Comparison of Clinicopathological Characteristics of BRCA1 Associated Breast Cancer Cases with BRCA1 Negative Breast Cancer Cases: A Prospective Study of 100 Women in a Tertiary Care Cancer Hospital

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Received: 9 December 2013 Accepted: 1 January 2014 Published: 15 January 2014

Abstract

Aims and Objectives: Mutations in BRCA1 gene are associated with greater risk of developing breast cancer. We determined whether clinicopathological characteristics of the tumour differ in patients with and without BRCA 1 expression.Materials and Methods: The study was conducted in 100 cases of breast carcinomas received as lumpectomy or mastectomy specimens in the

Index terms— breast carcinoma, immunohistochemistry, ER, PR, BRCA 1.

1 Introduction

reast cancer is the most common malignancy in women. It is the commonest cause of death in developed countries in middle aged women and is becoming frequent in developing countries as well. (1) It has been estimated that by 2020, 14% of the world's Author ? ? ? ? ¥: SGRDIMSAR, Amritsar. e-mails: manisha_salwan@yahoo.com, mriduvikram@yahoo.com, drmmgoyal113@gmail.com, manasmadaan@gmail.com, dr.gazalsra@yahoo.com cancer cases will be in India. An analysis of breast cases among women in Delhi, Mumbai, Chennai and Bangalore between 1982 and 2005 performed by the ICMR revealed that the number of breast cancer cases have more than doubled in the last 10 years. (2) Mutations in the tumour suppressor gene BRCA 1 are believed to be responsible for majority of hereditary breast cancers. It is estimated that women with BRCA 1 mutations have a lifetime risk of developing breast cancer as high as 87% and 50% of increased chances of its expression in blood relatives of BRCA1 positive patients. (3) Breast cancer susceptibility gene 1 (BRCA 1) associated breast cancers often occur in younger women and such tumours are of high grade and lack hormone receptors according to several studies. (4,5) Many studies have shown that BRCA 1 associated breast cancers are associated with adverse histopathological features suggestive of aggressive cancer phenotype (6,7) but other studies failed to demonstrate such association. (8) This study on 100 breast cancer patients has been done in an attempt to better define the relationship between BRCA1 mutation and tumour grade, tumour stage and Estrogen receptor (ER), Progesterone Receptor (PR) expression.

2 II.

3 Materials and Methods

4 a) Histopathology

Haematoxylin-Eosin stained sections from 100 formalin fixed breast cancer specimens received as mastectomy and lumpectomy specimens over the period of one year (Nov 2013-Oct 2014), were diagnosed in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and research, Amritsar. The tumours were graded

from grade I to grade III according to Nottingham Modification of Bloom-Richardson method taking into account the parameters-tubule formation, nuclear grading and number of mitosis /HPF. The tumours were evaluated for the histological types, lymphocytic stromal response, nuclear chromatin pattern and nucleoli. Lymph nodes

5 b) Immunohistochemistry

IHC was performed by using the antibodies against the estrogen receptors (ER), the progesterone receptors (PR) (Diagnostic Biosystem) and BRCA1(Biocare Medical).The antigen retrieval was done by using pressure cooker method with 10 mmol citrate buffer at pH 6.0. Tris buffer was used as the wash buffer and Diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. The endogenous activity was blocked by using hydrogen peroxide. After protein blocking, the slides were incubated overnight with the available ER, PR and BRCA-1primary antibodies and they were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy. ? 10% nuclei stained brown were taken positive for ER and even 1% stained were taken positive for PR. For BRCA-1 this value for positive stained nuclei was ? 30%.

6 III.

7 Results

The age of presentation ranged from 26-70 years with a mean of 52.3 years. The maximum number of patients were in the age group of 41-60 years (60% of the patients). Left sided breast cancer cases (55%) outnumbered the right sided cases (45%), and the upper outer quadrant being the most commonly involved site (61%).

The size varied from 1.5-5.0 cms with maximum number of cases being > 2cms (65%). All the tumours were infiltrating ductal carcinoma NOS (not otherwise specified). 4/100 cases were graded grade I, 67/100 as grade II and 29/100 as grade III. Lymph nodes were recovered in 86 cases out of which 50 cases showed lymph node involvement. 30 cases were ER + and PR + and 5 cases were ER + and PR-. They were taken together as positive for ERPR (35 cases). BRCA 1 positivity was seen in 36/100 cases. Out of these 36 BRCA1 positive cases, 06 cases also showed positivity for ERPR. Rest 29 positive ERPR cases were BRCA 1 negative. While correlating ER, PR and BRCA1 expression it was concluded that BRCA1 positive cases showed a significantly lower ER PR expression as compared to BRCA 1 negative cases with p=0.008 (Table 1).BRCA1 positivity was found to be associated with higher grade of tumour (p=0.015) (Table 2). Similarly lymph node involvement was significantly higher in BRCA 1 positive cases as compared to BRCA 1 negative cases.(p=0.001) (Table 3).BRCA 1 positivity was higher in cases where tumour size was >2 cms (p<0.001)(Table 4).

In contrast, ER PR expression was associated with favourable prognostic markers-lower tumour grade, smaller tumour size and less number of lymph nodes involved.

8 Figures

Figure 1 (A, B) Figure **??** (A, B, C, D) IV.

Figure Legends

9 Discussion

The incidence of breast cancer in India is on the rise and is rapidly becoming the number one cancer in females pushing cervical cancer to second spot. (2) It is widely acknowledged that breast cancers show several characteristics which play an important role in their diagnosis and treatment. So all the variables including age of the patient, grade of the tumor, immunohistochemistry and genetic profiles are important associated factors.

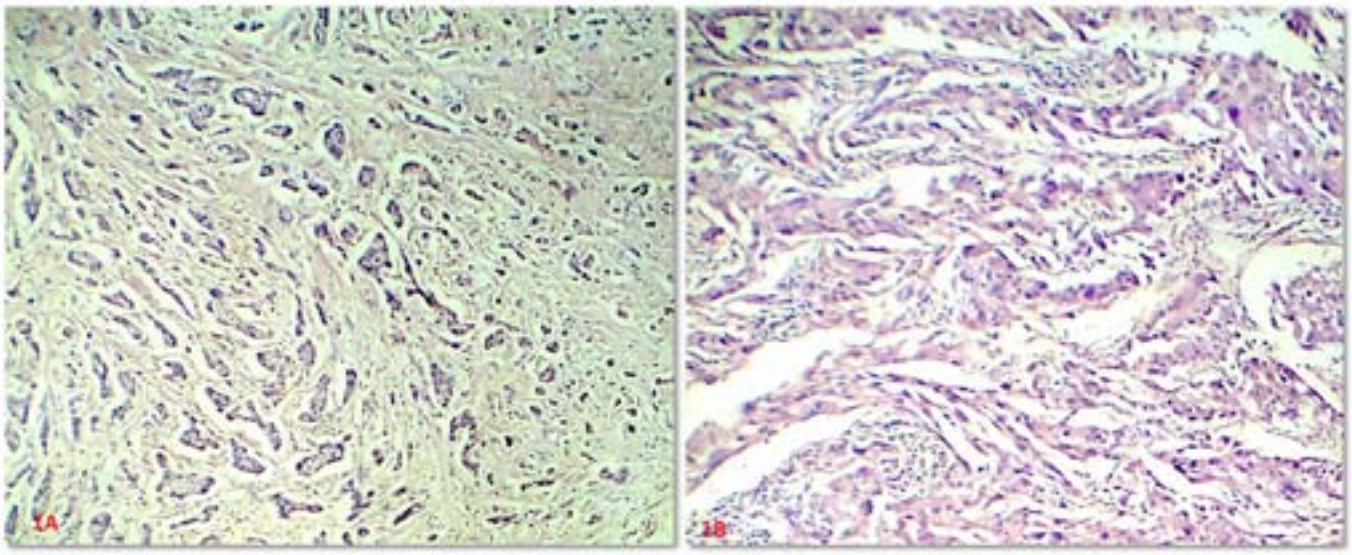
Age is an important factor as with the advancing age risk of development of breast cancer increases. Age of the patients varied from 26-70yrs with maximum number of cases in the age group of 41-60 yrs (60%) with mean age of 52.3 years. In different parts of the country similar results regarding maximum number of cases in this age group have been reported. In these studies mean age of breast cancer patient was found to be lower than the western countries counterpart by an average difference of one decade. (9,10) In the immunohistochemical profile -ER PR positivity was calculated to be 35 % which is in concordance with various studies conducted in this part of continent. (11,12) ER PR positivity was found to correlate significantly with favourable prognostic factors as lower grade of tumour (p=0.012), less number of lymph nodes involvement (p=0.009) and smaller size of the tumour at the time of presentation (p<0.001) .This result is consistent with previous reports. (13,14) BRCA1 positivity was seen in 36 out of 100 cases which is similar to other various studies where positivity varied from 25% to 35%. (15,16) In this study we compared the clinical and pathologic characteristics of BRCA 1 positive cases with BRCA 1 negative cases. Significantly more of the BRCA1 positive were ERPR negative (30/36-83%) than BRCA1 negative cases (35/64-54%). Several studies have evaluated decreased hormone expression in BRCA 1 mutation carriers. In these studies 60%-85% of BRCA 1 carriers were diagnosed ERPR negative as compared to 20%-40% of BRCA 1 non carriers. (16,17,18) Similarly more of the BRCA1 related cancers had higher tumour grade i.e. grade III (22/36-61%) as compared to BRCA1 negative cases (7/64-11%). This result is in concordance with previous studies which have shown that BRCA 1 related cancers were of higher histological grade. (16,17) In a study conducted in Jewish women, 76.5% of BRCA 1positive tumours had a higher nuclear grade as compared

to only 27.3% of BRCA 1 negative tumours. (17) The individuals with BRCA1 mutation were significantly less likely to present with stage1 disease. BRCA1positive cases had higher number of lymph nodes involvement (p= 0.001) and tumour size > 2 cms at the time of presentation (p<0.001). These results are similar to those reported previously in the literature. (18) It was proposed that there is some intriguing mechanism of interaction between BRCA 1 and other molecular markers. BRCA 1mutation is followed by p53 dysfunction and cancer cells become ERPR negative, hence favouring the unique profile of BRCA 1mutant tumours. (19) Despite the presence of association of BRCA 1with poor prognostic markers, several studies have failed to demonstrate a worse clinical outcome in such patients as compared to their counterparts. No significant difference was seen between BRCA 1 associated and non BRCA 1associated cases in five year relapse free survival, five year event free survival and five year overall survival. However women with germline BRCA 1mutation significantly belong to younger age group and more likely to develop contralateral breast cancer and ovarian cancers. (5) In the above study, several statistically significant correlations were found between BRCA 1positivity, clinical variables and molecular markers. Women with BRCA 1 mutations have a life time risk of developing breast cancer as high as 87% and 50% of increased chances of its expression in blood relatives of positive BRCA 1. (3) So all the blood relatives of patients with BRCA 1positivity on IHC should be screened for BRCA 1 gene mutation.

Our findings suggest that BRCA 1 positive tumours have a unique molecular profile and a different mechanism of tumorigenesis. These tumous are of high grade, higher stage at presentation and ERPR negative. Whether the more aggressive biological phenotype of BRCA1 tumours justifies more aggressive treatment is still a matter of debate. Further studies should determine whether treatment outcome differs for BRCA 1 mutation carriers as compared to BRCA 1negative counterparts.

10 Bibliography

Figure 1: BC



1

Figure 2: Figure 1 (

1

ER + PR-	02	03	05
ER-PR+	00	00	00
ER + PR +	04	26	30
ER-PR-	30	35	65

Figure 3: Table 1 :

2

1	02	-	02	04
2	28	14	39	53
3	05	22	24	07

Figure 4: Table 2 :

3

Year 2014
Volume XIV Issue VIII
Version I
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ER PR status	BRCA 1 Positive		Negative BRCA1	Total
Grade	ERPR+	BRCA 1+	ER-PR-	BRCA 1-
Lymph	ER+	ER-	BRCA 1 +	BRCA 1-
Nodes	PR+	PR-		
N0	13	23	05	31
N1 (1-3)	10	04	09	05
N2 (4-9)	07	19	16	10
N3 (?10)	03	07	06	04

Figure 5: Table 3 :

4

<2cm	22	13	09	26
>2cm	13	52	27	

Figure 6: Table 4 :

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