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Case Report
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Next Generation Sequencing in a Young Female with Invasive Ductal Carcinoma of Breast Associated with DCIS: A Medical Case Study and Recommendations for Practice

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Abstract

Patient: Female, 43.

Final diagnosis: Invasive Ductal Carcinoma with ductal carcinoma in-situ.

Symptoms: A hard palpable mass at the upper outer quadrant of right breast not associated with pain or discharge.

Medication: Adjuvant chemotherapy, adjuvant hormone therapy.

Procedure: Breast Conservation Surgery (BCS) with Oncoplasty, Adjuvant Radiotherapy.

Background: Incidence of Breast Cancer (BC) in young Indian women is on the rise in both urban and rural India. Causative factors for the upsurge of hormone positive as well as hormone negative cancers in young premenopausal women are yet to be identified. If National Comprehensive Cancer Centre Criteria (NCCN) guidelines are followed for genetic testing 50% patients who might be harboring pathogenic mutation of clinical significance might be missed. So, multigene testing should be regularly done in high-risk women to identify other genetic mutations that might contribute to the genesis, progression of breast cancer apart from BRCA1/2.

Case Report: This is a case report of a young woman (43 years) suffering from carcinoma of her right breast who underwent multiple gene panel testing as a part of her disease workup revealing interesting findings that might have implications for future recommendations.

A large right breast lump affecting upper and upper outer quadrant of right breast was found to have a small focus of invasive carcinoma associated with segmental DCIS. She underwent BCS with oncoplastic reconstruction, Sentinel Lymph node biopsy. She received adjuvant chemotherapy followed by adjuvant radiotherapy. She is now on tamoxifen and under follow up for more than a year.

Conclusion: Rising incidence of BC in young women in India needs further research to identify probable genetic factors that might be responsible for the disease. The multigene panel test findings in our case have shown that many other mutations might be causatively associated with breast cancer in young women apart from the common BRCA genes. Multigene testing by NGS has the potential to provide significant insight into hitherto unknown genetic factors and thus identify targets for chemoprophylaxis, chemotherapy and cancer management.

Keywords: Breast cancer; Young Indian women; Invasive ductal carcinoma; Next generation sequencing

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Introduction

The National Comprehensive Cancer Network (NCCN) has set up standards for genetic testing in breast cancer. Following these guidelines excludes many young Indian women with breast cancer who do not have a family history of breast or in fact any type of cancer. It has been reported that nearly 50% of women breast cancer patients are carriers of germline pathogenic/likely pathogenic mutations which will not be identified if genetic testing is done following NCCN

criteria [1]. The epidemiology of breast cancer in Indian women is significantly different when compared with the western population. Striking dissimilarities between them are with respect to median age of onset of the disease, stage at which the disease is detected and lack of awareness amongst the masses. In India, the peak age of women diagnosed with breast cancer is 40 to 50 years which is almost a decade earlier than the west [2]. Nearly 75% of the Indian population is less than 50 years of age when diagnosed with breast cancer [3]. Further, breast cancer is diagnosed in young Indian women at an advanced stage of the disease which is marked by high pathologic grade, rapid proliferation rate, large tumor size and predominance of triple negative breast cancer [4].

Advancement in the screening, imaging and diagnostic strategies have led to the early detection of the disease. However, early detection of breast cancer in women less than 40 years of age is more challenging as they are excluded from the standard breast screening program guidelines because of cost-benefit issues and higher mammographic breast density compared with older women.

The past decade has seen tremendous breakthrough in the field of oncologic research, be it in terms of therapy, screening or molecular research [5]. Genomic research and gene panel testing has opened new avenues for precision oncology. Breast cancer is an intricate and incomprehensible disease when it comes to analyzing the risk factors which can be genetic or environmental. Only 10% to 15% of breast cancers are inherited out of which *BRCA*1/2 mutations account for only 4% to 6% of it [6].

Next Generation Sequencing (NGS) is a new platform in genomic research that provides reading lengths as long as the entire genome, thus reducing the cost of sequencing and making gene panel testing an integral part of clinical diagnosis and treatment [7]. Next generation sequencing has greatly improved our understanding of breast cancer, especially highlighting on the identification of various molecular markers which control disease diagnosis, prognosis and treatment. Germline mutations in *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *AKTI*, *CDH1*, *GATA3*, *RB1* and *APC* identified to have higher incidence of breast cancer. However, not much information is available about the genetic basis of sporadic breast cancer. So, for young Indian women, without any family history of cancer, genetic testing has to be done to identify other genetic mutations that might be responsible for breast cancer.

Every case of BC is unique with respect to its genetic basis, pathological findings and molecular studies. The current study was undertaken to understand the cause and probable reasons of invasive breast cancer with Ductal Carcinoma *in situ* (DCIS) in a 43-year-old premenopausal woman with no family history of cancer, no comorbidities like hypertension, diabetes, and no habituations (alcohol and tobacco).

Clinical Findings

Patient history

A 43-year-old female with no known comorbidities was diagnosed with carcinoma of her right breast with DCIS. She did not have a family history suggestive of cancer. A mother of two children, she had her first child at 29. She had her second child at the later age of 39 years. On examination she had a swelling in the Upper Outer Quadrant (UOQ) of right breast in the 9 to 12 O'clock position. On palpation, a hard irregular mass about 7.5 cm × 5 cm adjacent to the Nipple Areola Complex (NAC) was observed. NAC appeared edematous with nipple deviation towards the mass. She was advised to do undergo bilateral breast mammography. The right and left breast were found to be heterogeneously dense ACR type-C, predominantly composed of fibroglandular element seen interspersed with fibrofatty element. Multiple coarse heterogeneous and linear ductal calcifications were primarily distributed in the upper outer quadrant of the right breast.

As part of standard mammographic examination high resolution ultrasound was performed. Hypoechoic mass with irregular spiculated margin was seen in the upper outer quadrant of the right breast. The lesion measuring 22 mm × 8 mm with microcalcification in 9 to 10 O'clock was found reaching up to the retroareolar region. An ill-defined heterogenous lesion with microcalcification measuring 19 mm × 9.2 mm was seen in 10 to 11 O'clock position in the upper quadrant of the right breast. The lesion was continuous with the above-mentioned lesion suspicious of malignancy, BIRADS 5/6.

Magnetic resonance imaging

Due to discrepancy in the results (dimensions) of mammography, CBE and ultrasound, the patient was asked to do contrast MRI of her breasts. An irregular T2STIR hyperintense mass lesion with spiculated margins was seen in the upper outer quadrant of the right breast. It showed early enhancement with rapid washout (Type-III) curve on contrast kinematics. The lesion measured approximately 7.2 cm × 2 cm on MRI.

Immunohistochemistry

USG guided core biopsy was performed which confirmed the suspicion of malignancy as invasive ductal carcinoma with DCIS. The receptors were ER positive and PR positive showing strong nuclear positivity in 60% and 50% of tumor cells respectively. HER-2/neu receptors were not found in tumor cells and Ki-67 was found to be 5%.

Positron emission tomography-computed tomography

Increased FDG uptake was observed in irregular heterogeneously enhancing lesion with spiculated margins measuring 4.9 cm × 3.1 cm with overlying skin thickening and absence of satellite nodules. No distant metastasis was seen.

Gene panel testing

The patient working in the field of molecular biology and mother of two daughters was interested to know the probable cause of the early onset of the disease and whether there is any familial risk. *BRCA*1/2 mutations were discussed as well as multigene panel testing. Due to absence of family history, the patient was more interested to do a gene panel testing. One hundred and fifty-two (152) gene panel testing were done by Genecore diagnostics New Delhi. Massively Parallel Sequencing (Next Generation Sequencing) Genomic DNA from the submitted specimen was enriched for the complete coding regions and splice site junctions of genes using custom bait-capture system. Paired End Sequencing was performed with 2×100/2×150 chemistry, on an Illumina platform. Reads were assembled and were aligned to reference sequences based on NCBI RefSeq transcripts and human genome builds GRCh37/UCSC hg19. Data was filtered and analyzed to identify variants of interest and interpreted in the context of a single most damaging, clinically relevant transcript for the purpose of the report, indicated as a part of variant details. Sequence and copy number variants were reported according to the Human Genome Variation Society (HGVS). Tools and Databases employed for analysis: ClinVar, OMIM, HGMD, UCSC genome browser, UniProt, Ensembl, dbSNP, gnomAD, ExAC, PubMed, DGAP,

ICGC, Kaviar, various bioinformatics analysis, predictive tools and disease specific databases used as appropriate. In the current study a missense mutation at codon 2505 of *APC* gene (C.7513) CGG to GGG substitution of arginine for glycine was observed. A single nucleotide variant was identified in the *PALL D* gene, where the amino acid Arg at position 896 is changed to a Gln.

Treatment

After detailed discussion in the multidisciplinary tumor board and multiple rounds of discussion with patient and her family members, she underwent BCS with sentinel lymph node biopsy utilizing Indocyanine Green (ICG). An infrared camera was used for sentinel lymph node biopsy with frozen section assistance taken for sentinel lymph node and lumpectomy margin assessment. Specimen mammography was done to confirm adequate excision of DCIS component. Reconstruction of defects of central and superior lateral quadrant was done with an extended LTAP (Lateral Thoracic Artery Perforator) flap. Histopathology report showed Invasive Ductal Carcinoma, NST with histological Grade I. DCIS was present in form of solid cribriform, papillary pattern and Comedonecrosis. Two weeks later chemoport placement was done. Three weeks post-surgery, adjuvant chemotherapy was started. She received 4 cycles of Epirubicin 160 mg and cyclophosphamide 860 mg and 4 cycles of docetaxel (Taxocare) 115 mg at 21 days interval for a span of 6 months. There were no pronounced side effects and the blood parameters and other vitals were normal throughout the chemotherapy cycles. After 21 days of completion of chemotherapy she received adjuvant Radiotherapy (RT) to right breast, SCF and axilla to a dose of 40 Gy/15 fractions for 30 days at 2.67 Gy/fraction with IGRT technique followed by boost to the postoperative cavity 12.5 Gy/5 in True Beam LINAC. Post RT, the patient was put on tamoxifen. She is now under follow up for more than one-year post-surgery.

Discussion

Breast Cancer (BC) was earlier thought to be a disease of the western world, but in the past two decades, it has had a significant rise in incidence in the Indian sub-continent. Several studies have predicted that the worldwide incidence of BC is likely to cross almost 2 million by the year 2030 [8]. According to the GLOBOCAN data 2020, in India, BC is the most common cancer in women comprising of 13.5% of all types of cancer with a mortality of 10.6%. The National Cancer Registry Program of India has predicted the total cases of breast cancer would be 2,30,000 per year by the year 2025 with a major portion of it being young women. According to the GLOBOCAN 2020 data, incidence of new cases was 34,65,951 with 11,21,413 deaths worldwide while India registered 12,04,532 new cases with 436,417 deaths [9]. The peak age of breast cancer in India is 40 to 50 years, while in UK it is 60 to 65 years. In United States only 5% to 7% cases of BC are detected in women below the age of 40 years [10]. So, the burden of the disease and the ratio of incidence to mortality have a completely different scenario in India as compared to the west. The clinicopathological profile of BC in Indian scenario is also completely different from the western population. Prognostic tests like MammaPrint and Oncotype DX used to predict a recurrence score for western women cannot be completely relied upon for Indian women.

Of further concern for young women is the fact that sensitivity of usual screening modalities like mammography and ultrasound is less than 70% for dense breasts. Hence, early detection of breast cancer in young women remains a challenge. Again, young age itself is a negative prognostic factor, because it increases the probability of local and distant recurrence and contralateral breast cancer [11].

A study carried by Yadav et al. [12] reported that a substantial portion of women with BC carrying pathogenic mutations do not qualify for testing if NCCN guidelines are followed. In addition, the spectrum of genetic mutations associated with breast cancer in India is yet to be defined at par with the western population as there is high prevalence of mutations or likely pathogenic mutations currently classified as Variants of Uncertain Significance (VUS) as per available data, most of which comes from the western population. It is the need of the hour that there should be radical change in the screening and treatment practices targeting young Indian women suffering from BC. It is thus important that germline testing should be a part of cancer management and treatment in India. With the reduction in cost of genetic testing and increasing availability, more and more patients should be advised for germline testing.

In the current study, the patient did not have any family history of cancer, nor any comorbidities and habituations like tobacco or alcohol. She opted for germline testing to understand the probable cause and identify genetic predisposition, any factors predictive of recurrence or metachronous cancers and response to adjuvant chemotherapy. A multigene panel testing was done to identify pathogenic/likely pathogenic variants. Multigene testing detects high rates of pathogenic mutants or (VUS) that has to be properly analyzed. VUS are variants usually found in non-coding regions as missense or synonymous mutations with very little clinical information due to insufficient data. With increasing access to genetic testing, the percentage of VUS will also increase which has to be properly addressed as a part of clinical cancer management protocol. Majority of data on VUS in international databases is from the research on western population with very few data from Indian population. There will also be a significant decrease in VUS as data from various ethnic population increases [13]. If more and more patients go for gene panel testing, huge data will be generated that will definitely contribute in building up Indian cancer data registry. Novel biomarkers might also be identified that is/are specific to the Indian population which can be used in preparing POC (Point of Care) diagnostics. Thus, genetic tests can help in understanding metachronous cancer in patients and disease recurrence which can be checked by timely surgical and nonsurgical prophylactic interventions [14]. Thus, genetic counselors can do pretest counseling focusing on VUS and give recommendations for risk reduction options.

Some commonly studied genes for breast cancer include *BRCA1*, *TP53*, *PTEN*, *AKTI*, *CDH1*, *GATA3*, *RB1* and *APC*. Few other genes have also been reported to cause somatic mutation in tumors, these include *APC*, *ARID1A*, *ARID2*, *SMAD4*, *ASXL1*, *MAP2K4*, *BAP1*, *MLL2*, *MLL3*, *KRAS*, *NF1*, *SETD2*, *SF3B1*, and *STK11*. The Adenomatous Polyposis Coli or *APC* gene is located at chromosome 5q22.2. *APC* gene is expressed in most tissues including lungs, liver, kidneys and mammary glands. *APC* mutations are major contributing factor for colorectal cancer but in breast cancer the mutation rate ranges from 0.4% to 18% [15]. Epigenetic change like hypermethylation of the CpG islands in the *APC* promoter region results in silencing of the transcript and loss of protein expression [16]. Silencing of this tumor suppressor gene results in the initiation of breast cancer. Somatic mutation in the *APC* gene has been observed in advanced stages of primary breast cancer majority of them found at

G residues, G to T change. Kashiwaba et al. [17] first reported the role of *APC* mutations in breast cancer. They isolated mutated *APC* gene from tumor tissue and by direct DNA sequencing found mutations at codon 1081 (AGC to ATC) resulting in substitution of serine for isoleucine and at codon 1096 (CUG to CAT) resulting in substitution of glutamine for histidine in 6% of the tumors. In the current study a missense mutation at codon 2505 of *APC* gene (C.7513) CGG to GGG substitution of arginine for glycine was observed. Some peculiarities that have been reported about *APC* mutation is that almost all mutations have occurred in the first half of the coding sequence and somatic mutations for colorectal cancer have been clustered in the Mutation Clustered Region (MCR) (codons 1286-1513) [18]. Further, Hayes et al. [19] reported only one mutation in two samples after analyzing exon 15 in twenty-seven metaplastic carcinoma cases. Similarly, Stephens et al. [20] used whole exome sequencing and detected only two somatic alterations in 100 breast cancer patients. Thus, *APC* gene mutation has already been reported for sporadic primary breast cancer cases, but the percentage is very less. Since the mutation is not in the MCR region it can be hypothesized that the patient's chances of getting colorectal cancer is minimal.

PALL D gene encodes for a cytoskeleton protein, which is a component of actin containing microfilaments that controls cell shape, adhesion and contraction. Polymorphisms in this gene are associated with pancreatic cancer type1. From the ClinVar database it has been identified as a single nucleotide variant, where the amino acid Arg at position 896 is changed to a Gln changing protein sequence. The missense c.2687G>A (p.Arg896Gln) variant in *PALL D* gene has not been reported previously as a pathogenic variant nor as a benign variant. Transcript variant has been identified to be palladin isoform 1. *PALL D* gene encodes for multiple isoforms of the Palladin actin associated protein of which nine distinct isoforms have been identified [21]. Palladin isoform 4 (90kDa) and isoform 3 (140Da) have been reported to control invasive behavior of metastatic breast cancer cells while isoform 1 (200kDa) and isoform 4 are not expressed in the pancreas. Since this isoform1 is not expressed in the pancreas so the chances of patient having pancreatic cancer is lowered. The analysis is completely based on the NGS data and it has to be further validated with Sanger Sequencing. The purpose of this multiple gene panel testing was done to identify high and low penetrance susceptible genes for this cancer. This would help the patient and her family to assess the hereditary risk management. In the current study plasma based NGS was done, as it detects genetic mutation at a higher frequency compared to tissue specimen [22-37].

Conclusion

Our study provides information on the VUS mutations and is likely to add to the growing body of information on different genetic variants from India. The novelty of the study is identification of two new missense mutation *APC* and *PALL D* gene by NGS sequencing, which has to be further validated by Sanger sequencing. As a roadmap for breast cancer management in young Indian women we recommend that multi-gene panel testing should be made mandatory for all young women with breast cancer patients. It is also very important that genetic counselors be a part of every multidisciplinary oncology team. All oncologists should be sensitized, so that they recommend muti gene panel testing for young women with breast cancer. However, it is important that the results have to be properly explained to the patients with respect to any implications on recurrence, metastasis and disease-free survival. Further, professional bodies associated with breast cancer management should negotiate with insurance companies to include multi-gene testing in the list of allowed tests for young women with breast cancer, so that more patients are able to afford it. If more and more patients go for gene panel testing, huge data will be generated that will definitely contribute in building up Indian cancer data registry. Novel biomarkers will also be identified that is specific to the Indian population which can be used in preparing POC (Point of Care) diagnostics.

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