen appears to facilitate such prolonged holding times of bile. All these combined increase the risk of malignancy [9].

K-ras and p53 mutations are the most common molecular alterations in gallbladder cancer [10]. Mutant p53 is found in 92 % of invasive carcinomas, 86 % of carcinoma in situ and 28 % of dysplastic epithelium. K-ras mutations are identified in 39 % of gallbladder carcinomas [11]. In one study, b-RAF mutations were evident in 33 % of gallbladder cancers. Overexpression of the c-erbB-2 gene and decreased expression of the nm23 gene product have been reported to play an important role in the development of gallbladder cancer. A few studies have demonstrated that CDKN2 (also known as MTS1 or p19^{ink4}) is an important gene in gallbladder carcinogenesis. Another candidate suppressor gene implicated in the development of gallbladder cancer is the fragile histidine triad (FHIT) gene. A recent study defined epigenetic inactivation of tumor suppressor genes SEMA3B and FHIT in gallbladder cancers, suggesting a role in gallbladder cancer pathogenesis [12].

Material and Methods

The present study was conducted in the Department of Surgery, Gandhi Medical College, and associated Hamidia Hospital Bhopal M.P. India, from October 2013 to October 2014.

A total of 50 patients were included in the study with a division into two respective arms of carcinoma breast and gallbladder diseases. Each arm included 25 patients. The diagnosis of the patients was made on the basis of available patient data. The sample of patients considered for inclusion in the study was of adult females with no history of any surgery or chemoradiation therapy.

Any patient who had a breast lump with/without axillary lymph nodes with histological proof of malignancy was

included in the study. Also, females with h/o of dyspepsia, right-upper-quadrant pain with ultrasound-proven gallstones or gallbladder mass were included in the study.

On admission, a detailed history regarding any previous surgery or chemoradiation therapy was taken. Patients with any such positive history were excluded from the study. All 50 patients were subjected to examination by the same surgeon, radiologist and pathologist.

Blood work was drawn on the day of admission for all basic routine investigations and karyotyped and processed on the very same day.

Observation

A total of 50 patients were enrolled in the study after fulfillment of inclusion criteria. We observed that 48 % of breast cancer patients were in the age group of 41–50 years while a total of 24 patients in this age group had gallstones. Synchronicity of the two diseases was seen in a total of 15 (60 %) patients. These 15 patients had breast cancer as well as gallbladder pathology (Figs. 1 and 2).

One of the variables studied was the presence or absence of gallbladder diseases in patients with breast cancer in respect to the size of the primary tumor. Amongst 25 patients, 15 patients (60 %) had a lump size more than 25 cm³, and out of these, 10 patients (66.7 %) had gallbladder disease.

In this study, ultrasound was used to assess the Breast Imaging Reporting and Data System (BIRADS) grading of the breast tumors. It was noticed during the course of the study that the incidence of gallbladder pathologies was higher in women with high-grade BIRADS lesions (Fig. 3).

Chromosomal aberrations during the study programme were analyzed using the standard karyotype technique. We studied 25 women with breast cancer and found 7 (28 %) to have chromosomal abnormalities. Out of these patients, 2 had chromosomal 13 involvement while 4 had chromosomal 17 aberrations. One patient, however, had involvement of 13q and 17p chromosomes (Fig. 4).

A comparison of BIRADS grade of the breast lesions with chromosomal aberrations was also done (Fig. 5).

Fig. 1 CA breast and GB disease

Fig. 2 Lump size of CA breast and GB disease

Discussion

In this case series with 50 patients enrolled, chromosomal aberrations were studied with respect to epidemiological factors known to medical science.

Amongst the 50 patients included in the study, the highest incidence of breast cancer was seen amongst women in the age group of 41–50 years (48 %). Ferlay et al., in their world-wide study on cancer incidence and mortality, demonstrated the mean age for breast cancer to be 40–50 years [13].

The study had a total of 40 patients with gallbladder diseases [25 patients with only gallbladder pathologies and 15 patients who had breast cancer and synchronous gallbladder diseases]; amongst them, we observed that gallbladder diseases were most commonly seen in the age group of 41–50 years (47.5 %). Gallbladder cancer was seen in one patient who was more than 60 years of age. Dutta et al. in their retrospective analysis of data from two tertiary centers of India reported a mean age of 30–50 years for patients with cholelithiasis and a mean age of 60 years for patients with gallbladder cancer [14].

We studied the synchronicity of the two diseases, and it was observed that amongst the 25 patients with breast cancer, 15 patients (60 %) had a co-existence of gallbladder diseases (14 had cholelithiasis and 1 had gallbladder cancer). Mohamed et al. in their study of 90 patients with invasive breast cancer reported an incidence of 30 % synchronicity of the diseases. However, their study included only post-menopausal women and our study had both groups viz. pre-menopausal and post-menopausal.

Fig. 3 BIRADS classification and GB diseases

Fig. 4 Chromosomal aberrations

BIRADS is the most commonly used radiological parameter to report breast lumps as benign or malignant. In our study, we used ultrasonography to establish the BIRADS grade for the breast tumor. We compared the BIRADS grade of the tumor with the presence of gallbladder pathologies. Estrogen is known to increase breast density, and thus, high serum estradiol levels are associated with higher BIRADS grading of the tumor. Mokbel established this fact in 2002 [15]. We studied the BIRADS grade of tumors and presence of gallbladder diseases. We observed in our study that women with higher-BIRADS-grade lesions had a higher incidence of gallbladder diseases. Amongst 40 women with gallbladder pathologies (25 with benign breast diseases and 15 with breast cancer), we observed that 18 (45 %) women had a BIRADS grade 4 breast disease. We suggest that women with higher-BIRADS lesions should be screened for synchronous gallbladder pathologies to detect early cancers.

One of the end points in this study was to establish an association between breast cancer and gallbladder diseases at the chromosomal level. Chromosomal analysis was done using standard karyotype to assess alterations. Although a primitive approach for genetic association, nevertheless, we observed a significant number of patients having anomalies when they had synchronous pathologies. With 15 synchronous cases, 7 patients had anomalies in their karyotype. Aberrations on chromosomes 13 and 17 involving both the short and long arms were observed. Deletions on 13q, 17q, and 17p were observed in 2 patients each, while 1 patient had deletions on 13q and 17p. This patient with two chromosomal aberrations was 70 years old and had breast cancer at the time of presentation along with histopathological evidence of

Fig. 5 No. of abnormal karyotypes and BIRADS class

adenocarcinoma bladder. Ultrasonography-guided biopsies were taken from the gallbladder and liver metastasis to establish the histopathology and differentiate these lesions from the possibility of breast cancer metastasis. We report the findings of the “Institute of Cytology and Preventive Oncology Annual report 2003-05”. They studied the patterns of mutations in sporadic breast cancers and reported alterations in BRCA1 and BRCA2 genes at transcriptional levels in sporadic breast cancer. These alterations reported were different from those observed in familial breast cancer. They also reported the role of altered p53 in 25–30 % of patients with sporadic breast cancer. As we know, literature is flooded with evidence of the dominant role of p53 mutations in gallbladder cancer. Thence, we postulate the role of p53, BRCA1 and BRCA2 mutations in the causality of breast cancer with synchronous gallbladder lesions.

We purport the alterations of genes located on chromosomes 13p, 13q, 17p and 17q to have a significant role in the development of breast cancer and also the synchronicity of gallbladder diseases. With knowledge of effects of estrogen on the p53 gene, we have hypothesized the co-existence of breast cancer and gallbladder diseases to be of significant importance that needs further scientific exploration.

Conclusion

With no causal and temporal relationship yet established between the two conditions, it is imperative that further investigation be done at the genomic level. It appears as though a diverse array of genomic aberrations yet to be mapped for breast cancer and also the genetics of gallbladder disease are not yet fully understood. We do not postulate a hypothesis here. Rather, we feel that there is a need to carry out detailed studies to identify exact coding errors to promote research in gene therapy. The authors also feel that genetic counselling be offered to patients of either disease.

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